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Bundle-imaging Fiber Photometry Cube with Targeted Optogenetics (BFTO)

User Manual

Version 1.0.0

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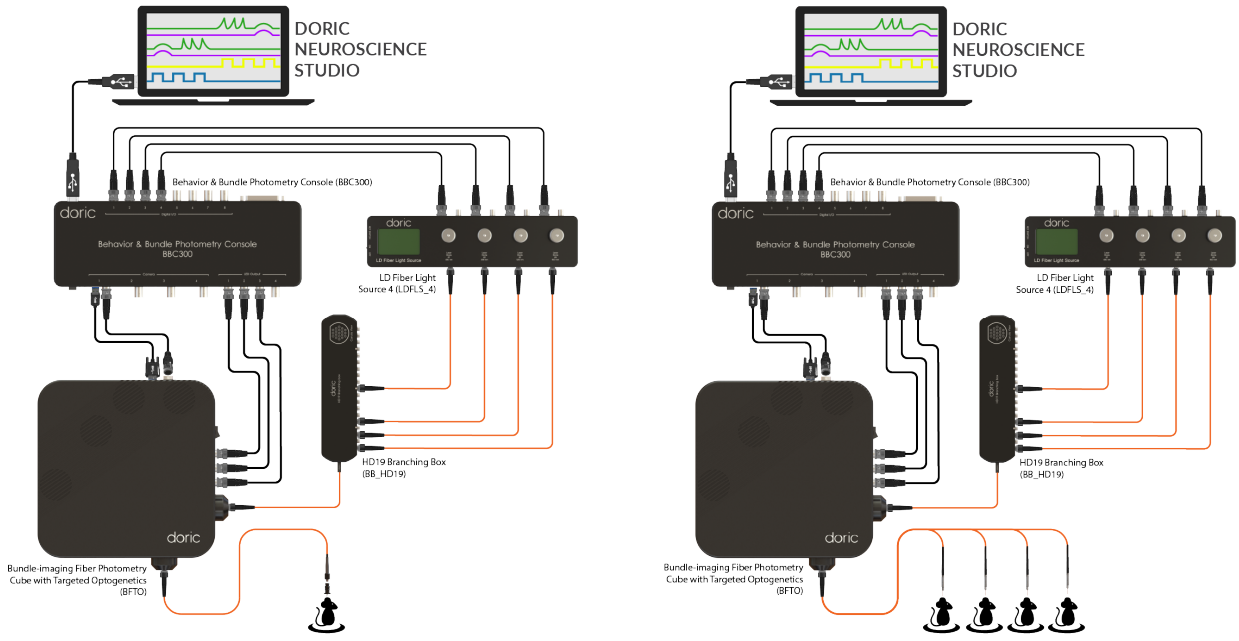
Introduction

The Bundle-imaging Fiber Photometry with Targeted Optogenetics (BFTO) is designed for multi-site experiments that combine photometry and optogenetics. Among all of Doric Lenses' photometry systems, the BFTO provides the most flexibility, as it supports dual-color fiber photometry in 7 to 19 sites, while independently controlling the optogenetic timing and wavelength at each of those sites.

The BFTO is interchangeably compatible with either **High Density** (Fig.1.1a) or **Fan Out** (Fig. 1.1b) output configuration. Both types of patch cords are available in 7, 9, or 19 fiber bundle patterns, and the connexion of either patch cord with the BFTO is designed for a single, reproducible orientation. However, note that the pattern of the high-density patch cords from the sample port must always match the pattern of the optogenetics port.

To record green and red photometry signals, the BFTO uses built-in LEDs to illuminate the entire sample port, and a CMOS sensor to simultaneously image the fluorescent signal from every fiber within the bundle. The fluorescent light collected from each fiber within the bundle creates circular spots on a CMOS sensor. The electrical read-out from pixels within each fiber image correlates with the calcium activity of the corresponding brain site.

To independently control the optogenetic stimulation, the optogenetics fiber bundle connects to a Branching Box that allows then to connect individual light sources, for both blue and red opsin activation. The created optogenetic pattern is transmitted through the corresponding fibers of the sample port connector for multi-site stimulations in a single animal (using a High-density cannula, Fig. 1.1a) or in multiple animals (using a branching patch cord, Fan Out configuration, Fig. 1.1b).



(a) *BFTO system (HD19 configuration)*

(b) *BFTO system (Fan Out configuration)*

Figure 1.1: *Bundle-Imaging Fiber Photometry System with Targeted Optogenetics overview*

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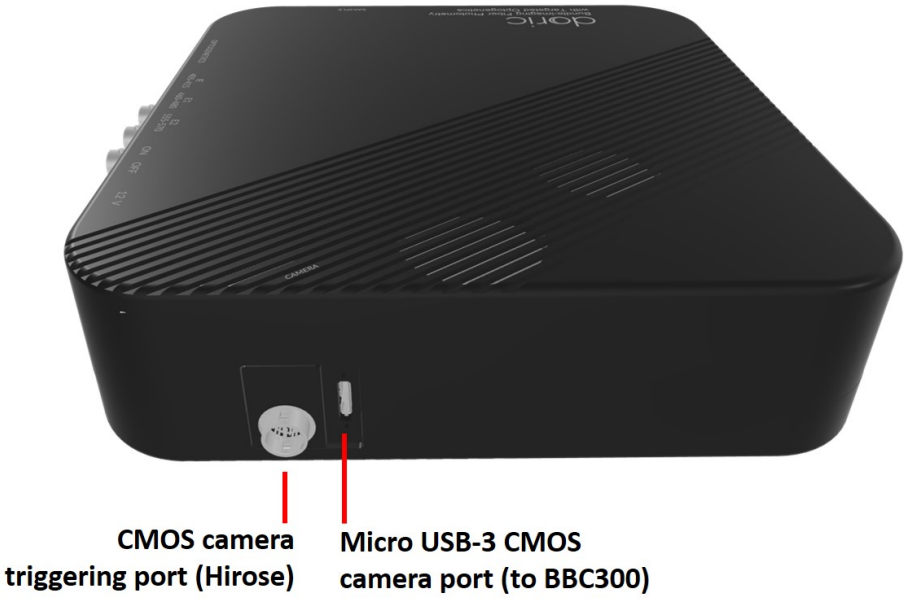
System Overview

- The **Bundle-imaging Fiber Photometry Cube with Targeted Optogenetics (BFTO)** enables multi-site measurements through High-density Fiber-optic Patch Cords (HDP). These patch cords are available in either square or hexagonal patterns of 7, 9, or 19 fibers connected to the **Sample Port** (Fig. 2.1a), allowing combined optogenetics and photometry recording. The sample port doesn't have spectral filtering, all wavelengths can pass freely through it. The sample port consists of a microscope lens and a fiber adapter to image fiber bundle onto the cameras. To accommodate larger fiber bundles (up to 2.5 mm of diameter), an SMA receptacle is used on the sample port. Two types of bundle can be connected to the SMA port: A Fan Out bundle where each fiber has an individual connector at its other end, or a High Density fiber that has multiple fiber-optic strands terminated on one side in the same high-density connector pattern as the corresponding High-Density Fiber-optic Cannula Array. Low autofluorescence materials and black epoxy are used to reduce background fluorescence and prevent cross-talk between each fiber.
- **Build-in LEDs** combine Isosbestic & Functional GCaMP excitations with Red Fluorophore excitation. Isosbestic and functional fluorescent signals are recorded with the same image sensor by interleaving the excitations. Both the LED driver and the CMOS detector are integrated within the system.
- The optogenetics stimulation pattern can vary in type, number, and emitted wavelengths. For this purpose, a second similar high-density fiber optic patch cord is connected to the **Optogenetics SMA Port** and connects on its other side to a 7-, 9- or 19-ports Branching Box. The Branching Box is one of the a key component within the BFTO system for the targeted optogenetic feature. Each port of the Branching Box corresponds to a single fiber within the bundle. Thus, the Branching Box arranges the independent optogenetic excitations (arising from multiple light sources) to the proper fibers within the bundle, which are then funneled into the Opsin port of the BFTO.
- **Excitation BNC Ports** are designated by E on the top engraving (Fig. 2.1a). Since there is more than one excitation port, they are labeled as E1 (for the green fluorescence) and E2 (for the red fluorescence). Isosbestic excitation port is labeled IE. Excitation ports contain a filter chosen to correspond to the excitation peaks of the fluorescent protein the BFTO is designed to measure, the wavelength bandwidth being indicated below the corresponding port name (Fig. 2.1a).
- **The Micro USB-3 Camera Port** (Fig. 2.1b) sends data from the CMOS sensor to a Behavior and Bundle Photometry Console (BBC300) via a USB-3 cable. The camera can be triggered by connecting a Hirose Trigger cable to the **Camera Trigger port**.
- The BFTO is also equipped with a **power switch** and a **status light** turning ON when the device is powered on (Fig. 2.1a).
- The **12 VDC power input port** is used to power the BFTO (Fig. 2.1a).

IMPORTANT NOTE: Aeration grids, located on top of the BFTO, are important for proper ventilation of the system. These grids must stay unobstructed at all time to garranty a good system operation.

(a) *BFTO system side view*



(b) *BFTO system camera view*

Figure 2.1: *Bundle-Imaging Fiber Photometry Cube with Targeted Optogenetics overview*

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Operations Guide

3.1 Connecting the Bundle-Imaging Fiber Photometry with Targeted Optogenetics System

Figure 3.1 and *Figure 3.2* illustrate the connections between all subsystems in 2 different configurations: High Density and Fan Out configurations.

1. **Connect** a Mini USB 3.0 to USB 3.0 cable between the BFTO and a camera port of the Behavior and Bundle Photometry Console (BBC300).
2. **Connect** a Hirose triggering cable between the camera trigger port of the BFTO and a BNC camera port of the Behavior and Bundle Photometry Console (BBC300). Note that the same port as the USB3.0 cable should be used on the console.
3. **Connect** the Built-in LEDs excitation ports of the BFTO to the LED Output ports of the Behavior and Bundle Photometry Console (BBC300) using BNC cables.
4. **Connect** a HD7, HD9 or HD19 Branching Box to the Optogenetics SMA port of the BFTO.
5. **Connect** the HD7, HD9 or HD19 Branching Box to a Laser Diode Fiber Light Source using FC/APC to FC fiber optic patchcords. The number of Laser Diodes used will depend on the experimental design.
6. **Connect** the LD fiber light source Input ports to the digital I/O ports of the BBC300 with BNC cables. The number of connections required between both devices depends on the configuration of your experiment and the number of Laser Diodes involved. To ease experiment configuration in Doric Neuroscience Studio, we recommend connecting LD1 with DI/O port 1...
7. **Connect** the LD fiber light source driver, the BFTO, and the BBC300 to the 12 V AC/DC and 60W power supply with the power supply splitters.
8. **Plug** the power supply to a power outlet.
9. **Connect** a USB 3.0 cable from the BBC300 to the PC.
10. **Connect** the SMA connector of the BFTO sample port to a High Density patchcord or a branching patchcord, depending on your configuration.
11. **Open** Doric Neuroscience Studio software and refer to its user manual to set up and experiment.

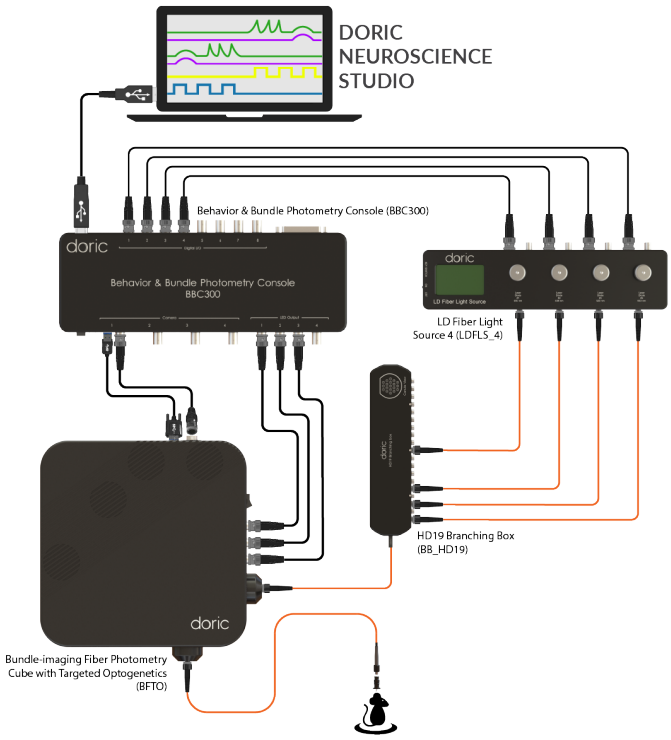

Figure 3.1: *BFTO system: HD19 configuration*

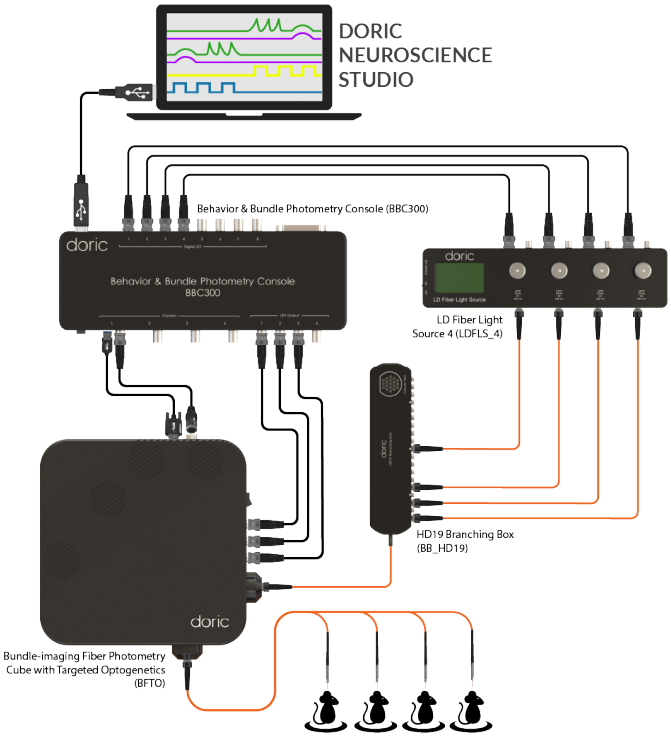

Figure 3.2: *BFTO system: Fan Out configuration*

3.2 Optical fiber patch cord

- **Clean** the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.
- With an FC connector, the **connector key must be oriented to enter within the receptacle slot** to ensure proper connection (Fig. 3.3).

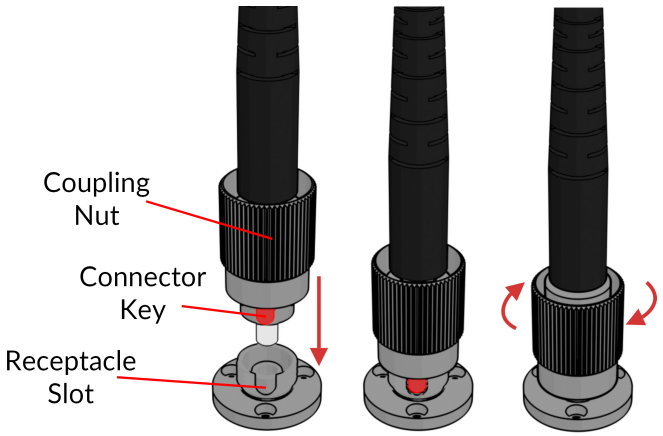

Figure 3.3: *FC connector, Fiber Installation*

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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Specifications

4.1 General specifications

Table 4.1: *General specifications for BFTO*

SPECIFICATION	VALUE	UNIT
Bundle-imaging Fiber Photometry Cube with Targeted Optogenetics		
Wavelength range	350 to 1100	nm
Field of view	Ø3	mm
Objective NA	0.40	-
Max. number of sites	- 7x core 400 µm NA0.37 - 9x core 400 µm NA0.37 - 19x core 200 µm NA0.37	-
LEDs Excitation Uniformity	90% over FOV	-
Optogenetics Excitation Uniformity	65% over FOV	-
Optogenetics Crosstalk between fibers	> 41	dB
Optical fiber compatibility	Core diameter 200 or 400 µm NA 0.37	-
Optical filter attenuation	> OD 5 outside band	-
Optical fiber connector	SMA	-
Built-in LEDs		
Max Current	500	mA
Maximum Output Power	See Table 4.3	-
Sensor		
CMOS image sensor	Sony IMX174LLJ	-
Pixel Size	5.86 x 5.86	µm
Resolution	1024 x 1024	pixels
Quantum Efficiency	82% at 520nm	
Frame Rate	up to 60	Hz
Power consumption (supplied by USB)	200	mA
Physical properties		
Size	220x220x59	mm
Mass	2700	g
Power supply		
Voltage	110 - 240	VAC
DC power supply	12	VDC
Power	36 (or 60 if splitted power w/ other devices)	W
Output current	3	A

Table 4.2: *Computer requirements for BFMC Gen3 installation*

Operating system	Microsoft 10, 64 bit
Memory	8 GB RAM minimum (16 GB recommended)
Processor speed	3 GHz and 8 cores
Hard drive	500 MB of free hard disk space (SSD recommended)
Data link	USB3.0 (cable included)

4.2 Optical specifications

Table 4.3: *Typical Built-in LED Output Power vs Optical Fiber Core Diameter*

LED		TYPICAL OUTPUT POWER @200 mA, CW (mW)	
Central Wavelength (nm)	Bandwidth FWHM (nm)	Core 200 µm 0.37 NA	Core 400 µm 0.37 NA
405	10	~0.100	~0.700
415	10	~0.130	~0.500
474	23	~0.180	~0.700
563	9	~0.020	~0.130

Table 4.4: *Typical filter configuration of BFTO*

Fluorescence Mini Cubes	Excitation (nm)	Fluorescence (nm)
GCaMP + red fluorophore		
Excitation 1 (isosbestic)	400-410 or 410-420	
Excitation 2 (functional)	460-490	500-540
Excitation 3 (red fluorophore)	555-570	580-600

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Support

5.1 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.

5.2 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.

5.3 Disposition

Figure 5.1: *WEEE directive logo*

According with the directive 2012/19/EU of the European Parliament and the Council of the European Union regarding Waste Electrical and Electronic Equipment (WEEE), when the product will reach its end-of-life phase, it must not be disposed with regular waste. Make sure to dispose of it with regards of your local regulations. For more information about how and where to dispose of the product, please contact Doric Lenses.

5.4 Contact us

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

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