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

User Manual

Version 1.0.1

Contents

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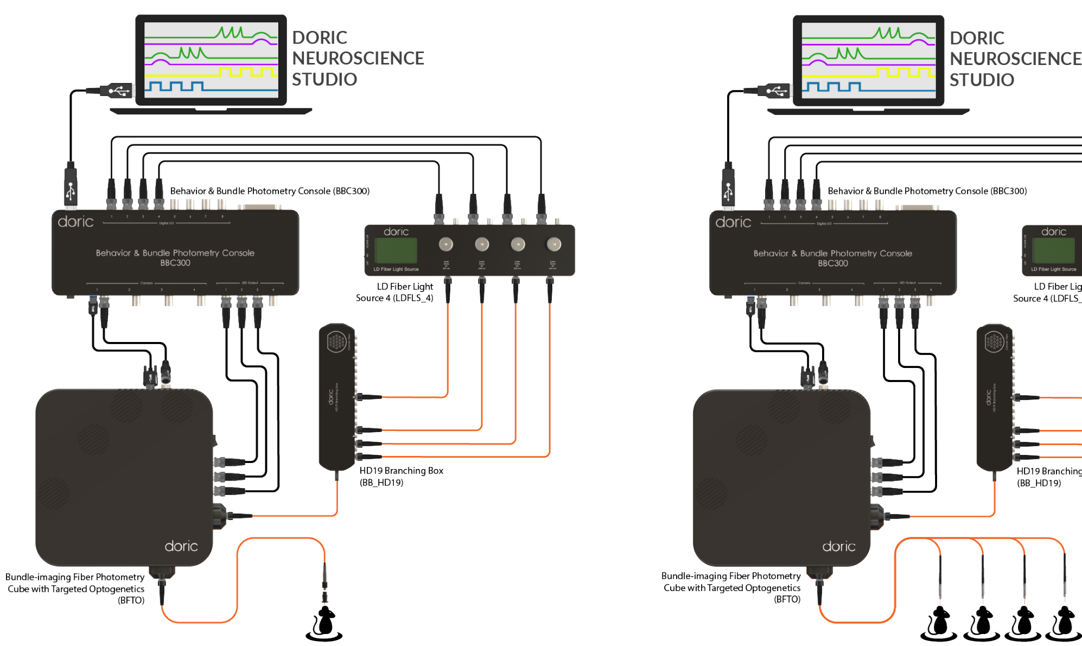
Introduction

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.

1.1 Fiber Photometry requirements

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 connection 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.



(a) *BFTO system (HD19 configuration)* (b) *BFTO system (Fan Out configuration)*

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

1.2 Optogenetics requirements

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).

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System Overview

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

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.

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.

Breakdown of the BFTO components:

- **Sample Port**
- 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 HD 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.
- **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.
- **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).

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*

2.2 Branching Box

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Getting Started

This chapter provides a step-by-step guide to wire the BFTO photometry system in your lab for the first time. If you require additional assistance or have any questions, please don't hesitate to contact the Doric team for support at **sales@doriclenses.com**.

The Bundle Fiber Photometry system is compatible with both the NC500 and BBC300 consoles. Follow the steps below to set up the hardware according to your console:

3.1 Wire up the system with the BBC300 console

Figure 3.2 provides a general overview of the system wiring with the BBC300 console. Initially, we will connect the BBC300 console to the computer and then wire the console to the camera detector (inside the BFTO box). This step ensures that the detector camera will record photometry signal:

1. Use the blue USB 3.0 cable to connect the USB 3.0 port of the BBC300 console (beside the power port) to a USB 3.0 port on your computer. **USB 3.0 connection is important to ensure reliable high-speed data transmission.**

   Note: If you do not have USB 3.0 port, use the PCI card that comes with the system to add USB ports to the desktop computer.

   Note: Avoid Using USB hubs, and replacing Doric USB 3.0 cable for a very long one.

2. Use a USB 3.0 cable to connect one of the BBC300 camera port to the BFTO Micro USB 3.0 CMOS camera port (Figure 2.1). **USB 3.0 connection is important to ensure reliable high-speed data transmission.**

3. Use a Hirose triggering cable to connect the corresponding BBC300 camera BNC port to the BFTO box CMOS camera triggering port (Figure 2.1). **Note** that the same port as the USB 3.0 cable should be used on the BBC300 console.

4. Do not forget to plug the power supply of both the BBC300 console and the BFTO box to a power outlet.

Now we need to connect the integrated LEDs of BFTO box to the BBC300 console to provide the excitation light that are needed to activate the fluorescent signal from biosensors during photometry recording:

5. Use three BNC cables to connect the **LED Output** ports of the BBC300 to the **E1** (isosbestic), **E2** (Blue light) and **E3** (Yellow light) LED excitation ports of the BFTO box. For standard wiring connect:

   1- BBC300 LED Output 1 -> BFTO box **E1** port

   2- BBC300 LED Output 2 -> BFTO box **E2** port

   3- BBC300 LED Output 3 -> BFTO box **E3** port

6. To connect the BFTO to the animal, plug your Fan Out (branching) or HD connectors to the sample port of the BFTO box.

Note: After inserting the HD connector, gently continue rotating it until you feel a click. This click indicates that the notch (Fig. 3.1) has locked into place and the correct orientation has been achieved. This ensures alignment the optogenetics HD port, and you will opto-target the right ROIs during experiment.

Note: If the patch cord is old, clean the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.

Figure 3.1: *HD connector Notch*

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

To use with a rotary joint (commutator), you will need a branching patch cord with FC output connectors. At each FC branch of your patch cord, you can then connect a pigtailed rotary joint. Then pair another FC-ZF patch cord between the output of the rotary joint and the implanted cannula.
Note: You can use a rotary joint only with the fan-out configuration where you use one rotary joint per fan-out branch, see figure 3.2.

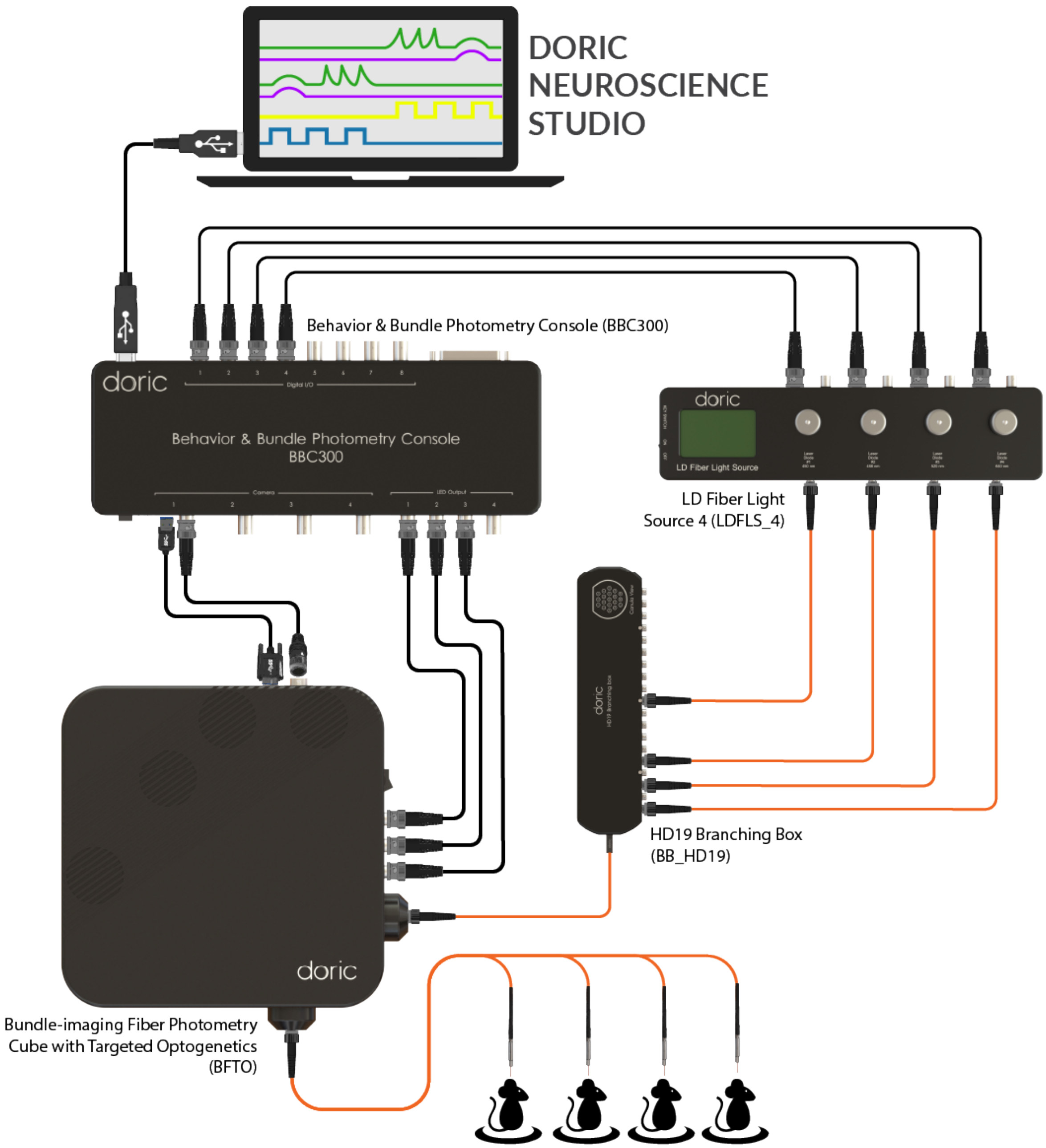

Figure 3.2: *BFTO system with BBC300 console: Fan Out configuration*

3.2 Wire up the optogenetics with BBC300 console

So far, the wiring for the photometry recording has been completed. We can now assemble the optogenetics components, the Laser Diode Fiber Light Source (LDFLS) and HD branching box (Fig. 3.3) . Follow the steps below to connect them:

1. Begin with the LDFLS. Depending on the number of ROIs that you want to deliver optogenetics, your LDFLS can have different number of channels. Here, we will give an example for a 4-channel LDFLS (Fig. 3.3). Using BNC cables, connect 4 Digital I/O channels of BBC300 console to 4 **"Input"** channels at the back of the LDFLS. We recommend connecting as:

    - BBC300 DIO port 1 -> LDFLS Input 1
    - BBC300 DIO port 2 -> LDFLS Input 2
    - BBC300 DIO port 3 -> LDFLS Input 3
    - BBC300 DIO port 4 -> LDFLS Input 4

    Note: The **"Output"** BNC channels behind the LDFLS can be used to record a copy of the TTL pulses that are send to the ROIs, if ever needed.

2. On the LDFLS, there are 4 control knobs. These knobs allow you to manually change the channel mode or adjust the output power. However, since the system will be configured through the Doric Neuroscience Studio (DNS) software, manual adjustment using these knobs is not necessary here.

3. The FC/APC connectors at the front of the LDFLS are the laser output ports. Use FC-FC patch cords to connect each laser output to the corresponding ROI ports on the HD Branching Box where optogenetic stimulation will be delivered.

    In Fig. 3.3 image, the branching patch cord outputs are labeled as 3, 7, 13, and 19. Therefore, the LDFLS ports are connected as follow:

    - LDFLS Channel 1 -> Branching Box Chanel 3
    - LDFLS Channel 2 -> Branching Box Chanel 7
    - LDFLS Channel 3 -> Branching Box Chanel 13
    - LDFLS Channel 4 -> Branching Box Chanel 19

4. The HD Branching Box has a pigtailed HD fiber optic patch cord. Connect this fiber to the Optogenetics HD port of the BFTO.

    Note: After inserting the HD connector, gently continue rotating it until you feel a click. This click indicates that the notch (Fig. 3.1) has locked into place and the correct orientation has been achieved. This ensures optogenetics alignment with the HD Sample port, and you will opto-target the right ROIs during experiment.

5. Do not forget to plug the LDFLS power supply into a power outlet.

Note: All required cables are included with the system shipment.

At this point all the system wiring is complete. To make configurations for optogenetics stimulation in DNS, please refer to this Tutorial Video, or contact us at sales@doriclenses.com.

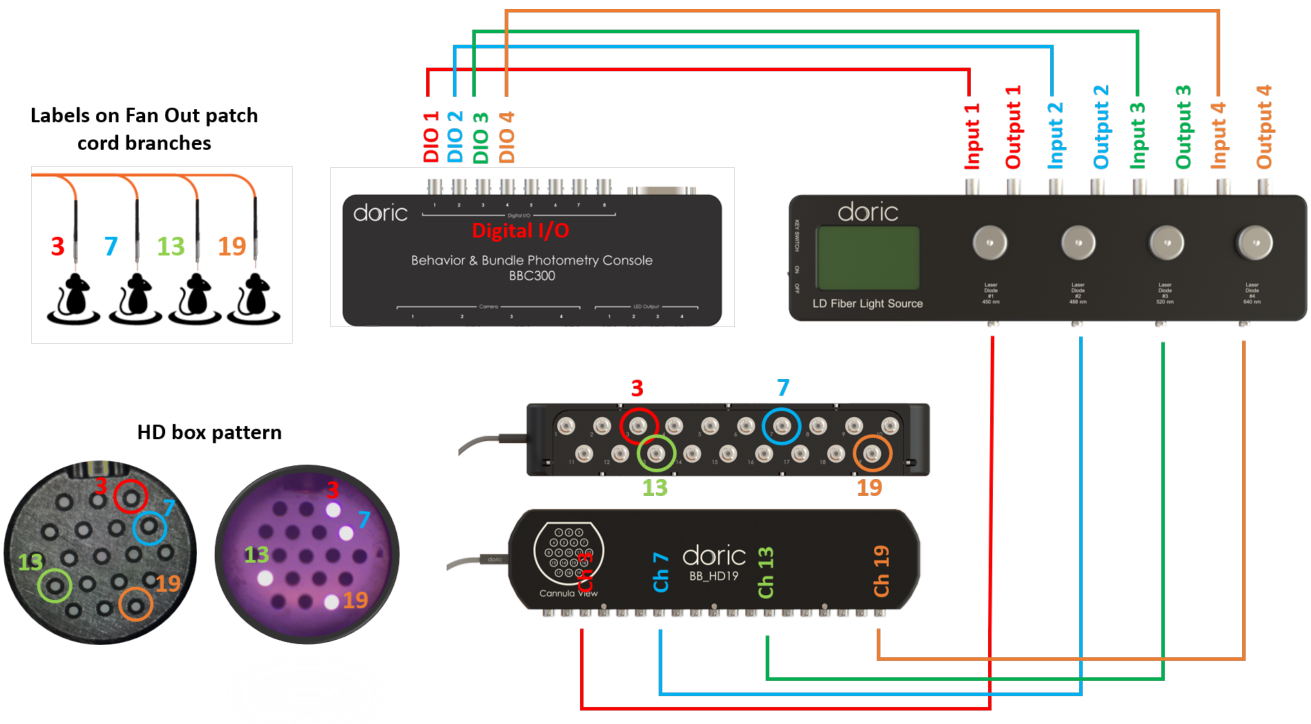

Figure 3.3: *Optogenetics Wiring*

3.3 Wire up the system with NC500 console

Figure 3.5 provides a general overview of the system wiring with the NC500 console. Initially, we will connect the NC500 console and camera detector (inside the BFTO box). This step ensures that the detector camera will record photometry signal:

1. Use the blue USB 3.0 cable to connect the USB 3.0 port on the NC500 console (at the back of the console) to a USB 3.0 port on your computer. **USB 3.0 connection is important to ensure reliable high-speed data transmission.**

   Note: If you do not have USB 3.0 port on your computer, install the PCI card that comes with the system to add USB port to the desktop computer.

   Note: Avoid Using USB hubs, or replacing Doric USB 3.0 cable for a very long one.

2. Use a blue USB 3.0 cable to connect one of your computer USB 3.0 port to the BFTO Micro USB 3.0 CMOS camera port (Figure 2.1). **USB 3.0 connection is important to ensure reliable high-speed data transmission.**

3. Use a Hirose triggering cable to connect the NC500 **Analog Out port 1** to the BFTO box CMOS camera triggering port (Figure 2.1).

4. Do not forget to plug the power supply of both NC500 console and BFTO box to a power outlet.

Now we need to connect the integrated LEDs of BFTO box to the console **Analog Out** ports, to provide the excitation light that are needed to activate the fluorescent signal from biosensors during photometry recording:

5. Use three BNC cables to wire as follow:

   1- NC500 Analog out 3 -> BFTO **E1** port

   2- NC500 Analog out 4 -> BFTO **E2** port

   3- NC500 Analog out 5 -> BFTO **E3** port

6. To connect the BFTO to the animal, plug your Fan Out (branching) or HD connectors to the sample port of the BFTO box.

Note: After inserting the HD connector, gently continue rotating it until you feel a click. This click indicates that the notch (Fig. 3.4) has locked into place and the correct orientation has been achieved. This ensures optogenetics alignment with the HD Sample port, and you will opto-target the right ROIs during experiment.
Note If the patch cord is old, clean the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.

Figure 3.4: *HD connector Notch*

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

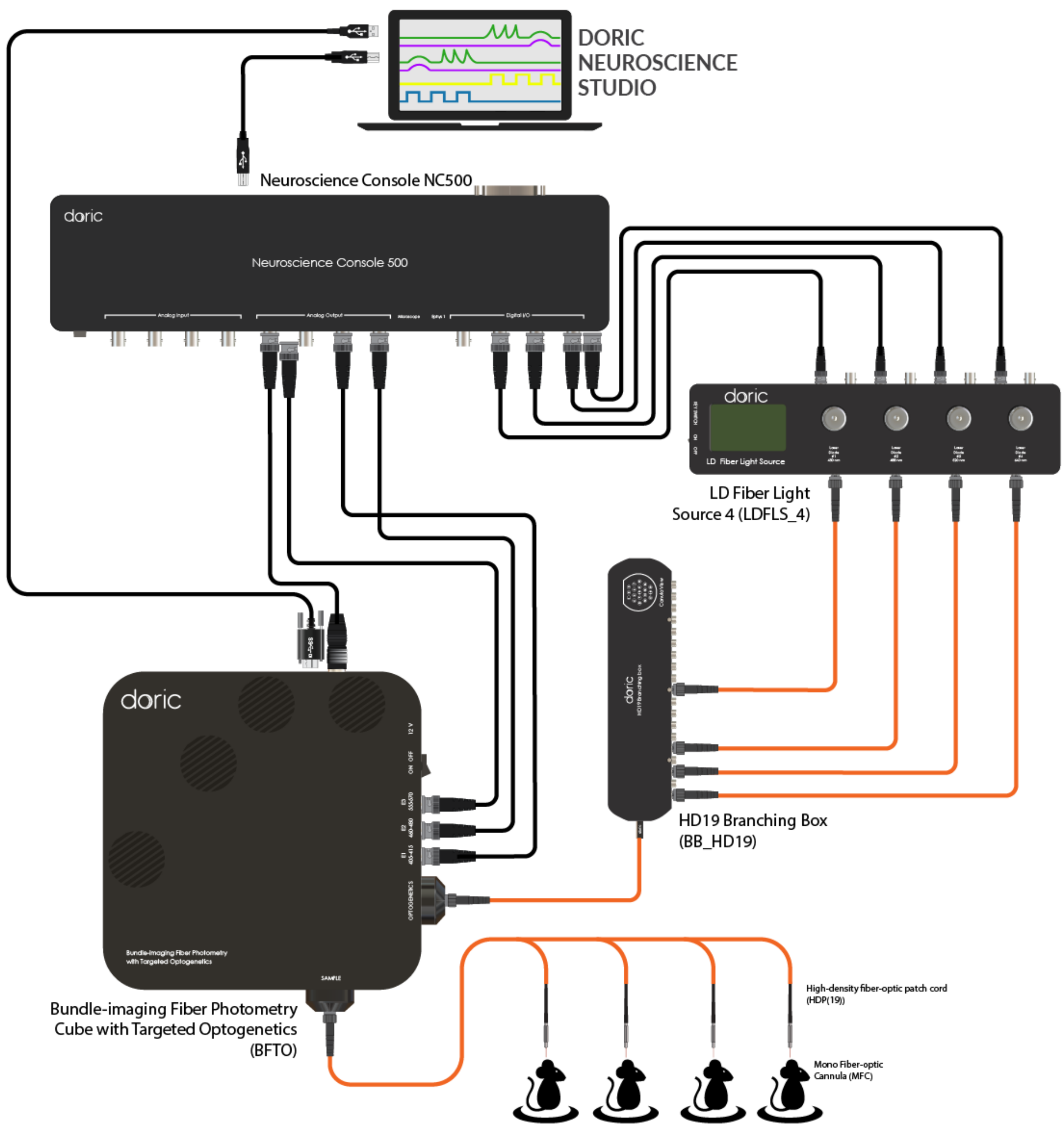

Figure 3.5: *BFTO system with the NC500: Fan Out configuration*

3.4 Wire up the optogenetics with NC500 console

So far, the wiring for the photometry recording has been completed. We can now assemble the optogenetics components, the Laser Diode Fiber Light Source (LDFLS) and HD branching box (Fig. 3.6) . Follow the steps below to connect them:

1. Begin with the LDFLS. Depending on the number of ROIs that you want to deliver optogenetics, your LDFLS can have different number of channels. Here, we will give an example for a 4-channel LDFLS (Fig. 3.6). Using BNC cables, connect 4 Digital I/O channels of NC500 console to 4 **"Input"** channels at the back of the LDFLS. We recommend connecting as:

   - NC500 DIO port 1 -> LDFLS Input 1
   - NC500 DIO port 2 -> LDFLS Input 2
   - NC500 DIO port 3 -> LDFLS Input 3
   - NC500 DIO port 4 -> LDFLS Input 4

   Note: The **"Output"** BNC channels behind the LDFLS can be used to record a copy of the TTL pulses that are send to the ROIs, if ever needed.

2. On the LDFLS, there are 4 control knobs. These knobs allow you to manually change the channel mode or adjust the output power. However, since the system will be configured through the Doric Neuroscience Studio (DNS) software, manual adjustment using these knobs is not necessary here.

3. The FC/APC connectors at the front of the LDFLS are the laser output ports. Use FC-FC patch cords to connect each laser output to the corresponding ROI ports on the HD Branching Box where optogenetic stimulation will be delivered.

   In Fig. 3.6 image, the branching patch cord outputs are labeled as 3, 7, 13, and 19. Therefore, the LDFLS ports are connected as follow:

   - LDFLS Channel 1 -> Branching Box Chanel 3
   - LDFLS Channel 2 -> Branching Box Chanel 7
   - LDFLS Channel 3 -> Branching Box Chanel 13
   - LDFLS Channel 4 -> Branching Box Chanel 19

4. The HD Branching Box has a pigtailed HD fiber optic patch cord. Connect this fiber to the Optogenetics HD port of the BFTO.

   Note: After inserting the HD connector, gently continue rotating it until you feel a click. This click indicates that the notch (Fig. 3.4) has locked into place and the correct orientation has been achieved. his ensures optogenetics alignment with the HD Sample port, and you will opto-target the right ROIs during experiment.

5. Do not forget to plug the LDFLS power supply into a power outlet.

Note: All required cables are included with the system shipment.

At this point all the system wiring is complete. To make configurations for optogenetics stimulation in DNS, please refer to this Tutorial Video, or contact us at sales@doriclenses.com.

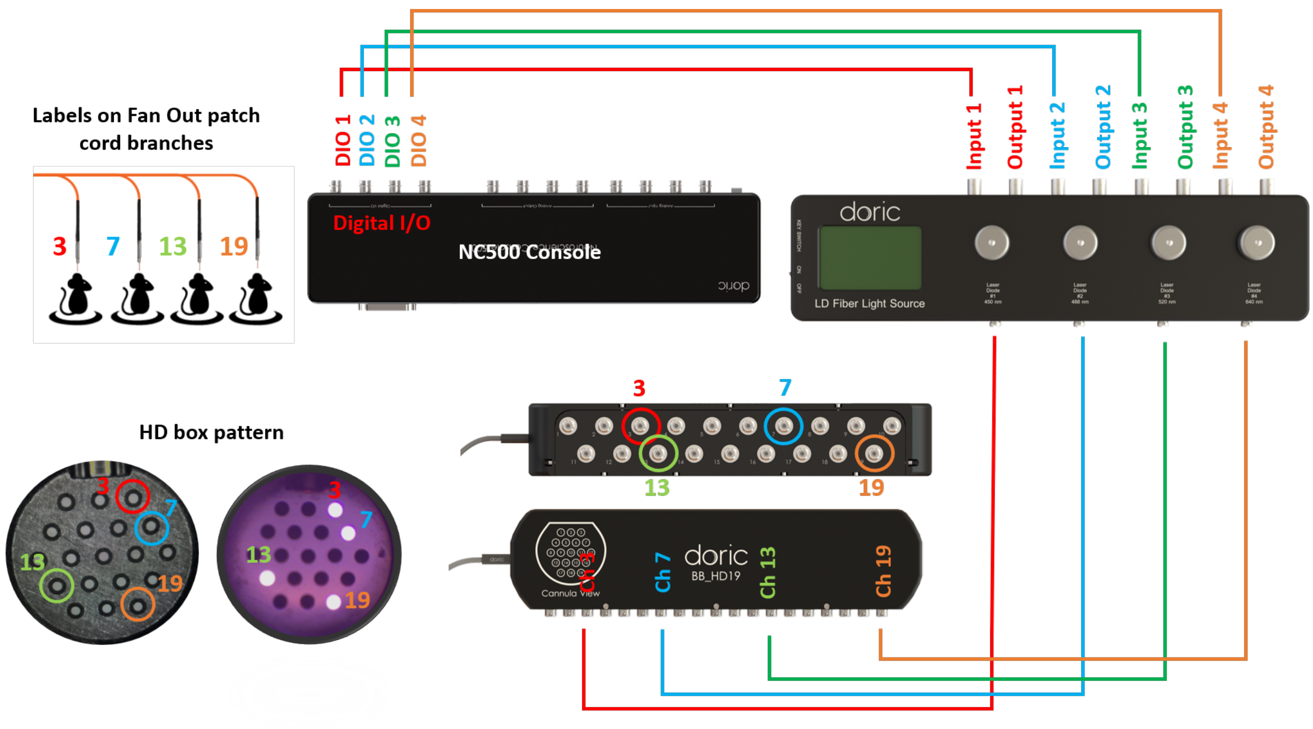

Figure 3.6: *Optogenetics Wiring*

4

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:

Phone 1-418-877-5600

Email sales@doriclenses.com

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