doric

Fiber Photometry System

Getting Started Guide

Version 2.2.1

Contents

Fiber Photometry Systems at a Glance

1.1 Systems Overview

In neuroscience, **Fiber photometry** denotes a method whereby chronically implanted optical fibers deliver excitation light to neurons tagged with a fluorescent calcium indicator and collect their overall activity-induced fluorescence. **Fiber photometry** sums up the activity-induced fluorescence of all neurons expressing the indicator(s).

In addition to systems custom-designed for your needs, we offer systems for typical modular fiber photometry measurement setups. The *Locked-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations* system measures the 405 nm (isosbestic point) excited GCaMP fluorescence, and the 465 nm excited calcium-dependent GCaMP fluorescence, on a single photodetector. The *Separated Two Fluorophores Fluorescence* contains all the items necessary to perform photometry measurements of two independent colors in freely-moving animals for GFP-like and RFP-like fluorophores.

A typical set-up for freely behaving animals contains of the following elements (Fig. 1.1).

- A *Fiber photometry console* to synchronize output control and the acquisition of data.

- An *Integrated fluorescence mini cube* where beam splitters combine the excitation wavelengths and separate the emission wavelengths. This *Fluorescence Mini Cube* comes with the following.

    - One or more *Built-in fluorescence detector heads* capable of sensing low-intensity light. The fluorescence emission can be collected with one photodetector and subsequently demodulated or, after spectral separation, collected with respective photodetectors.

    - One or more *Built-in LED optical heads* that provide light for the experiment.

- An optional *Fiber-optic rotary joint* can be used to allow free movement of the experimental subject.

- An *Optical cannula*, with connecting *Fiber-optic patch cords*, to deliver light to the subject.

- A *Low Autofluorescence Patch Cord* to allow light collection with minimal fluorescence noise.

The following section describes these standard elements and their purpose.
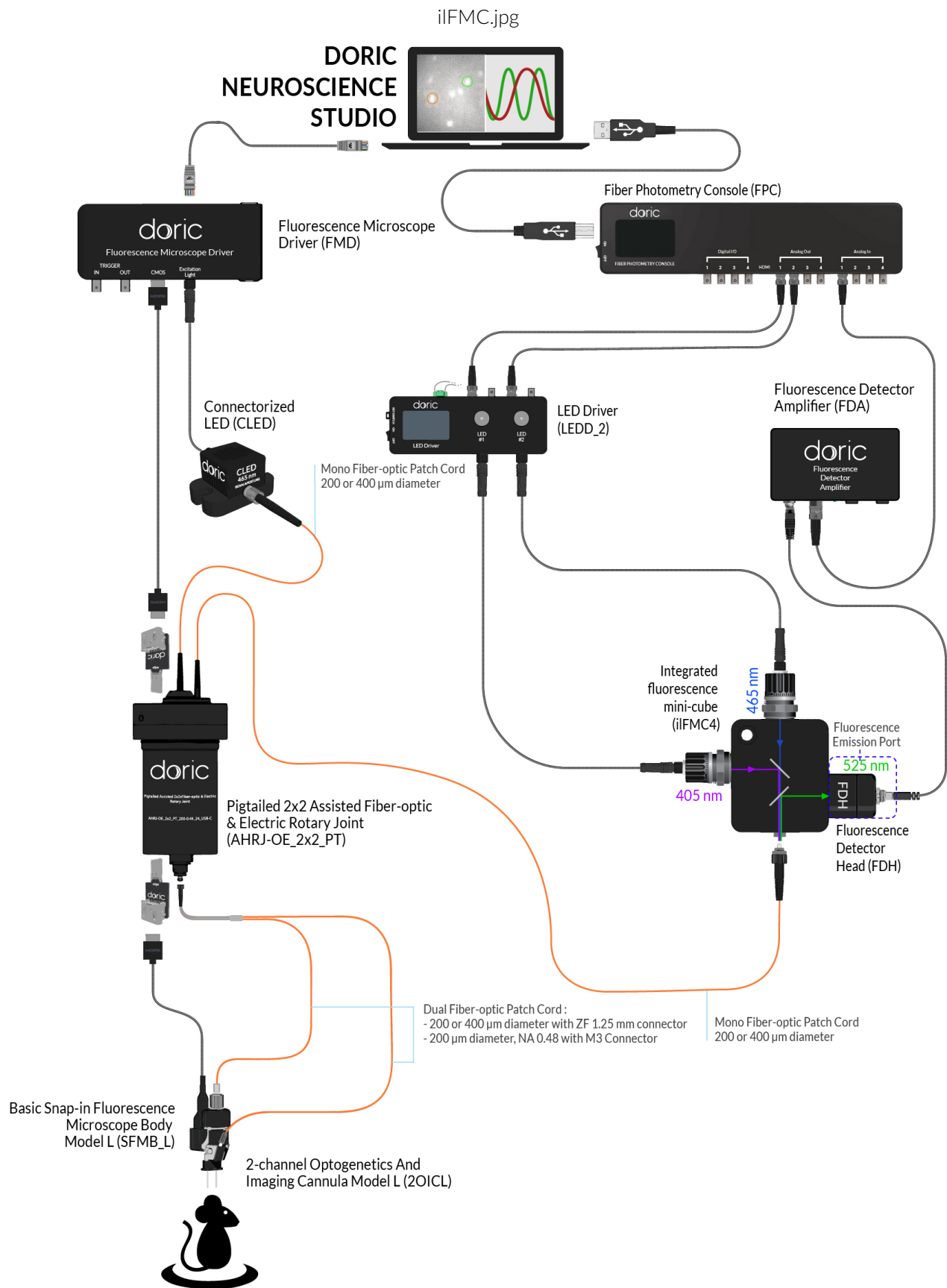
Figure 1.1: *Typical Modular Fiber Photometry Measurement Setup*

1.2 Fiber Photometry Console

This FPGA based data acquisition unit synchronizes the output control and the acquisition of the input data. This device seamlessly integrates with Doric Neuroscience Studio software, which provides the user-interface for multi-channel photometry experiments.

The software interface enables control over the CW excitation light pulses, square or sinusoidal waveform of an external source (i.e. LED driver) with 4 digital input/outputs and 4 analog voltage outputs. The software interface displays real-time data of up to 4 detectors input signals and performs data acquisition. The other new functionalities are being developed and users can upload them as they become available.

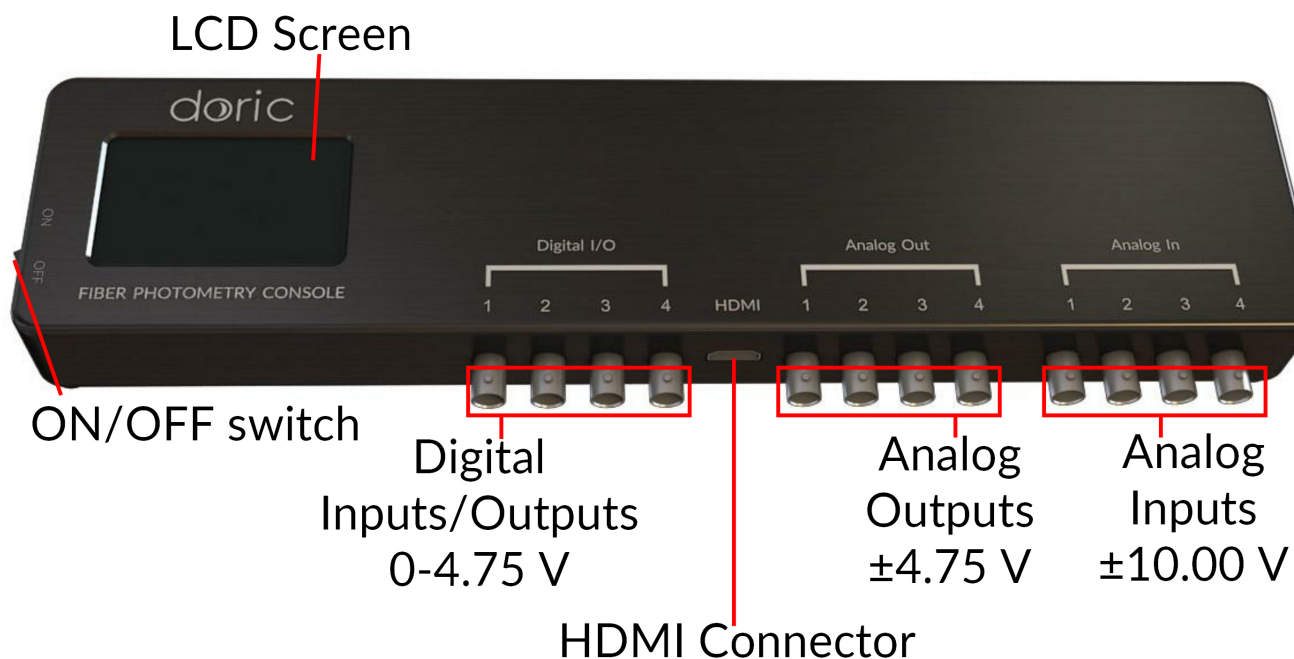The Fiber Photometry Console inputs and outputs are shown in figures 1.2 and 1.3.

Figure 1.2: *Front view of the Fiber Photometry Console: Inputs and outputs*

- The **LCD Screen** displays console information.

- The **Digital/IO** ports sends 0-4.75 V TTL pulses.

- The **HDMI** port acquires digital signals and digital communication SPI and LVDS via a custom pinout HDMI connector.

- The **Analog-out** ports send a variable ± 4.75 V analog signal signal.

- The **Analog-in** ports acquire analog signals up to ±10 V.

- The **Power On/Off** opens and closes the device.

Figure 1.3: *Back view: Maintenance and Power Supply*

- The **12V port** connects to the 12 VDC Power supply.

- The **USB port** allows a USB-B connection to a computer.

- The **Service** port is a USB-B port through which the firmware of the device can be updated.

- The **USB-3** charging port is currently disabled.

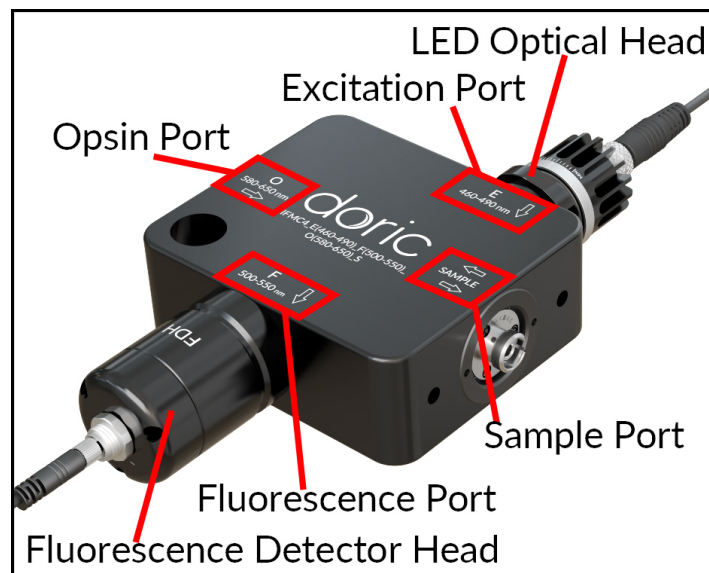## 1.3 Integrated Connectorized Fluorescence Mini Cube

Figure 1.4: *4-port Fluorescence Mini Cube*

The *integrated Fluorescence Mini Cube* is an optical assembly that allows the combination of multiple excitation and detection signals. The cubes are classified by their number of ports, with 3, 4, 5, 6 and 7 port cubes available. The ports are each classified according to their usage, and are also qualified by a wavelength band corresponding to the bandwidth of optical filters within the cube.

- **E** and **IE** (Fig. 1.4) represent entry ports for fluorescence and isosbestic point excitation light. Each port of this type comes with a *Built-in LED Optical Head* that contains the following elements.

    - The **Intensity Adjustment Ring** (Fig. 1.5a) allows additional adjustment of the overall output intensity of light.
    - The **M8 connector port** (Fig. 1.5a) is used to connect the optical head to the *LED Driver* using a *Male-Female M8 Cable*.

- The **Heat-sink Fins** (Fig. 1.5a) are used to evacuate heat from the light source, allowing stable output power. Ensure the fins are not blocked to allow proper cooling.

- **F** (Fig. 1.4) represents ports for fluorescence emission light. Each port of this type comes with a *Built-in Doric Fluorescence Detector Head*.

  - The **M5 Connector** (Fig. 1.5b) allows the *Fluorescence Detector Head* to be connected to the *Fluorescence Detector Amplifier* using an M5 male/male connection cable.

  - For extremely low light level applications, the fluorescence ports (F, F1, etc.) can have a *Built-in Photomultiplier Tube* rather than a *Built-in Fluorescence Detector Head*.

- **O** (Fig. 1.4) represents optogenetic activation or silencing ports. These are always FC receptacles to allow connection to laser or fluorescence light sources.

- **S** (Fig. 1.4) represents the exit port to the sample. These are always FC receptacles to allow connection to an experimental subject.
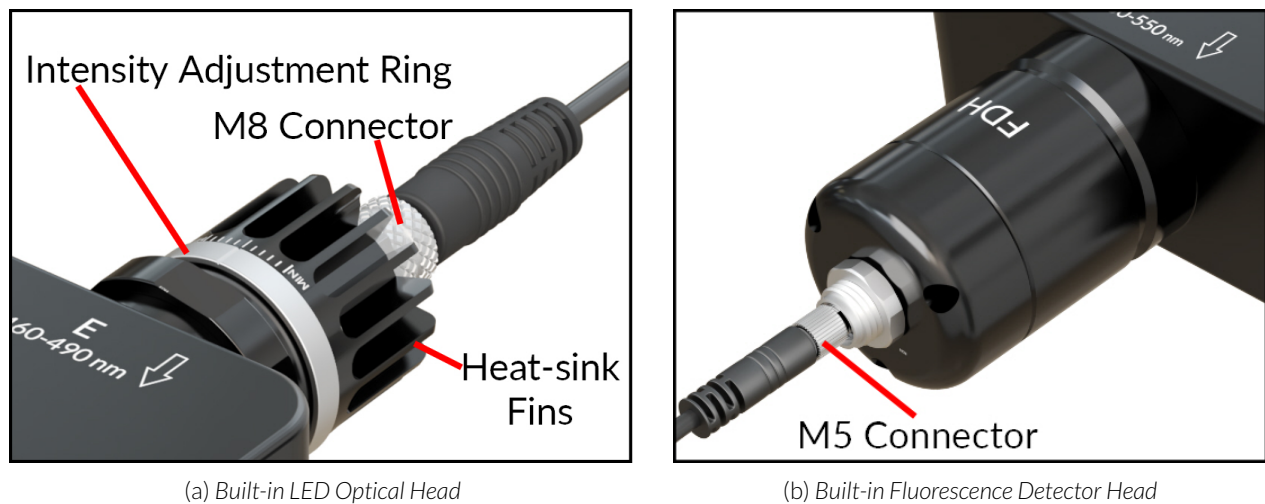

(a) *Built-in LED Optical Head*   (b) *Built-in Fluorescence Detector Head*

Figure 1.5: *ilFMC Built-in components*

1.4   Light Sources

1.4.1   LED Driver

Doric *LED Drivers* can be used as a stand-alone device or controlled via USB port. Each channel connects to a single *Connectorized LED* which can be controlled manually or via the Doric Neuroscience Studio Software.
The LEDs drivers can be used as a stand alone device. During stand-alone operation, it is possible to change the operating mode (CW or external analog mode) and the current sent to the **Connectorized LED**. These changes can be done directly on the device with the control knobs and the LCD display.
Connecting the LED drivers to a computer provides the user with more options. Doric Neuroscience Studio Software allows the access to more operating modes like CW, external TTL, external analog, internal TTL and internal Complex modes. Doric Neuroscience Studio Software enables the creation of different sequences of light source activation. It also provides the possibility to let these sequences be triggered or paused by an external signal. If more power is needed, it is possible to overdrive the LED driver with the software. Our LED driver has a live pulse capability allowing the visualization of the signal modulation on the input BNC in a scope-like manner.

- The **LCD display** (Fig. 1.7) allows easy operation and monitoring. For each channel, the LCD displays the type of light source (LED), the operating mode, the center wavelength in nm and the current setting.
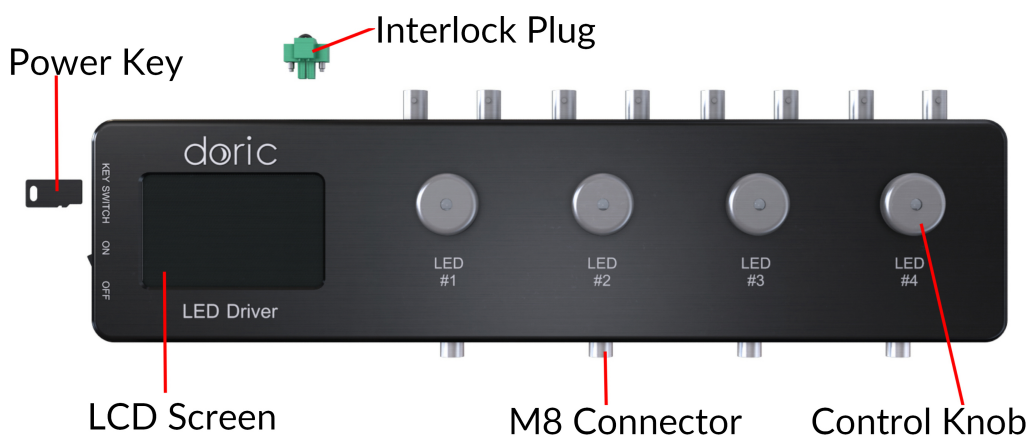
Figure 1.6: *LED Drivers; 1-, 2- and 4-channel*

Figure 1.7: *Front view of a 4-channel LED Driver*

- The **Power key** must be properly inserted into the key switch to enable operation of the light source(s) connected to the driver. Note that, despite its similar shape, the power key is not a standard micro SD card such as those used in some digital cameras. Do not attach the Key to a key fob or similar holder; this may prevent proper insertion of the **Power key**.

- The **M8 connector** is used to link the driver and LED.

- The **Interlock Connector Plug** (Fig. 1.8) allows the user to connect the driver to an interlock system. It is recommended to connect the interlock plug to a laboratory interlock system. This is critical when using LEDs in the UV or Infrared spectrum, as they are invisible to the naked eye.

- The **Input BNC** allows the control of the LED driving current of the corresponding source with an analog or TTL signal.

- The **Output BNC** are used to monitor the driving current of the corresponding light source.

- The **12 VDC power input** connects the driver to its 12 VDC power supply.

- The **USB-B Connector** allows the driver to be connected to a computer using a USB-A/USB-B cable.

12 VDC power input

Interlock Connector Plug

Output BNC

Input BNC

USB-B connection to computer

Figure 1.8: *Rear view of a 4-channel LED Driver*

- The **On/Off switch** (Fig. 1.9) turns on/off the driver.
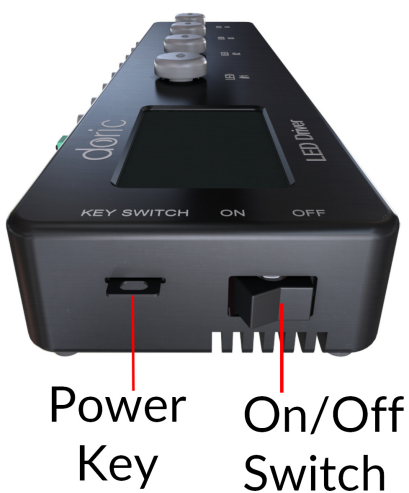

Power Key

On/Off Switch

Figure 1.9: *Side view of a 4-channel LED Driver*

1.5   Rotary Joints

The various cables and fibers used to connect a **Fiber Photometry system** to an animal can be problematic when used with a freely-moving animal subject. Rigid cables can limit animal freedom and comfort, while excessive torque applied to the cables by animal movement can break them. A rotary joint allows the cables and fibers to move with the animal, without effect on signal quality. When used for fiber photometry, *Pigtailed Rotary Joints* are favored.

1.5.1   Pigtailed 1x1 Fiber-optic Rotary Joint

The pigtailed variant of the **1x1 Fiber-optic Rotary Joint** (Fig. 1.10) includes FC connectorized fiber-optic patch cords pigtailed on both sides of the rotary joint. This involves pre-alignment of the optical fibers, which reduces intensity variation in rotation normally observed. They have been designed for applications where optical power variation must be minimized, such as fiber photometry. These rotary joints are optimized for use with 400 μm and 200 μm diameter core optical fibers, with an NA of 0.57.
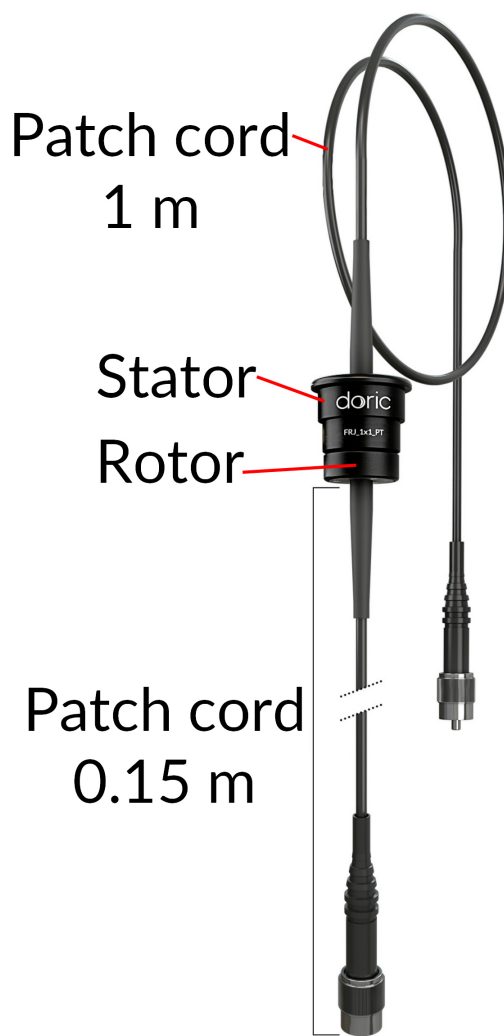

Figure 1.10: *Pigtailed 1x1 Fiber-optic Rotary Joint*

1.6   Low Autofluorescence Patch Cords

All optical fibers posses a certain innate fluorescence due to their chemical composition. Often called Autofluorescence, this light can interfere in photometry measurements by overwhelming them. The *Doric Lenses Low Autofluorescence (LAF) Patch Cords* have minimal autofluorescence and are thus ideal for fiber photometry. All **LAF** patch cords are identified by the red **Coupling Nut** on the connector.

1.7   Photodetectors

1.7.1   Doric Fluorescence Detector

The **Doric Fluorescence Detector** is the first picowatt range sensor developed for fiber photometry. The detector is directly integrated into our line of *ilFMC* photometry cubes, though it can be used separately. As the *Fluorescence Detector Head* is already described alongside the *Integrated Fluorescence Mini Cube*, only the *Fluorescence Detector Amplifier* is described here.

Fluorescence Detector Amplifier

The Fluorescence Detector Amplifier amplifies the signal coming from the detector head and transmits it to a recording system using a BNC output. It contains the following elements.
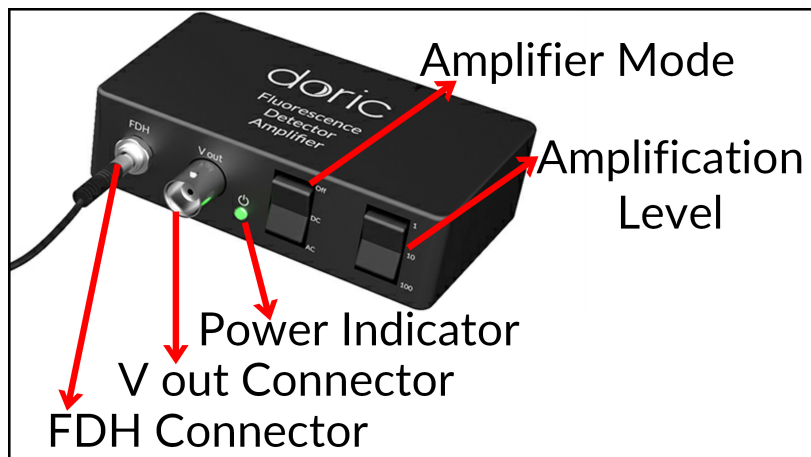

Figure 1.11: *Doric Fluorescence Detector Amplifier Elements*

- The **FDH Connector** (Fig. 1.11) is an M5 type connector used to link the amplifier and the head using a shielded M5 cable.

- The **V out Connector** (Fig. 1.11) is a BNC type connector used to connect the fluorescence detector with a DAQ system.

- The **Power Indicator** light (Fig. 1.11) shines green when the detector is on.

- The **Amplifier Mode** switch (Fig. 1.11) is used to switch the detection mode from **Off** to **AC** or **DC**.

- The **Amplification Level** switch (Fig. 1.11) allows the choice of amplification levels at 1, 10 or 100 times.

- The **Power Supply** connector, located on the back of the unit, is used to connect the 12 V power supply to the amplifier.

1.7.2 Newport Visible Femtowatt Photoreceiver Module Model 2151

This battery-operated photodetector has high gain and detects CW light signals in the picowatt range. When used in conjunction with a modulated light source and a lock-in amplifier, it achieves sensitivity levels in the femtowatt range. For this Newport product Doric offers an add-on *Fiber-optic adapter* that improves coupling efficiency between the large core, high NA optical fibers used in fiber photometry and the relatively small detector area. Its output analog voltage (-3 to 7.5 V) can be monitored with the *Fiber photometry console* or any other analog data acquisition box.

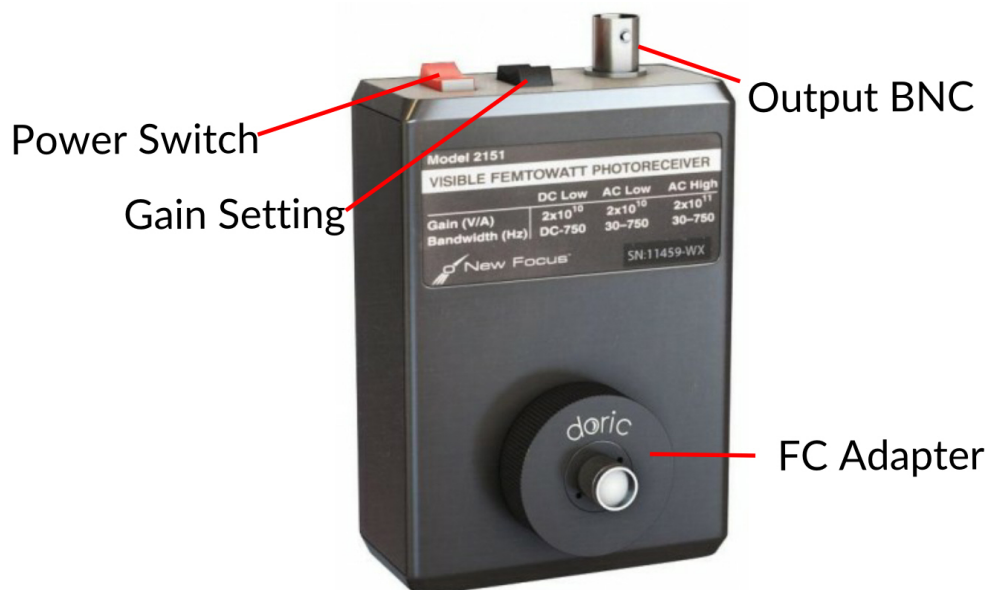For additional details about this device, see the Newport website.

Figure 1.12: *Newport Visible Femtowatt Photoreceiver Module Model 2151 + Doric FC Adapter*

1.7.3 Hamamatsu H10722-20 Photosensor Module

Warning: The *Hamamatsu H10722-20 Photosensor Module* is highly sensitive and can be easily damaged if exposed to high optical power.

For low light level applications that require higher bandwidth, the fluorescence port can be replaced by the photomultiplier tube directly attached to the mini-cube. The *Hamamatsu H10722-20 Photosensor Module* is compatible with our cubes and is the most sensitive detector we offer for very low light level detection. Unlike other ports of our mini cubes that have receptacles and focusing lens, the port for this sensor has a lens that adjusts the beam size to fit the size of photomultiplier tube (PMT) instead of a receptacle. The PMT is highly sensitive and can be easily damaged if exposed to high optical power.

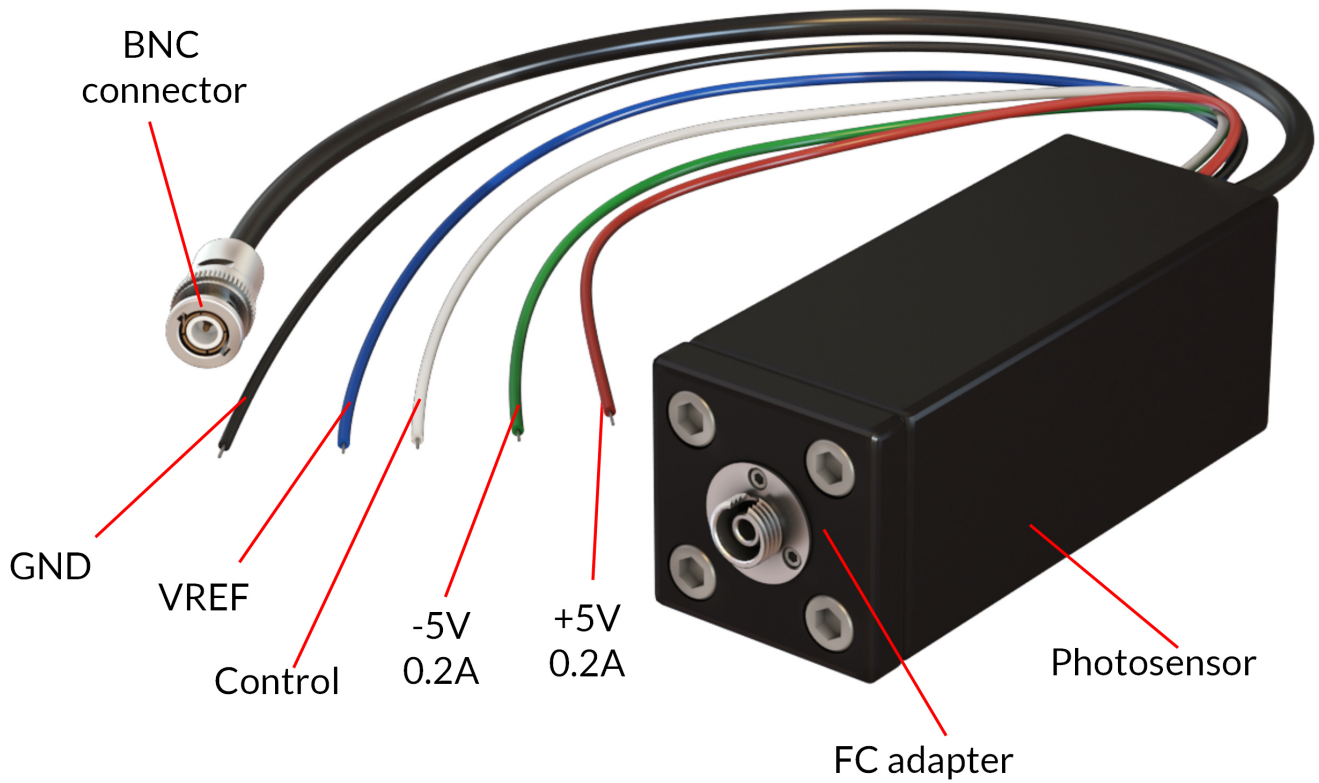For more information about the product, go to the Hamamatsu website.

Figure 1.13: *Hamamatsu H10722-20 Photosensor Module + Doric FC Adapter*

1.7.4 Power Supply for PMT Module C10709

(a) *Front view*

(b) *Back view*

Figure 1.14: *Power Supply for PMT Module C10709*

The photosensor module requires a power supply and the C10709 model is recommended. Both drive voltages and control voltages can be supplied from this single unit.

For more information about this device, go to the Hamamatsu website.

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Getting Started
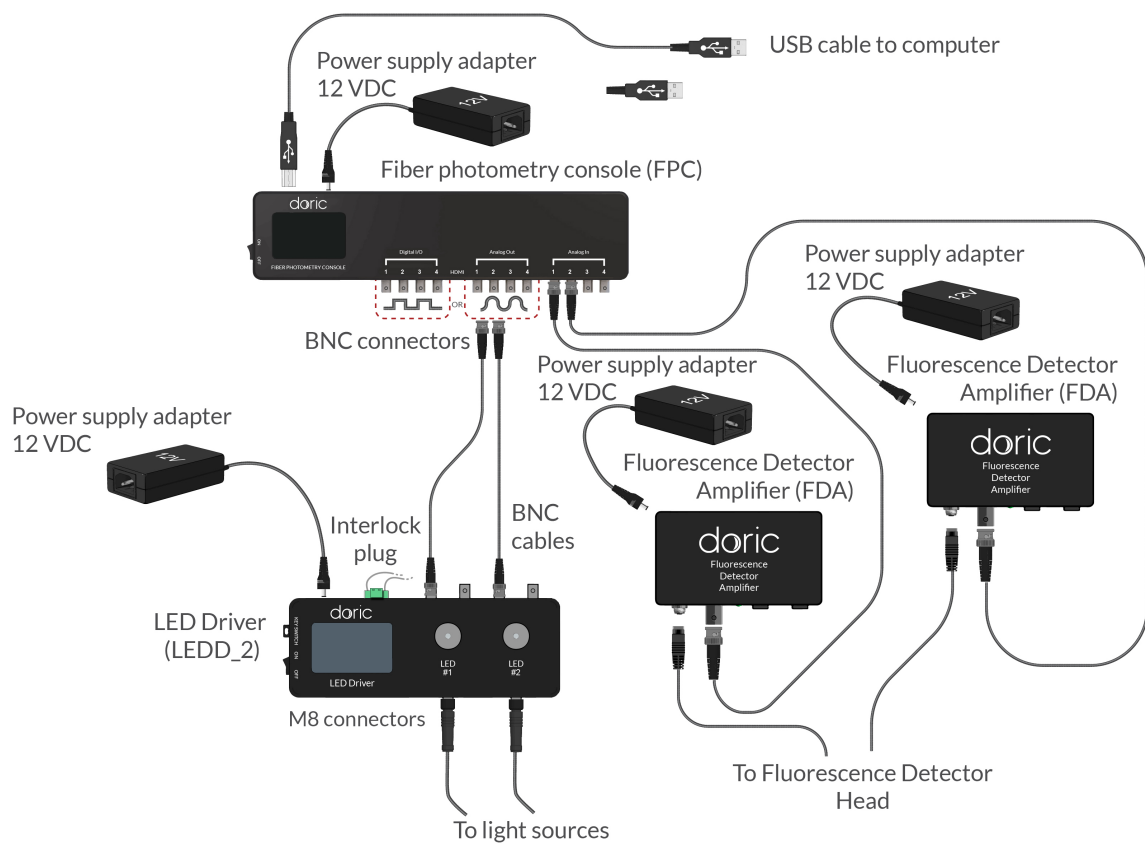
2.1 Connecting the Fiber Photometry System

Figure 2.1: *Connections to the Fiber Photometry Console*

Follow this quick start procedure to install and connect the system. We recommend the following order to avoid device and driver detection problems. If you need more information about each device or the software, refer to the User Manual of each device.

1. **Install the Doric Neuroscience Studio software.** Follow the on-screen instructions to install the *Doric Neuroscience Studio* on the hard drive of your computer. For more details, refer to the Doric Neuroscience Studio Software Manual.

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2. **Connect the Fiber Photometry Console.** The console unit is operated with a 12 VDC power supply adapter. When it is powered, turn on the switch, then connect the console to the computer via a USB cable. A Windows USB driver will be automatically installed. Open the software to complete the installation of other devices.

3. **Connect the Programmable LED Driver.** Connect the LED Driver to the power outlet with the included 12 V AC-DC adapter. When the LED is controlled by the console, light stimulation can be synchronized with the data acquisition.

   - Digital outputs can activate the light source driver with TTL pulses (0 - 5 V square pulses). Connect the *LED Driver* **Input ports** to the **Digital I/O**. Turn the *LED driver* switch ON and click on the knob twice for the **Ext. TTL** mode. Set a maximal current value corresponding to the excitation power required for the experiment.

   - Analog outputs can activate the light source driver with analog pulse sequence. Connect the *LED Driver* **Input ports** to the **Analog Out** ports. Turn the *LED driver* switch ON and click on the knob thrice for the **MOD** mode (For the *LEDD*) or ANALOG mode (for the *LEDRV*). Here, the maximal current value acts as a current ceiling, meaning even analog signals which would normally provide a higher current will be clipped.

4. **Connect the LED Module.** The *Built-in LED Optical Heads* come pre-installed on the *Integrated Fluorescence Mini Cube*. Connect the *LED Driver* to the *Built-in LED Optical Heads* using the *M8 Cables* (Fig. 2.1).
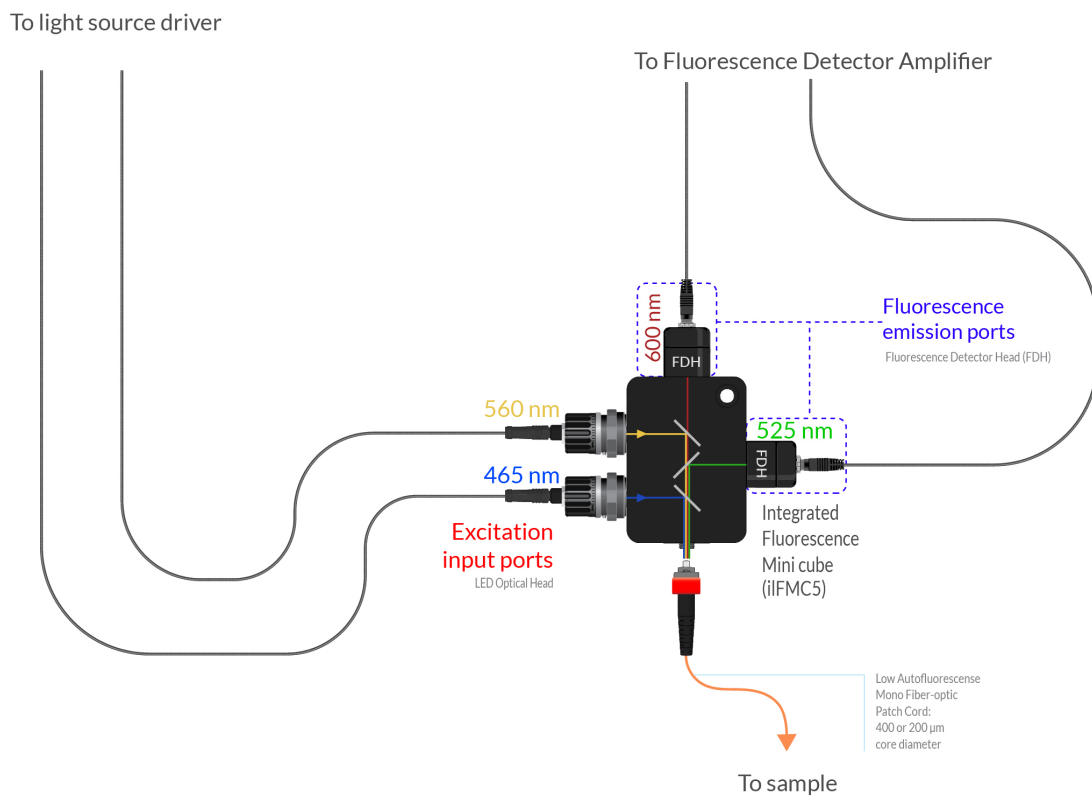

Figure 2.2: *Integrated Fluorescence Mini-cube Connections*

5. **Connect the Fluorescence Mini Cube.** The sample ports send light to the experimental subject through a *LAF Patch Cord* (Fig. 2.2). Please note that the use of a rotary joint may cause some signal variations and loss of light. In this case, extra care is required during the data analysis and a reference channel is helpful. Perform the following steps to connect the patch cord to the sample port.

   a) Clean the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.
   b) With an FC connector, the connector key must be oriented to enter within the receptacle slot to ensure proper connection (Fig. 2.3).
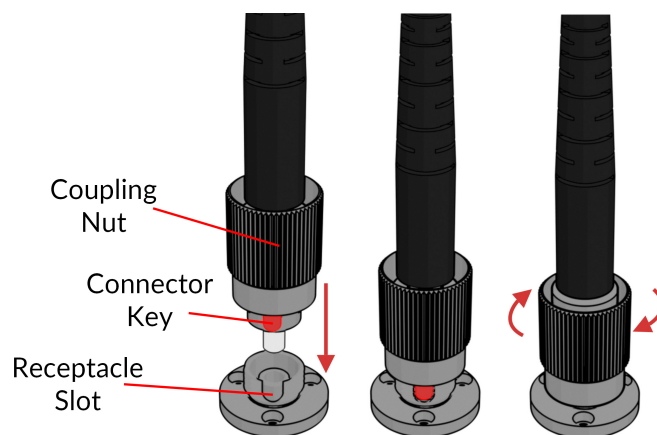
Figure 2.3: *FC connector, Fiber Installation*

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

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6. **Connect the Photodetectors.**

   - The *Built-in Fluorescence Detector Head* comes pre-installed into the *Integrated Fluorescence Mini-Cube*. Connect the head to the *Fluorescence Detector Amplifier* (Fig. 1.11) using the *M5 Detector Cable*[1]. Use a BNC cable to connect the amplifier to the analog input port of the *Fiber Photometry Console* (Fig. 2.1). We recommend the verification of gain settings, as signals may vary from one experiment to another and can require different parameters. In fiber photometry, the DC amplifier mode is needed. The amplification level can be adjusted depending on the experiment.

   - The **Newport Visible Femtowatt Photoreceiver Module Model 2151** (Fig. 1.12)is connected to the Fluorescence Mini Cube emission ports with a ***600 µm core, NA 0.48 optical fiber patch cord*** (Fig. 2.2). Use a BNC cable to connect the Newport photoreceiver module in the Analog input port of the Fiber Photometry console (Fig. 2.1). The Newport photoreceiver module is battery operated and is turned on with the red switch. Ensure the module is switched off when the experiment is over to preserve battery power. We recommend the verification of gain settings, as signals may vary from one experiment to another and can require different parameters. In any case, the DC low gain setting should be used.

   - The **Hamamatsu H10722-20 Photosensor Module** is linked to the *Fluorescence mini cube* emission port by an optical fiber or directly installed on the FMC emission port by an FC connection. The BNC cable of the photosensor module can be plugged to a power meter to measure the detection signal. **The photomultiplier tube (PMT) is highly sensitive and can be easily damaged if exposed to high optical power.**

     The Hamamatsu H10722-20 Photosensor Module requires a **Power Supply Hamamatsu Model C10709**. On the back of the Photosensor Module, 5 wires of different colors are used to supply operation voltage and control gain. Each colored wire should be connected to the connector of the same color on the power supply (Fig. 1.13).

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[1]See the application note if there is difficulty installing the M5 connector

2.2 Configuring the Fiber Photometry Console

To configure the *Fiber photometry console*, use the *Doric Neuroscience Studio*. The software allows for the selection of acquisition modes and parameters. Data acqustion parameters are set on **Analog input** channels, while the illumination parameters are set on **Digital** or **Analog** outputs, depending on the output signal required. In every mode, the **LIVE** button starts all programmed sequences. We recommend the activation of the **AUTOSCROLL** option that follows the trace over a given length of time. The length of the displayed trace can be changed. As the **LIVE** mode keeps up to only a small amount of data in memory, *it is not recommended to use this mode for important data*.

To automatically save data, use the **RECORD** button. This button activates a measurement mode that periodically saves the data to the hard drive, allowing longer acquisition sessions. To specify the location where data is saved, as well as which traces are saved, use the **Saving options** button to open the **Saving options** window. In this mode it is also possible to decimate the results, reducing the amount of data points saved and the size of the saved files.

2.2.1 Setting up Continuous acquisition mode

Data acquisition

On an **Analog input** channel, change the channel mode to **Linear**. Select an **Acquisition Rate** from 0.3 to 12 kS/s, depending on the temporal resolution desired. Keep in mind that a higher sampling rate will produce bigger files. For standard calcium activity measurements, the lowest sampling rate (0.3 kS/s) will give the best signal-to-noise ratio. It is mandatory to set the photodetector in DC mode for continuous signal acquisition.

Light excitation

When using **Digital outputs**, set the channel in **Continuous Wave (CW)** mode. In this configuration, the **Digital output** will be 5 V when enabled, and 0 V when disabled. Connect the proper *Fiber photometry console* outputs to the *LED driver* inputs, and set the *LED driver* in *External TTL* mode. In this mode, set the output current directly on the LED driver.

When using **Analog outputs**, set the channel in **Continuous Wave (CW)** mode. The voltage output is defined in the **Voltage (V)** box, and can be set between 0 V and 4.75 V. Set the *LED driver* in **MOD** mode. In this mode, the LED driver will output a current proportional to the voltage with a conversion factor of 400 mA/V in standard operation mode, and 40 mA/V in low-power mode.

2.2.2 Setting up Time Series mode

Data acquisition

Time series mode allows multiple recording sessions with predefined durations and a delay between recordings; e.g. 1 minute of recording every hour for 24 hours.

Specify the target file parameters in the **Saving options** window. To activate this mode, click the **Time Series** button on the **Acquisition** tab. Set the time series configuration, **Time ON** and **Interval between series** as required.
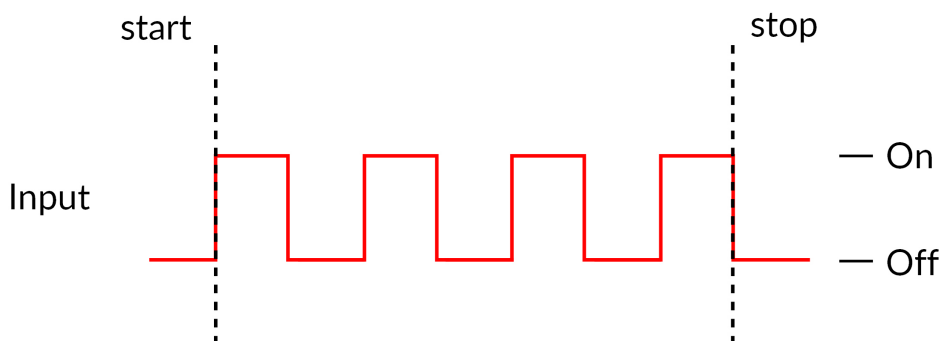

Figure 2.4: *Time Series acquisition timing diagram*

2.2.3   Setting up Interleaved acquisition mode

Data acquisition

This acquisition mode allows for interleaved detection of two fluorescence signals on a single detector. The two excitation light sources are rapidly switched ON and OFF sequentially at a user-defined frequency, and the *Doric Neuroscience Studio* demodulates the signals. This type of setup can be used to detect two signals at different wavelengths using a single photodetector.
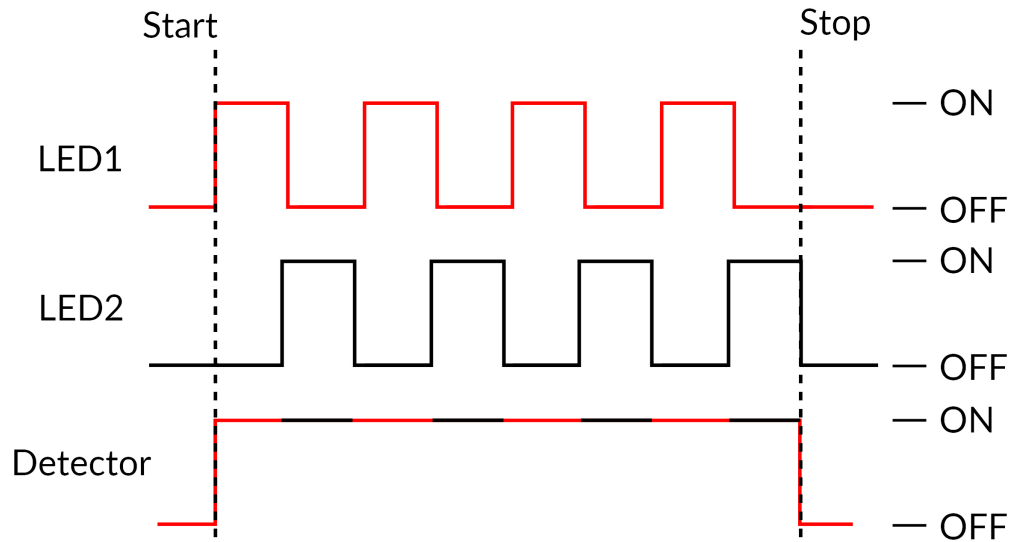
Figure 2.5: *Interleaved acquisition timing diagram*

Software Configuration

The following section describes the usage of the **Interleaved** measurement mode for **Analog Input** channels. Once the **Interleaved** channel mode is chosen, the the **Interleaved/Sequential options** box (Fig. 2.6) appears, containing the following parameters.

1. The **Channel #** drop-down lists allows the choice of interleaved outputs. Once **Channel #1** has been selected, **Channel #2** only allows the same type of output (analog or digital) to be selected.

2. The **Preconfiguration** drop-down list allows the choice of a pre-configured frequency for the interleaved channels. The previously selected channels are configured to function at the chosen frequency.
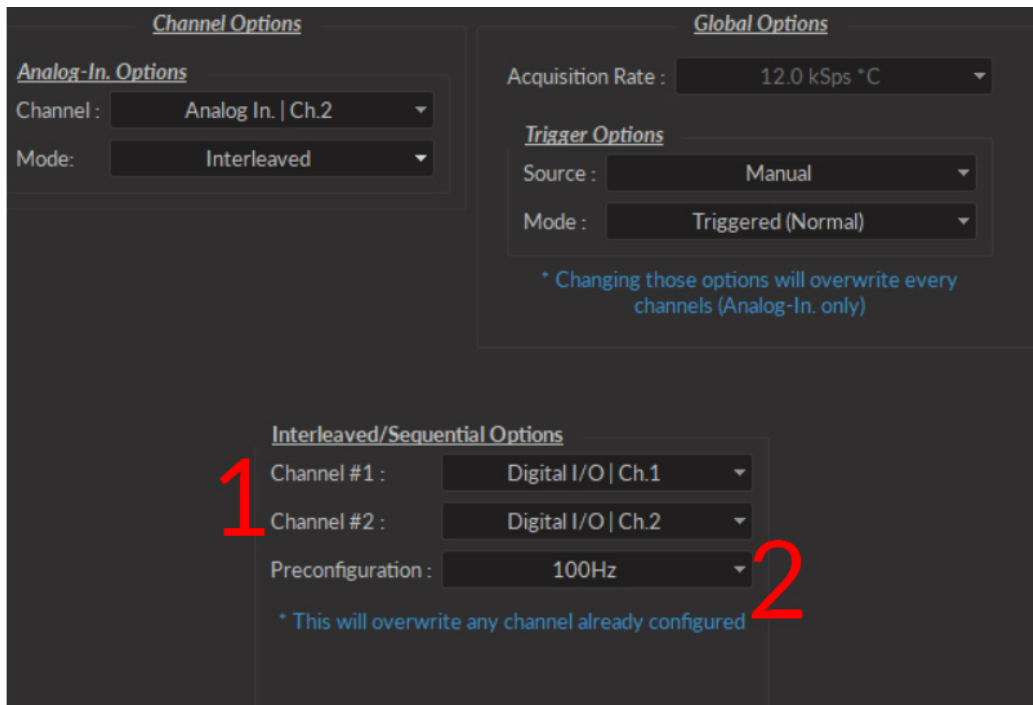
Figure 2.6: *Channels configuration, Analog Input Interleaved Mode*

The following steps describe the basic manner in which to use **Interleaved Mode**.

1. Select two channels from the **Channels #** lists.

2. Select a frequency from the **Preconfiguration** list.

3. Connect the *Fiber Photometry Console* to at least two different light sources using the outputs preconfigured for interleaved mode in the previous step. Ensure the driver(s) are in **External TTL** mode (for digital channels) or **MOD** mode (for analog channels), with the driver set at the desired maximum current level (in mA).

4. Connect the detector to the appropriate analog input channel(s) corresponding to the input.

5. Start measurement using **Play** or **Record** on the **Acquisition** tab.

6. After a measurement is made, 5 traces are available in the **Graphics/Traces List**.
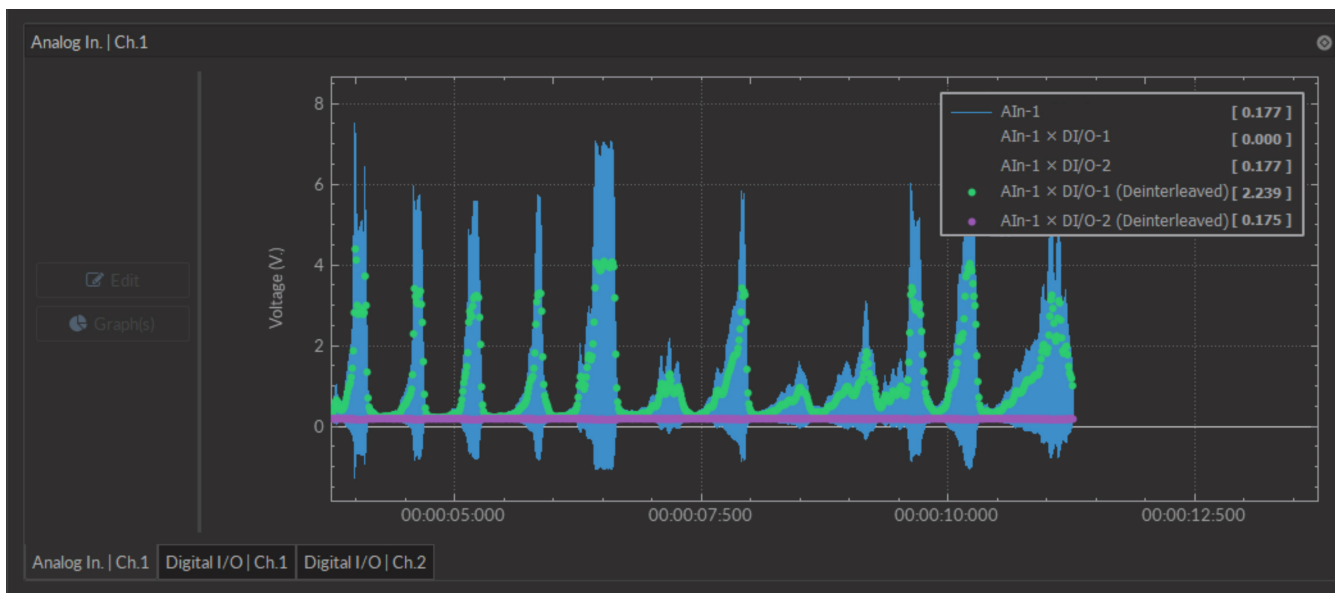
Figure 2.7: *Acquisition View, Interleaved Mode Traces*

- The **Raw Data** (AIn-1) (Fig. 2.6) shows the raw signal received on the input channel.
- The **Intermediate Data** (AIn-1 x DI/O-1) (Fig. 2.6) traces show the fluorescence signal caused by each light source, obtained by multiplying the output signals by the input signal.
- The **Deinterleaved Data** (AIn-1 x DI/O-1(mean)) (Fig. 2.6) shows the averaged signal of each demodulated pulse. This leaves a single averaged point per pulse sent by the output channels.

System Configuration

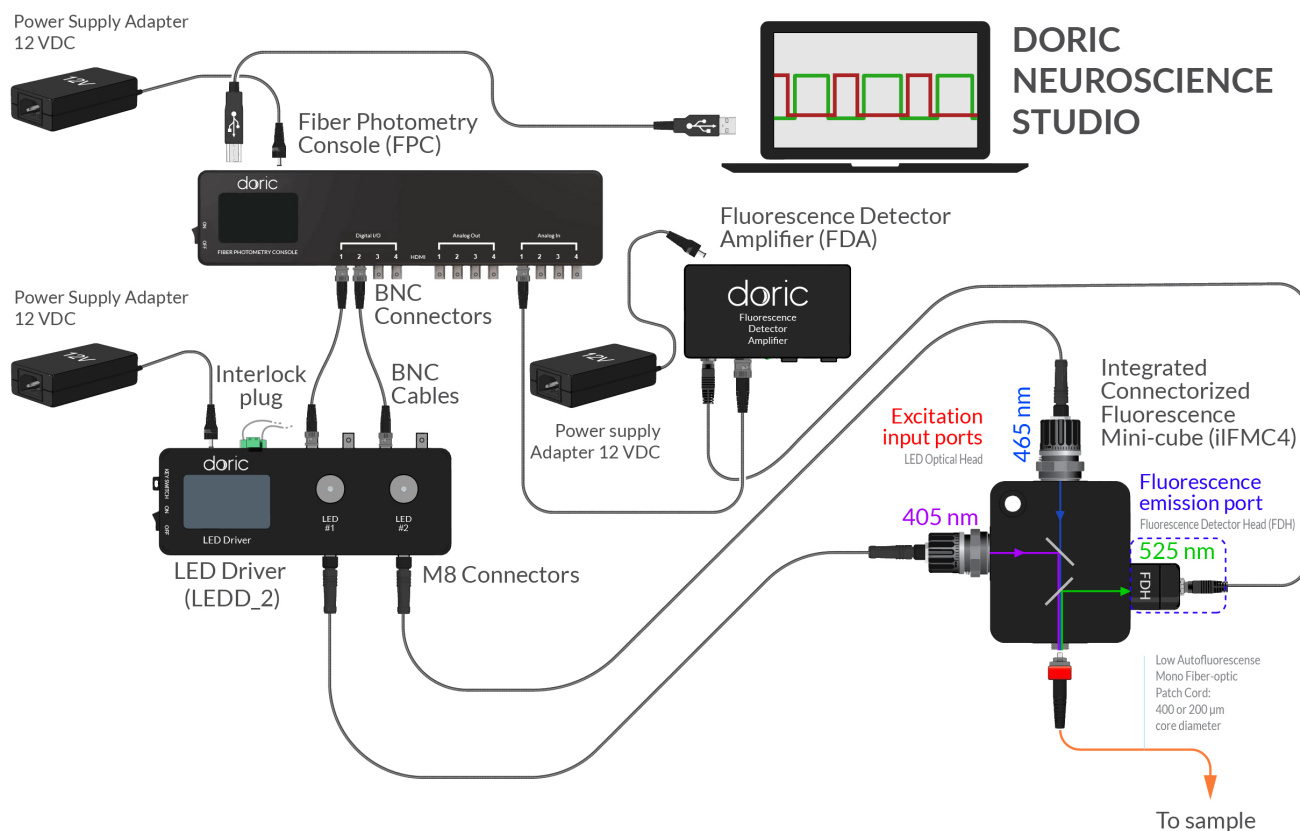The following section shows how to set up a simple interleaved detection setup.

Figure 2.8: *Interleaved detection setup*

For details on connecting specific elements and basic installation of devices, see section 2.2. The following describes the installation of a basic interleaved detection setup (Fig. 2.8). This procedure assumes the software has been properly configured as shown in section 2.2.3.

1. This configuration requires a 2-channel *LED Driver*, which will connect to the *Integrated Fluorescence Mini Cube*. The wavelength of the *Built-in LED Optical Heads* are chosen for the given application.

2. Connect the output **Interleaved** channel to the inputs of the *LED Driver* using BNC cables.

3. Connect the BNC port of the *Fluorescence Detector Amplifier* to the input **Interleaved** channel using a BNC cable.

4. Connect both *Built-in LED Optical Heads* to the *LED driver*.

5. The **Sample (S)** port of the *4-channel fluorescence mini cube* connects to the subject using an *LAF patch cord*.

6. Connect the *Built-in Fluorescence Detector Heads* of the **Fluorescence (F)** ports to the *Fluorescence Detector Amplifier*.

7. Once the setup is installed, the system is ready for interleaved detection.

2.2.4   Setting up Lock-In mode

Using a lock-in detection scheme, the *Fiber Photometry Console* can be used to detect fluorescence signals within strong noise or separate multiple signals using a single detector[2]. An LED light source emits a sinusoidal signal at a given frequency (called the **Carrier**), which is used as a reference. A detector is used to receive the resulting fluorescence. By targeting the **Carrier frequency** of the reference signal (known as **demodulation**), the result is separated from ambient noise at different frequencies. It is even possible to separate multiple carrier frequencies from a single signal.
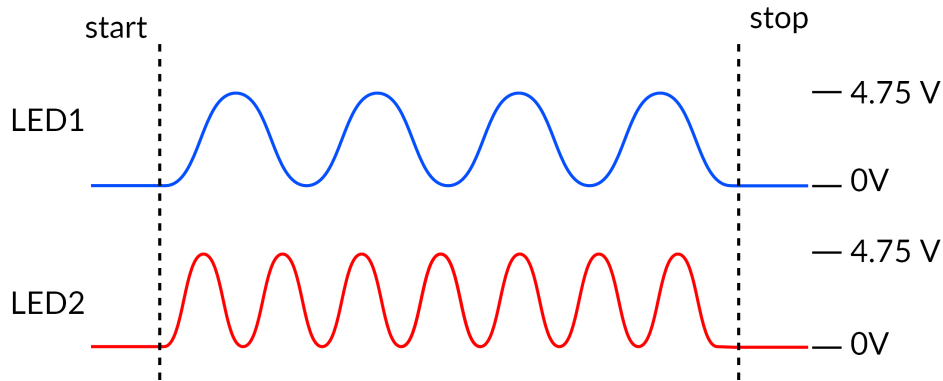

Figure 2.9: *Lock-in modulation timing diagram*

Software Configuration

The following section describes the usage of the **Lock-in** mode for **Analog Input** channels. Once the **Lock-in** channel mode is chosen, the **Lock-in options** box (Fig. 2.10) appears, containing the following parameters.
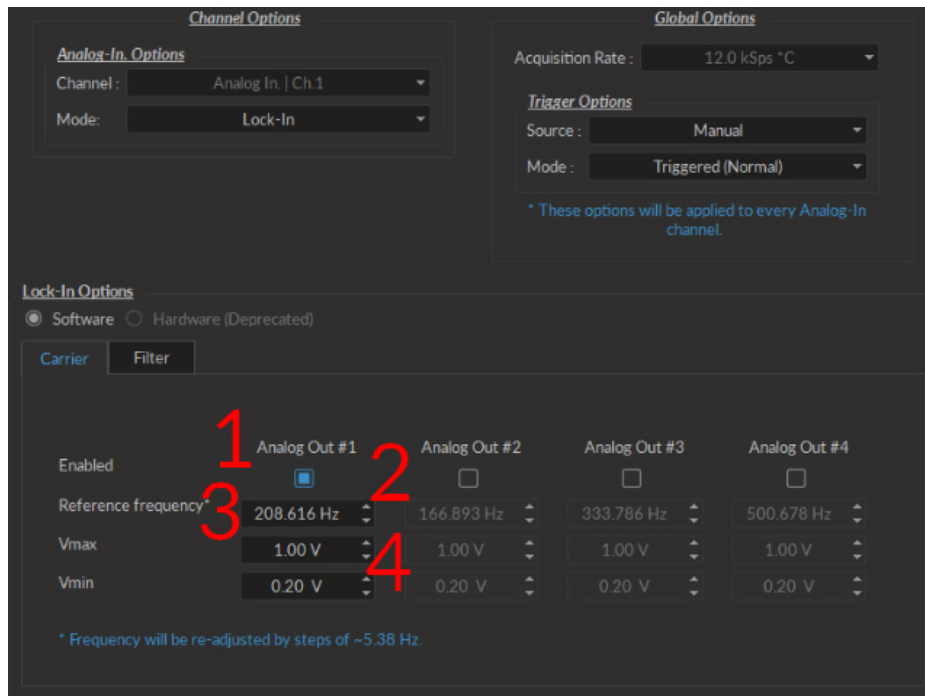

Figure 2.10: *Channels configuration, Analog Input Lock-in Mode, Carrier Tab*

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[2]Natural Neural Projection Dynamics Underlying Social Behavior Gunaydin LA, Grosenick L, Finkelstein JC, Kauvar IV*, Fenno LE, Adhikari A, Lammel S, Mirzabekov JJ, Airan RD, Zalocusky KA, Tye KM, Anikeeva P, Malenka RC, Deisseroth K. Cell 157:15351551 (June 2014).

1. The **Channel List** (Fig. 2.10) indicates which channels are used to output a reference frequency. Multiple output channels can be selected for a single input channel. Any output channels already configured by a different input channel will be usable, but not configurable. The output configuration must be changed in the initial input channel.

2. The **Enabled** checkboxes are used to select which output channels are in use.

3. The **Reference frequency** (Fig. 2.10) allows the choice of a reference frequency. The reference frequency should not be a multiple a of known noise frequency (e.g. 60 Hz), or a multiple of another reference frequency.

4. The **Vmax/Vmin** (Fig. 2.10) defines the voltage sent from the console to the device. The minimum possible difference is of 0.1 V for the **LEDD**. The **Vmin** value is 0.1 V for the *LEDD* and 0.2 V for the *LEDRV*.

5. The **Cutoff Frequency** (Fig. 2.11) (the frequency at which a -3 dB attenuation will occur), found on the **Filter** tab is kept fixed at 12 Hz for optimal filtering results.

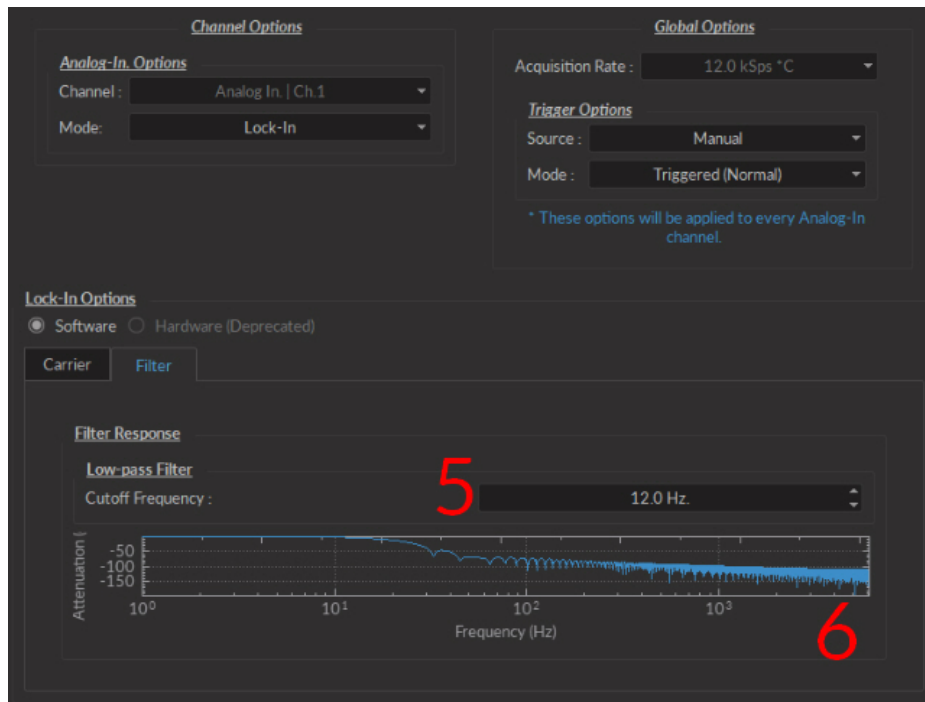6. The **Filter response** is displayed on the **Filter** tab.

Figure 2.11: *Channels configuration, Analog Input Lock-in Mode, Filter Tab*

2-signal demodulation System Configuration

By using 2 different carrier frequencies, 2 signals can be demodulated using a single detector. This configuration is often used in the recording of GCaMP fluorescence which is excited at 405 nm (calcium independent) and at 465 nm (calcium dependent). By modulating each wavelength at a different carrier frequency, it is possible to separate both signals using a single detector.
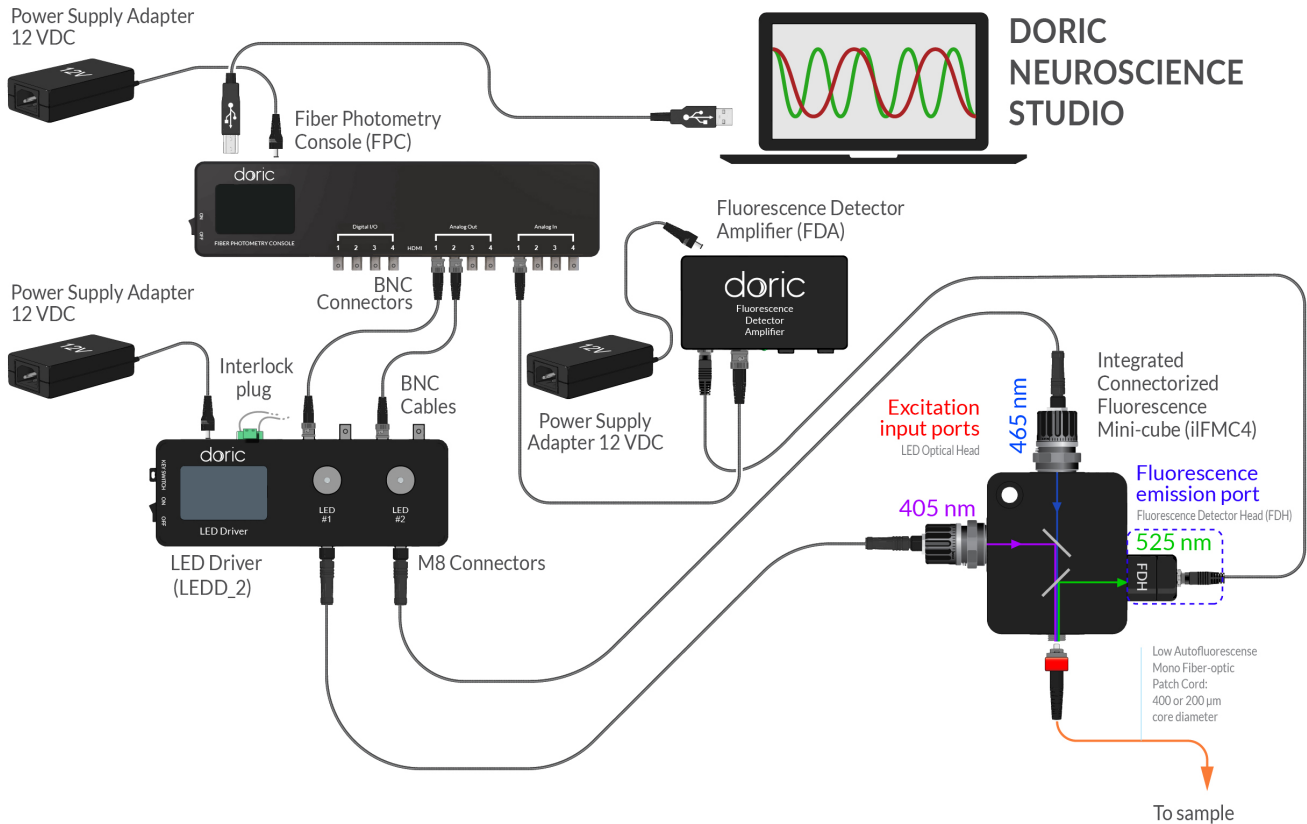

Figure 2.12: *2-signal demodulation setup*

1. This configuration requires a 2-channel *LED driver* and an *Integrated Fluorescence Mini Cube* with two *Built-in LED Optical Heads*. The wavelength of these light sources are chosen to match the given application.

2. Prepare two channels in **Lock-in** mode on the **ANALOG-IN** tab with different reference frequencies. These frequencies must not be a multiple of one another, or a multiple of a noise source (e.g. 60 Hz for electrical lighting).

3. Using BNC cables, connect each **ANALOG-OUT** channel to the corresponding **IN** port on the *LED driver*.

4. Do not connect the LED driver to the computer. The driver is used here in stand-alone mode and is controlled by the **Photometry Console**.

5. Connect the *LED Driver* to the *Built-in LED Optical Heads*.

6. Using the driver knobs, push until each channel indicates they are in **MOD** mode.

7. Turn the knobs until the channels reach maximum current. This should be 500 mA for a 405 nm, and 1000 mA for all others. In **MOD** mode this acts as a maximum limit for the output current. The real current sent to the LED (*CLED* or *Built-in LED Optical Head*) is determined by the voltage detected on the **IN port** of each *LED driver channel*, with a conversion factor of 400 mA/V in standard mode and 40 mA/V in low-power mode.

8. The **Sample (S)** port of the *4-channel Fluorescence Mini Cube* connects to the subject using an optical fiber.

9. Connect the *Built-in Fluorescence Detector Head* of the **Fluorescence (F)** port to the *Fluorescence Detector Amplifier*.

10. Connect the *Fluorescence Detector Amplifier* BNC port to the corresponding input on the *Fiber photometry console* using a BNC cable (Fig. 2.12).

11. Once the setup is installed, the system is ready for 2-channel demodulation.

Lock-in device guidelines

- Depending on the light source driver used, there is a minimum **V-** value to avoid clipping.

   - The *LEDRV* has a minimum current to avoid clipping of 80 mA. This corresponds to a **V-** of 0.20 V

   - The *LEDD* in normal mode has a minimum current to avoid clipping of 40 mA. This corresponds to a **V-** of 0.1 V.

   - The *LEDD* in low-power mode has a minimum current to avoid clipping of 4 mA. This corresponds to a **V-** of 0.10 V.

- When using the *Doric Fluorescence Detector* or the *Integrated Fluorescence Mini-Cube*:

   - Recommended carrier frequencies are between 200 Hz and 600 Hz. Lower frequencies will not function with the **Lock-in** algorithm, and the maximum sampling rate of the photodetector is 1000 Hz.

   - In **DC** mode, the photodetector has a range from 0-5 VDC. In **AC** modes, there is no offset, but the detector will saturate at ±5 V (10 V peak to peak). In **AC** mode, it is recommended to avoid **1x Amplification** as it saturates too easily.

- When using the *Visible femtowatt photoreceiver module*:

   - Recommended carrier frequencies are between 200 Hz and 600 Hz. Lower frequencies will not function with the **Lock-in** algorithm, and the maximum sampling rate of the photodetector is 750 Hz

   - In **DC-low** mode, the photodetector will saturate at +7 V. In **AC** modes, there will be no offset, but the sensor will saturate at ±3.5 V (7 V peak to peak). If the signal on the photodetector in **DC-low** mode is high, the **AC-low** mode can be used to reduce the signal offset.

2.3   Using the Fiber Photometry Analysis Module

The **Fiber photometry analysis module** provides the user with an easy mean to extract the relevant data from the data acquired by the *Fiber photometry console*. The software loads data in .CSV format, implements signal processing functions and saves the traces in .CSV format. This module does not replace standard analysis tools as Matlab, ImageJ or Excel, but aims to offer useful processing algorithms developed for the photometry data. For additional information, see the *Neuroscience Studio Manual*.

Support

3.1 Contact us

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

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Email sales@doriclenses.com