# Fiber Photometry

In neuroscience, fiber photometry denotes a method whereby a chronically implanted optical fiber delivers excitation light to neurons tagged with a fluorescent calcium indicator(s) and collects their overall activity-induced fluorescence. Within the field of view, the fluorescence microscopy indicates activity of each tagged neuron, while the fiber photometry sums up the activity-induced fluorescence of all neurons expressing the indicator(s).

Distinguishing the very weak fluorescence variations from relatively high noise levels requires careful selection of components within the system, from light sources to detectors. The connectorized LED module (CLED) as excitation source offers sufficient spectral intensity for most fluorescent markers, stable power and speckle-free illumination. An interesting alternative is a combination of UV or blue LEDs with the Ce:YAG source filtered to a required wavelength. The latter offers all the advantages of LED illumination, but with higher intensity in the 500-600 nm range. Laser sources could be considered when using small diameter core fibers with low NA and/or multiple color excitations requiring narrow spectral filtering.

The heart of the Fiber Photometry System is the Fluorescence Mini Cube (FMC) that directs excitation light into an optical fiber leading to the fiber-optic cannula. The fluorescence of the sample captured by the cannula is returned into the FMC, filtered and redirected into a detection fiber that goes to the high sensitivity photodetector. The opto-mechanical design of the fluorescence mini-cube, the filter selection and the coupling optics alignment play an important role in increasing the signal to noise ratio.

Typically, excitation optical power in mW range produces fluorescence responses in nW range. The detection of such a low level signal requires a low-noise amplified photodetector. As the optical isolation of each component is essential in this power range, the optical fibers must have protective jackets to avoid possible effects of ambient light on the measurement. The worst DC noise might be coming from the autofluorescence of the probe or the patch cord itself. To keep this noise in check, low autofluorescence optical fibers must be used and their length kept to a bare minimum. To prevent injecting light into optical fiber cladding, the fiber optic collimator must under fill the fiber NA and the light spot on the fiber's end face should be smaller than its core diameter.

# Fiber Photometry Systems

## **1-site Fiber Photometry Systems**

#### GCaMP Isosbestic and Functional Excitations - 405 and 465 nm

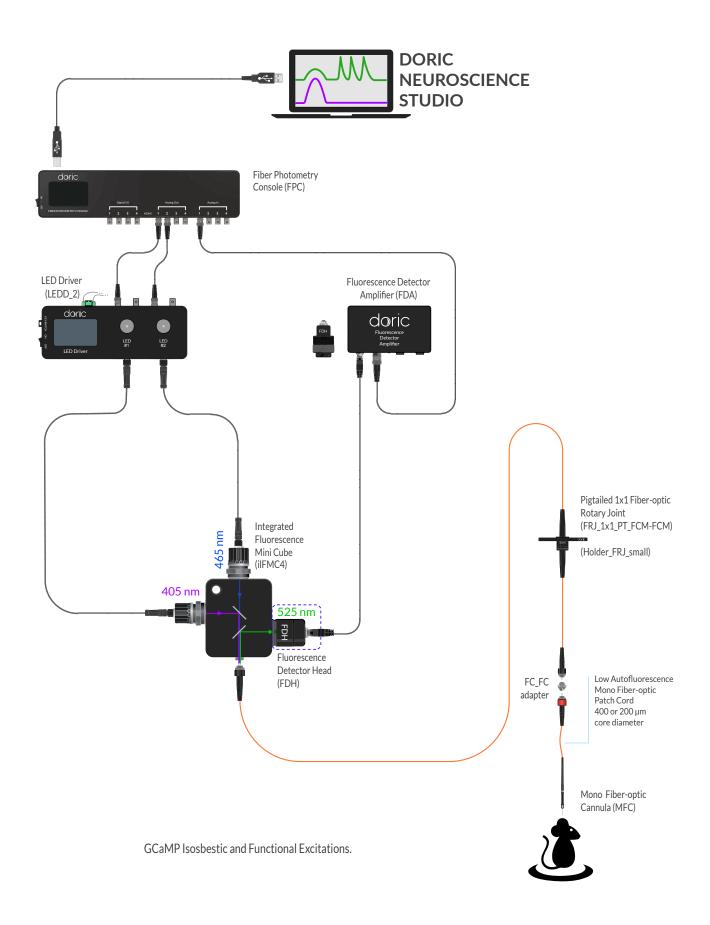
This 1-site Fiber Photometry System measures the 405 nm (isosbestic point) excited GCaMP fluorescence, and the 465 nm excited calcium-dependent GCaMP fluorescence, on a single photodetector. The fluorescence emission can be demodulated by lock-in detection, or by sequential acquisition. The GCaMP Isosbestic and Functional Excitations - 405 and 465 nm System contains:

- 2-channel LED Driver
- Integrated Fluorescence Mini Cube with 4 ports Lock-in or Sequential Detection of GCaMP Isosbestic and Functional Excitations
- Pigtailed 1x1 Fiber-optic Rotary Joint
- 1x1 Fiber-optic Rotary Joint Holder
- Rotary Joint Gimbal Holder
- Mono Fiber-optic Cannulas (10x)
- Fiber Photometry Cannula Holder
- Fiber Photometry Console for data acquisition and illumination control
- Doric Neuroscience Studio Software
- Fiber Photometry Rack to mount the whole system
- All required electrical cables and optical patch cords

## ORDERING CODE: FPS\_1S\_405/GFP\_00.57

Cannula fiber diameter (µm) <b>400</b> or <b>200</b>	
Cannula numerical aperture	

<u>Note</u>: Other light sources and different fluorophore combinations are possible. Please do not hesitate to request your preferences.



## GCaMP Isosbestic and Functional Excitations, and Red Fluorophore Fluorescence

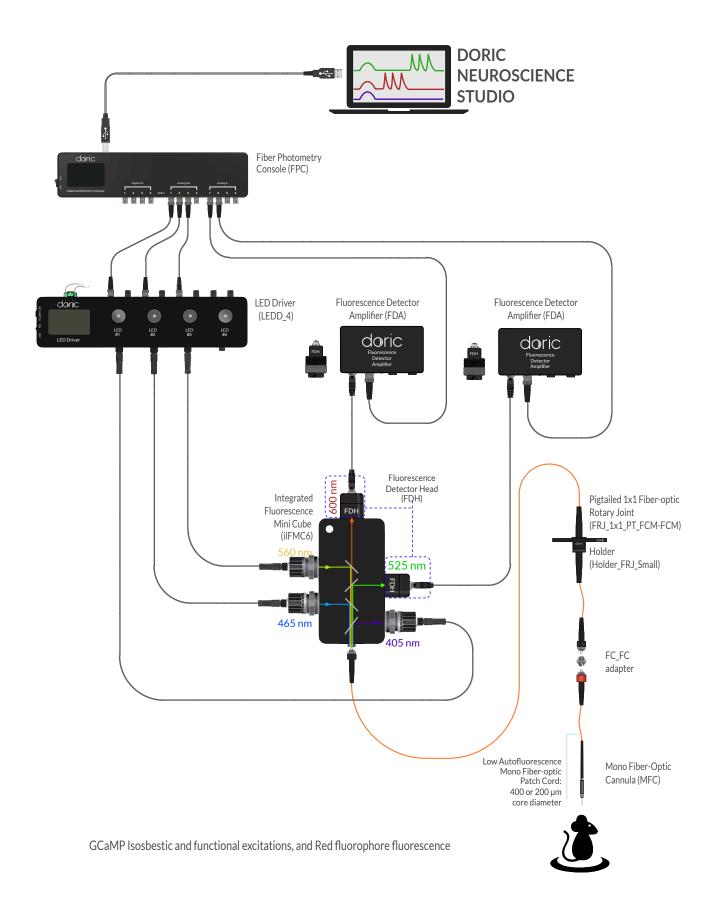
This 1-site Fiber Photometry System contains all the items necessary to do photometry measurements of two independent colors in freely-moving animals like GFP-like and RFP-like fluorophores. It also measures the 405 nm (isosbestic point) excitation of GCaMP fluorescence. The GCaMP fluorescence emission can be demodulated by lock-in detection or by sequential acquisition. It is also possible to use lock-in demodulation to ensure a proper spectral separation of the green and red fluorophore emissions. The GCaMP Isosbestic and Functional Excitations, and Red Fluorophore Fluorescence System contains:

- 4-channel LED Driver
- Integrated Fluorescence Mini Cube with 6 ports Two Fluorophores Fluorescence and GCaMP Isosbestic Excitation
- Pigtailed 1x1 Fiber-optic Rotary Joint
- 1x1 Fiber-optic Rotary Joint Holder
- Rotary Joint Gimbal Holder
- Mono Fiber-optic Cannulas (10x)
- Fiber Photometry Cannula Holder
- Fiber Photometry Console for data acquisition and illumination control
- Doric Neuroscience Studio Software
- Fiber Photometry Rack to mount the whole system
- All required electrical cables and optical patch cords

**Note**: Other light sources and different fluorophore combinations are possible. Please do not hesitate to request your preferences.

## ORDERING CODE: FPS\_1S\_405/GFP/RFP\_00.57

Cannula fiber diameter (µm) <b></b> <b>400</b> or <b>200</b>
Cannula numerical aperture



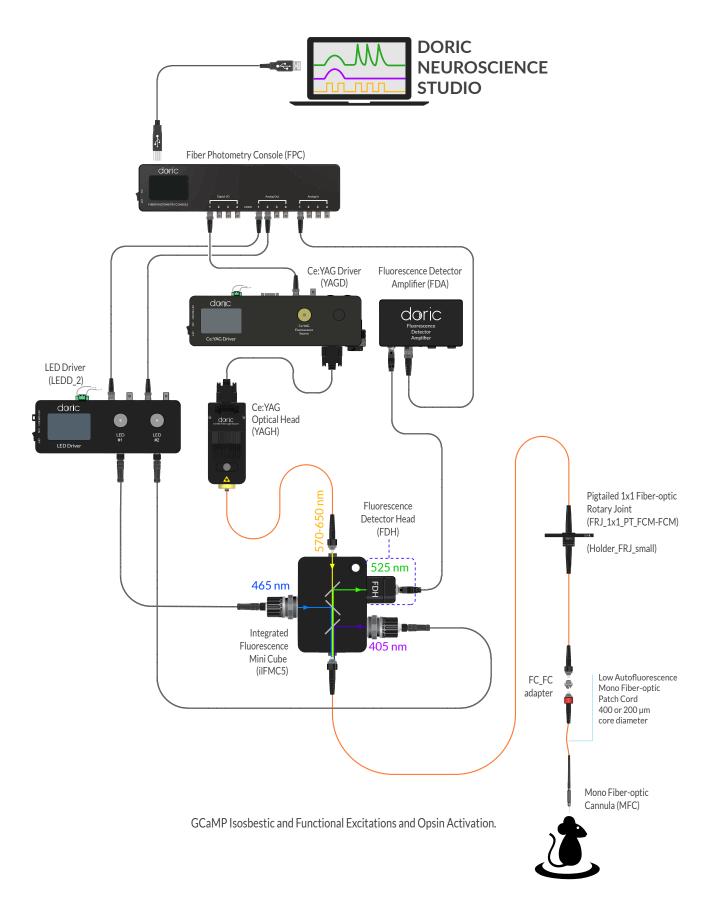
## GCaMP Isosbestic and Functional Excitations and Opsin Activation

This 1-site Fiber Photometry System measures the GCaMP isosbestic point and the GCaMP functional calcium-dependent fluorescence, on a single photodetector. The fluorescence emission can be demodulated by lock-in detection, or by sequential acquisition. This system allows the activation of an opsin (570-650 nm). The GCaMP Isosbestic and Functional Excitations, and Opsin Activation System contains:

- 2-channel LED Driver
- Ce:YAG Optical Head
- Ce:YAG Driver
- Bandpass Filter For Ce:YAG Fiber Light Sources
- Integrated Fluorescence Mini Cube with 4 ports Excitation, Fluorescence and Opsin Activation
- Pigtailed 1x1 Fiber-optic Rotary Joint
- 1x1 Fiber-optic Rotary Joint Holder
- Rotary Joint Gimbal Holder
- Mono Fiber-optic Cannulas (10x)
- Fiber Photometry Cannula Holder
- Fiber Photometry Console for data acquisition and illumination control
- Doric Neuroscience Studio Software
- All required electrical cables and optical patch cords

<u>Note</u>: Other light sources, such as laser diodes, and different fluorophore combinations are possible. Please do not hesitate to request your preferences.

ORDERING CODE: FPS_1S_405/GFP/opsin_00.	57
Cannula fiber diameter (µm) <b></b> <b>400</b> or <b>200</b>	
Cannula numerical aperture	



## 2-site Fiber Photometry Systems

#### 2-site GCaMP Isosbestic and Functional Excitations

This 2-site Fiber Photometry System measures the 405 nm (isosbestic point) excited GCaMP fluorescence, and the 465 nm excited calcium-dependent GCaMP fluorescence, on a single photodetector. The fluorescence emission can be demodulated by lock-in detection, or by sequential acquisition. The measures are done in two different brains areas.

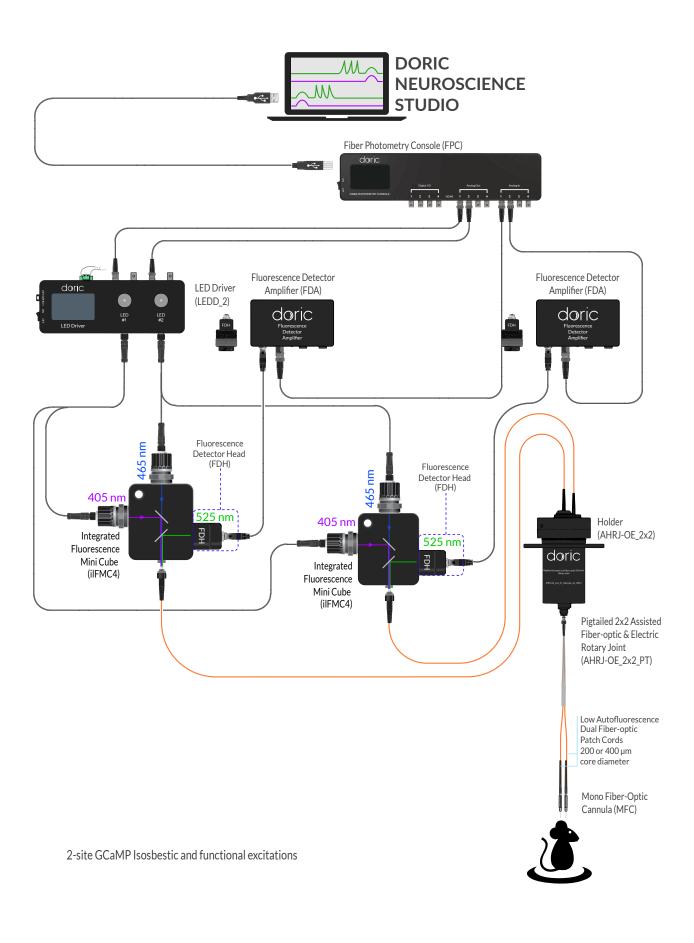
The 2-site GCaMP Isosbestic and Functional Excitations contains:

- 2-channel LED Driver
- Integrated Fluorescence Mini Cube with 4 ports Lock-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations (filter set optimized for 405 nm excitation and GFP; (2x)
- Pigtailed 2x2 Assisted Fiber-optic & Electric Rotary Joint
- 2x2 Fiber-optic Rotary Joint Holder
- Mono or Dual Fiber-optic Cannulas (10x)
- Fiber Photometry Cannula Holder
- Fiber Photometry Console for data acquisition and illumination control
- Doric Neuroscience Studio Software
- Fiber Photometry Rack to mount the whole system
- All required electrical cables and optical patch cords

## ORDERING CODE: FPS\_2S\_405/GFP\_00.57

Cannula fiber diameter (µm) —— **400** or **200** 

Cannula numerical aperture



## **Bundle-imaging Fiber Photometry Systems**

Scaling up fiber photometry measurement with photodiode based systems to a large number of samples can be difficult. This is due to the increasing number of components required, such as mini cubes, cables and sensors. With some compromises on time resolution and sensitivity, an alternative approach is to relay the image of an optical fiber bundle to an image sensor. The opposite end of the bundle can be split, with each connected to different samples. On the camera, the signal of each sample can be distinguished by measuring the average count coming from each individual fiber in the bundle.

#### GCaMP Isosbestic & Functional Excitations

The GCaMP Isosbestic & Functional Excitations Bundle-imaging Fiber Photometry System contains all items necessary to perform fiber photometry measurements on a large number of animals and sites with GFP-like fluorophores. It also measures the 405 nm (isosbestic point) excitation of GCaMP fluorescence. The GCaMP fluorescence emission can be demodulated by sequential acquisition.

The base GCaMP Isosbestic & Functional Excitations Bundle-imaging Fiber Photometry System contains the following elements.

- 2-channel LED driver
- Bundle-imaging Fluorescence Mini Cube with 4 ports Isosbestic and Functional Excitations
- Bundle-imaging Fiber Photometry Driver
- 4-port USB3.0 Hub
- Photometry Rack for BFPS
- All required electrical cables

<u>Note</u>: Cannulae, rotary joints and other elements used are to be defined for each application, and are not specified here.

## **ORDERING CODE:** BFPS\_405/GFP

#### GCaMP Isosbestic & Functional Excitations, & Red Fluorophore Fluorescence

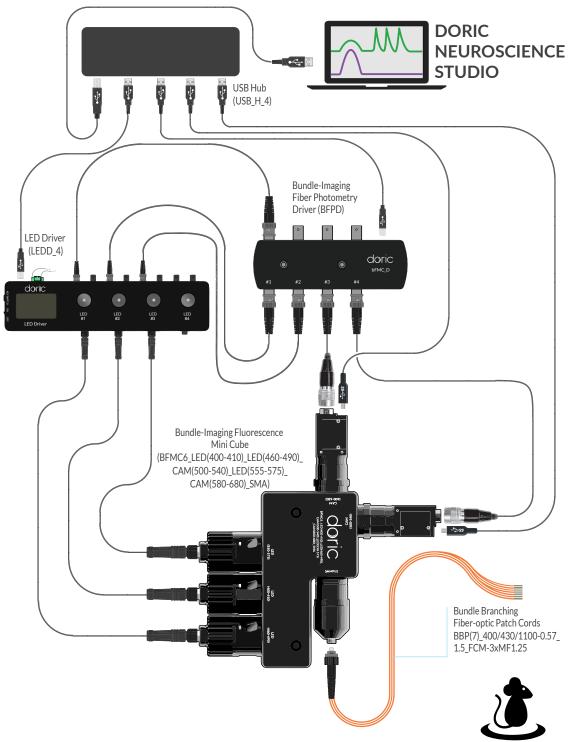
The GCaMP Isosbestic & Functional Excitations, & Red Fluorophore Fluorescence Bundleimaging Fiber Photometry System contains all items necessary to perform fiber photometry measurements on a large number of animals and sites with GFP-like and RFP-like fluorophores. It also measures the 405 nm (isosbestic point) excitation of GCaMP fluorescence. The GCaMP fluorescence emission can be demodulated by sequential acquisition.

The base GCaMP Isosbestic & Functional Excitations, & Red Fluorophore Fluorescence Bundle-imaging Fiber Photometry System contains the following elements.

- 4-channel LED driver
- Bundle-imaging Fluorescence Mini Cube with 6 ports GCaMP Isosbestic and Functional Excitations, & Red Fluorophore Fluorescence
- Bundle-imaging Fiber Photometry Driver
- 4-port USB3.0 Hub
- All required electrical cables
- Photometry Rack for BFPS

**Note**: Cannulas, rotary joints and other elements used are to be defined for each application, and are not specified here.

#### **ORDERING CODE:** BFPS\_405/GFP/RFP



GCaMP Isosbestic & Functional Excitations, & Red Fluorophore Photometry System

# Fiber Photometry Console



Fiber Photometry Console

This FPGA based data acquisition unit synchronizes the control of excitation light and the detection of the induced fluorescence. This device seamlessly integrates with the Doric Neuroscience Studio that provides user interface for multi-channel photometry experiments. The software interface enables control over the CW excitation light pulses, or the sinusoidal waveform trig of an external source (i.e. LED driver) with 4 TTL and 4 analog voltage outputs. The software interface displays real-time recording data of up to 4 detector input signals. Signal processing such as averaging, subtraction, multiplication to calculate the  $\Delta$ F/F<sub>0</sub> and other new functionalities are being developed. Updates will be freely available as they are released. Main features:

- 4 Digital Input/Output TTL, 25 MS/s, via 4 BNC connector; In : 3 k $\Omega$ , Out : 30  $\Omega$
- + 4 Analog Input  $\pm 10$  V, 17 bits, 15 kS/s, via 4 BNC connector; 124 k $\Omega$
- 4 Analog Output  $\pm$ 5V, 16 bits, 25 MS/s, via 4 BNC connector; 6  $\Omega$
- 1 digital communication SPI and LVDS via custom pinout HDMI connector
- USB2 connection to computer, cable included
- Compatible with Doric Neuroscience Studio with photometry-oriented interface
- All software updates included

## **ORDERING CODE: FPC**

# Connectorized Fluorescence Mini Cubes

The fiber photometry experiments may require a different number of excitation and detection channels, an optional optogenetically synchronized activation/silencing channel etc., directly affecting the number of fluorescence cube ports. The sample itself requires fixed or rotating port. As there are number of different possibilities of assigning these ports, our cube classification is based on a number of ports. So far, we offer fluorescence mini cube models with 3, 4, 5, 6 and 7 ports where each port is assigned one of the following functions: E for tagged neurons excitation band or IE for GCaMP isosbestic excitation band, F for fluorescence band, O for optogenetics activation or silencing and S for the sample. For extremely low light level applications, the fluorescence port code letters F, F1, F2, etc., can be replaced by PMT, meaning that the fiber-optic receptacle is replaced by a photomultiplier tube attached directly to the mini cube.

## Fluorescence Mini Cube with 3 ports

Single excitation band fiber photometry measurements use a *Fluorescence Mini Cube with 3 ports*, with one port for the excitation light, one for the fluorescence detection and one for the sample being tested. The cube has a dichroic mirror to separate the excitation light from the fluorescence emission and may incorporate narrow bandpass filters that limit the excitation or fluorescence spectrum. Currently we offer configurations for GFPlike or RFP-like fluorophores. The 3 ports' mini cube filters can be customized on request.

On the image E is for excitation, F for fluorescence and S is for the fixed sample port.



Fluorescence Mini Cube 3 ports

Filter Set	Excitation Band (nm)	Detection Band (nm)	Ordering Code
GFP-like	460-490	500-550	FMC3_E(460-490)_F(500-550)_S <sup>*</sup>
RFP-like	540-570	580-680	FMC3_E(540-570)_F(580-680)_S <sup>*</sup>

Table 101: Fluorescence Mini Cube 3 ports Ordering Codes

<sup>\*</sup>To use with a PMT, in the ordering code replace **F** for **PMT**, *e.g.* FMC3\_E(460-490)\_PMT(500-550)\_S

## Fluorescence Mini Cube with 4 ports

## • Excitation, Fluorescence and Opsin Activation

This cube is for measurements involving an excitation, an optogenetic activation/silencing, fluorescence detection and sample ports. Such a cube can be used for GCaMP fluorescence measurements combined with the activation of red opsins in the 580-650 nm band.

On the image E is for excitation, F for fluorescence, O for opsin activation/silencing and S is for the fixed sample port. The numbers in the brackets of the ordering code are for the corresponding wavelength bands.

## **ORDERING CODE:**

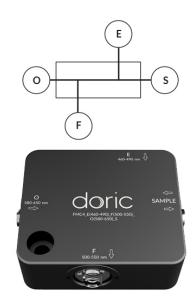
FMC4\_E(460-490)\_F(500-550)\_O(580-650)\_S\*

## Lock-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations

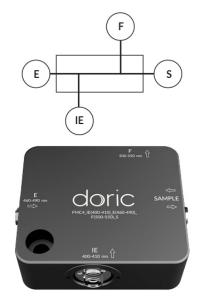
This cube permits excitation of the GCaMP isosbestic point with 400-410 nm light and fluorophores with 460-490 nm light. The single detector measures both signals within the fluorescence detection window from 500-540 nm. The separation of GCaMP isosbestic and functional excitations is possible if both excitations are modulated.

On the image IE and E are ports for excitations, F is for fluorescence detection and S is the for fixed sample port. The numbers in the brackets of the ordering code denote the corresponding wavelength bands.

## ORDERING CODE: FMC4\_IE(400-410)\_E(460-490)\_F(500-550)\_S\*



FMC4, Excitation, Fluorescence and Opsin Activation



FMC4, Lock-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations

<sup>\*</sup>To use with a PMT, in the ordering code replace **F** for **PMT**, *e.g.* FMC4\_E(460-490)\_PMT(500-550)\_O(580-650)\_S

## • FRET Cube (One Excitation and Two Fluorescence Detection Ports)

This cube is used to excite the donor fluorophore with a 420-445 nm excitation wavelength band. The donor fluorophore loses part of that energy to fluorescence in the 460-500 nm band, while the rest is transferred in a distance dependent radiationless manner to the acceptor fluorophore. The fluorescence emitted by the acceptor is detected in the 528-556 nm window.

#### ORDERING CODE: FMC4\_E(420-445)\_F1(460-500)\_F2(528-556)\_S\*



FMC4, FRET Cube

On the image E is for excitation, F1 and F2 for two spectrally different fluorescences and S is for the fixed sample

port. The numbers in the brackets are for the corresponding wavelength bands.

## Fluorescence Mini Cube with 5 ports

#### • GCaMP Isosbestic and Functional Excitations, and Opsin Activation

The GCaMP isosbestic point is excited by 400-410 nm bandwidth light and the 460-490 nm spectral window excites its functional fluorescence. The 500-550 nm spectral window collects the functional fluorescence of GCaMP. At the same time, an opsin is excited with 580-650 nm light.

#### **ORDERING CODE:**

FMC5\_IE(400-410)\_E(460-490)\_F(500-550)\_O(580-650)\_S\*



IE

FMC5, GCaMP Isosbestic and Functional Excitations, and Opsin Activation

\*To use with a PMT, in the ordering code replace **F** for **PMT**, *e.g.* FMC5\_IE(400-410)\_E(460-490)\_PMT(500-550)\_O(580-650)\_S

## Separated Two Fluorophores Fluorescence

This cube is used for green and red fluorophore excitation and their respective detection wavelengths. Other fluorophore combinations are possible.

#### ORDERING CODE:

FMC5\_E1(460-490)\_F1(500-540)\_E2(555-570)\_F2(580-680)\_S\*

## Fluorescence Mini Cube with 6 ports

## • Two Fluorophores Fluorescence and GCaMP Isosbestic Excitation

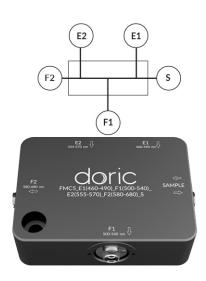
This cube can be used to detect the fluorescence from two calcium indicators and the GCaMP isosbestic point. The separation of functional and isosbestic fluorescence signals of GCaMP is possible if the light sources are modulated.

#### ORDERING CODE:

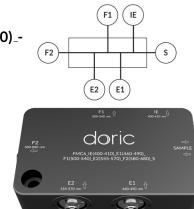
FMC6\_IE(400-410)\_E1(460-490)\_F1(500-540)\_E2(555-570)\_F2(580-680)\_-S\*

<sup>\*</sup>To use with a PMT, in the ordering code replace **F** for **PMT**, *e.g.* FMC5\_E1(460-490)\_PMT(500-540)\_E2(555-570)\_PMT(580-680)\_S

FMC6, Two Fluorophores Fluorescence and GCaMP Isosbestic Excitation



FMC5, Separated Two Fluorophores Fluorescence



## Fluorescence Mini Cube with 7 ports

## Three Fluorophores Fluorescence

This mini cube separates three different indicators simultaneously.



FMC7, Three Fluorophores Fluorescence

## ORDERING CODE: FMC7\_E1(400-410)\_F1(420-450)\_E2(460-490)\_F2(500-540)\_E3(555-570)\_F3(580-680)\_S\*

<sup>&</sup>lt;sup>\*</sup>To use with a PMT, in the ordering code replace **F** for **PMT**, *e.g.* FMC7\_E1(400-410)\_PMT(420-450)\_E2(460-490)\_PMT(500-540)\_E3(555-570)\_PMT(580-680)\_S

## Integrated Fluorescence Mini Cubes

In order to optimize signal detection and simplify usage, it is desirable to minimize the number of fiber optic connections to a Fluorescence Mini Cube. By taking advantages of the new Doric Fluorescence Detector, and our Built-in LED Optical Heads, it is possible to have an Integrated Fluorescence Mini Cube. The Integrated Fluorescence Mini Cubes include a number of built-in detectors and LED light sources on various ports to improve Fluorescence Mini Cube performance.

- The Built-in Fluorescence Detector Head allows for an increase in signal transmission of 30% due to the proximity of the detector active surface and signal source, as well as the reduced number of optical connections. A Fluorescence Detector Amplifier is provided for each detector head. All Fluorescence Mini Cube
  F ports come with a Built-in Fluorescence Detector Head.
- The Built-in LED Optical Head removes the need for light source patch cords. The Intensity Adjustment Ring allows additional fine control of light intensity, ideal for low-power fiber photometry experiments. All Fluorescence Mini Cube E and IE ports come with a Built-in LED Optical Head.
- All ports used for opsin activation/silencing, identified with **O**, have an FC receptacle.
- Any built-in device can be replaced by an *FC Receptacle* if requested. To order a cube without *Built-in LED Optical Heads*, use the ordering code for the iFMC: *e.g.* iFMC3\_E(460-490)\_F(500-550)\_S for the iIFMC3\_E(460-490)\_F(500-550)\_-S



Integrated Fluorescence Mini Cubes with 4 ports - LED & Doric Fluorescence Detector Head

# Integrated Fluorescence Mini Cubes with 3 ports - LED & Doric Fluorescence Detector Head

Single excitation band fiber photometry measurements use a *Fluorescence Mini Cube with 3 ports*, with one port for the excitation light, one for the fluorescence detection and one for the sample being tested. The cube has a dichroic mirror to separate the excitation light from the fluorescence emission and may incorporate narrow bandpass filters that limit the excitation or fluorescence spectrum. Currently we offer configurations for GFP-like or RFP-like fluorophores. The 3 ports' mini cube filters can be customized on request.



Integrated Fluorescence Mini Cube 3 ports

On the image E is for excitation, F for fluorescence and S is for the fixed sample port.

Table 102: Integrated Fluorescence Mini Cube 3 ports Ordering Codes

Filter Set	Excitation Band (nm)	Detection Band (nm)	Ordering Code
GFP-like	460-490	500-550	ilFMC3_E(460-490)_F(500-550)_S
RFP-like	540-570	580-680	ilFMC3_E(540-570)_F(580-680)_S

# Integrated Fluorescence Mini Cubes with 4 ports - LED & Doric Fluorescence Detector Head

## • Excitation, Fluorescence and Opsin Activation

This cube is for measurements involving an excitation, an optogenetic activation/silencing, fluorescence detection and sample ports. Such a cube can be used for GCaMP fluorescence measurements combined with the activation of red opsins in the 580-650 nm band.

On the image E is for excitation, F for fluorescence, O for opsin activation/silencing and S is for the fixed sample port. The numbers in the brackets of the ordering code are for the corresponding wavelength bands.

## ORDERING CODE:

iIFMC4\_E(460-490)\_F(500-550)\_O(580-650)\_S

## • Lock-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations

This cube permits excitation of the GCaMP isosbestic point with 400-410 nm light and fluorophores with 460-490 nm light. The single detector measures both signals within the fluorescence detection window from 500-550 nm. The separation of GCaMP isosbestic and functional excitations is possible if both excitations are modulated.

On the image IE and E are ports for excitations, F is for fluorescence detection and S is the for fixed sample port. The numbers in the brackets of the ordering code denote the corresponding wavelength bands.

## ORDERING CODE: iIFMC4\_IE(400-410)\_E(460-490)\_F(500-550)\_S



iIFMC4, Excitation, Fluorescence and Opsin Activation



ilFMC4, Lock-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations

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# • FRET Cube (One Excitation and Two Fluoresce Detection Ports)

This cube is used to excite the donor fluorophore with a 420-445 nm excitation wavelength band. The donor fluorophore loses part of that energy to fluorescence in the 460-500 nm band, while the rest is transferred in a distance dependent radiationless manner to the acceptor fluorophore. The fluorescence emitted by the acceptor is detected in the 528-556 nm window.



ilFMC4, FRET Cube

#### **ORDERING CODE:**

#### iIFMC4\_E(420-445)\_F1(460-500)\_F2(528-556)\_S

On the image E is for excitation, F1 and F2 for two spectrally different fluorescences and S is for the fixed sample port. The numbers in the brackets are for the corresponding wavelength bands.

Fiber Photometry

## Integrated Fluorescence Mini Cubes with 5 ports -LED & Doric Fluorescence Detector Heads

• Separated Two Fluorophores Fluorescence

This cube is used for green and red fluorophore excitation and their respective detection wavelengths. Other fluorophore combinations are possible.

#### **ORDERING CODE:**

iIFMC5\_E1(460-490)\_F1(500-540)\_E2(555-570)\_F2(580-680)\_-S



iIFMC5, Separated Two Fluorophores Fluorescence Cube

## GCaMP Isosbestic and Functional Excitations, an

The GCaMP isosbestic point is excited by 400-410 nm bandwidth light and the 460-490 nm spectral window excites its functional fluorescence. The 500-550 nm spectral window collects the functional fluorescence of GCaMP. At the same time, an opsin is excited with 580-650 nm light.

#### ORDERING CODE:

iIFMC5\_IE(400-410)\_E(460-490)\_F(500-550)\_O(580-650)\_S



ilFMC5, GCaMP Isosbestic and Functional Excitations, and Opsin Activation

## Integrated Fluorescence Mini Cubes with 6 ports -LED & Doric Fluorescence Detector Heads

## • Two Fluorophores Fluorescence and GCaMP Isosbestic Excitation

This cube can be used to detect the fluorescence from two calcium indicators and the GCaMP isosbestic point. The separation of functional and isosbestic fluorescence signals of GCaMP is possible if the light sources are modulated.



ilFMC6, Two Fluorophores Fluorescence and GCaMP Isosbestic Excitation Cube

#### ORDERING CODE:

iIFMC6\_IE(400-410)\_E1(460-490)\_F1(500-540)\_E2(555-570)\_F2(580-680)\_S <u>Notes</u>: The E2(555-570) Built-in LED Optical Head has no Intensity Adjustment Ring.

# Integrated Fluorescence Mini Cubes with 7 ports - LED & Doric Fluorescence Detector Heads

## Three Fluorophores Fluorescence

This mini cube separates three different indicators simultaneously.



iIFMC7, Three Fluorophores Fluorescence

#### ORDERING CODE:

iIFMC7\_E1(400-410)\_F1(420-450)\_E2(460-490)\_F2(500-540)\_E3(555-570)\_F3(580-680)\_S <u>Notes</u>: The E3(555-570) Built-in LED Optical Head has no Intensity Adjustment Ring.

# Rotary Fluorescence Mini Cubes

Fluorescence recording of freely moving subjects is a challenge for neuroscience researchers due to low signal and external sources of noise. Here we present a modified version of our *Doric Integrated Fluorescence Mini Cube* that can be mounted on the rotor of a specialized version of our 24-channel Assisted Electrical Rotary Joint. This configuration increases collection efficiency by removing one optical connection while reducing motion related signal variation.



Unmounted Rotary Fluorescence Mini Cube with 6 ports

- LED light sources are built directly into the fluorescence mini cube, and are optimized to deliver low excitation power.
- The integrated detector is an amplified photodiode circuit with comparable specifications to our *Doric Fluorescence Detector* (See section).
- To connect these devices to an LED Driver and a Fluorescence Detector Amplifier, a 24-channel Assisted Electrical Rotary Joint for Fluorescence Mini Cube (See section ) is required. Each rotary joint can hold up to two fluorescence mini cube.
- The *Rotary Fluorescence Mini Cubes* are modular, allowing them to be removed from a rotary joint and replaced. They can be ordered individually or added onto an existing system for added flexibility.



Two Rotary Fluorescence Mini Cubes with 6 ports- Integrated on an Assisted Electrical Rotary Joint

## **Rotary Fluorescence Mini Cubes with 3 ports**

## • 1 LED & 1 Integrated Fluorescence Detector-For GFP-like Fluorophore

Single excitation band fiber photometry measurements use a *Fluorescence Mini Cube with 3 ports*, with one port for the excitation light, one for the fluorescence detection and one for the sample being tested. The cube has a dichroic mirror to separate the excitation light from the fluorescence emission and may incorporate narrow bandpass filters that limit the excitation or fluorescence spectrum. Currently we offer configurations for GFP-like fluorophores.

## ORDERING CODE: RFMC3\_E(460-490)\_F(500-550)

## **Rotary Fluorescence Mini Cubes with 4 ports**

## 2 LED & 1 Integrated Fluorescence Detector-Lock-in or Sequential Detection for GCaMP Isosbestic and Functional Excitations

This cube permits excitation of the GCaMP isosbestic point with 400-410 nm light and fluorophores with 460-490 nm light. The single detector measures both signals within the fluorescence detection window from 500-550 nm. The separation of GCaMP isosbestic and functional excitations using lock-in demodulation or sequential detection.

## ORDERING CODE: RFMC4\_IE(400-410)\_E(460-490)\_F(500-550)\_S

## • 1 LED & 2 Integrated Detectors-FRET (Fluorescence Resonance Energy Transfer)

This cube is used to excite the donor fluorophore in a 420-445 nm excitation wavelength band. The donor fluorophore loses part of that energy to fluorescence in the 460-500 nm band, while the rest is transferred in a distance dependent radiationless manner to the acceptor fluorophore. The fluorescence emitted by the acceptor is detected in the 528-556 nm window.

#### ORDERING CODE:

RFMC4\_E(420-445)\_F1(460-500)\_F2(528-556)\_S

## **Rotary Fluorescence Mini Cubes with 6 ports**

 3 LED & 2 Integrated Detectors-GCaMP Isosbestic & Functional Excitations, & Red Fluorophore Fluorescence

This cube can be used to detect the fluorescence from two calcium indicators and the GCaMP isosbestic point. The separation of functional and isosbestic fluorescence signals of GCaMP is possible if the light sources are modulated using lock-in demodulation or sequential detection.

#### ORDERING CODE:

RFMC6\_IE(400-410)\_E1(460-490)\_F1(500-540)\_E2(555-570)\_F2(580-680)\_S

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## Bundle-imaging Fluorescence Mini Cubes



6-port Bundle-imaging Fluorescence Mini Cube

Performing fiber photometry on a large number of sites and animals can be inconvenient due to the large number of photoreceivers and patch cords required. By bundling the connective patch cords together and using a camera, it is possible to measure a great number of sites or animals at once.

The *Bundle-imaging Fluorescence Mini Cube* is able to perform such measurements. This is done by imaging a fiber bundle onto a camera using a microscope objective. These otherwise function as any fluorescence mini cube.

- The light source ports can be shipped with integrated LED light sources (**LED** ports) or with FC receptacles (**FC** ports).
- The camera ports can be shipped with integrated cameras (**CAM** ports) or with standard C-mounts (**CM** ports).
- To accomodate larger fiber bundles, an SMA fiber optic receptacle (**SMA** ports) is used on the sample port.

## Bundle-imaging Fluorescence Mini Cubes with 4 ports-Sequential Detection for GCaMP Isosbestic and Function

This cube permits excitation of the GCaMP isosbestic point with 400-410 nm light and fluorophores with 460-490 nm light. The camera measures both signals within the fluorescence detection window from 500-540 nm. The separation of GCaMP isosbestic and functional excitations is possible if both excitations are interleaved.



4-port Bundle-imaging Fluorescence Mini Cube

## ORDERING CODE:

• Integrated Cube:

## BFMC4\_LED(400-410)\_LED(460-490)\_CAM(500-550)\_SMA

• Connectorized Cube:

## BFMC4\_FC(400-410)\_FC(460-490)\_CM(500-550)\_SMA

## Bundle-imaging Fluorescence Mini Cubes with 6 ports-GCaMP Isosbestic & Functional Excitations, & Red Fluorophore Fluorescence

This cube can be used to detect the fluorescence from two calcium indicators and the GCaMP isosbestic point. The separation of functional and isosbestic fluorescence signals of GCaMP is possible if the light sources are interleaved. This cube contains two cameras, one for each fluorescence port. **ORDERING CODE:** 

#### ORDERING CODE.

• Integrated Cube:

BFMC6\_LED(410-420)\_LED(460-490)\_CAM(500-540)\_LED(555-575)\_CAM(580-680)\_SMA

## • Connectorized Cube:

BFMC6\_FC(410-420)\_FC(460-490)\_CM(500-540)\_FC(555-575)\_CM(580-680)\_SMA

## Photodetectors

### **Doric Fluorescence Detector**

This photodetector system designed specifically for use in fiber photometry experiments consists of the detector head and the detector amplifier. Its high gain and low noise allow for detection of signals in the sub-picowatt to nanowatt range. When used with a modulated light source and a lock-in amplifier it can detect signals in the femtowatt range.



Doric Fluorescence Detector

#### Fluorescence Detector Head

When the detector is not integrated into the iFMC, the Fluorescence Detector Head is fitted with an FC adapter to connect it to a signal source. The detector uses a specialized shielded cable to connect to the amplifier, keeping noise to a minimum.

#### 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. The amplifier has two detection modes (AC/DC), each selectable for all three (1x, 10x, 100x) amplification levels, allowing 6 different amplification configurations. The DC detection mode is optimal for use with interleaved measurements or during large-bandwidth

continuous measurements. The AC detection mode is optimal for lock-in modulation/demodulation and the elimination of low-frequency noise. Its output analog voltage (5 V) can be monitored with a DAQ board for data recording or an oscilloscope for live signal.

## Notes:

- The Fluorescence Detector Head and the Fluorescence Detector Amplifier are always shipped together with a Detector Cable.
- A battery pack is included with each Fluorescence Detector for cordless use.

SPECIFICATION		VALUE	
 Wavelength Range (nm)	320-1100		
Peak Sensitivity Wavelength (nm)		960	
Peak Responsivity (A/W)		0.6	
Output Impedance ( $\Omega$ )		50	
NEP (₩/√Hz)		<12	
CW Saturation Power (nW)	4.75		
DC Bandwidth (Hz)	0-1000		
AC Bandwidth (Hz)	30-1000		
Output Connector	Male BNC		
Detector Material	Si		
Detector Size (mm x mm)		1.1 × 1.1	
Amplification level	1x	10x	100x
Transimpedance Gain (V/A)	2×10 <sup>9</sup>	2x10 <sup>10</sup>	2x10 <sup>11</sup>
Maximum Conversion Gain (V/W)	$1.2 \times 10^{9}$	$1.2 \times 10^{10}$	$1.2 \times 10^{11}$

Table 103: Doric Fluorescence Detector Specifications

## ORDERING CODE: DFD\_FOA\_FC

## Newport Visible Femtowatt Photoreceiver Module

This battery-operated photoreceiver has high gain and detects CW light signals in the sub-picowatt to nanowatt range. When used in conjunction with a modulated light source and a lockin amplifier to reduce the measurement bandwidth, 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 op-



Newport Visible Femtowatt Photoreceiver Model 2151 + Doric FC Adapter tical fibers used in fiber photometry and the relatively small detector area. Its output analog voltage (0-5 V) can be monitored with an oscilloscope or with a DAQ board to record the data with a computer.

SPECIFICATION	VALUE
Model	2151
Wavelength Range (nm)	320-1050
Bandwidth (-3 dB)	DC-750 Hz (DC), 30-750 Hz (AC)
Conversion Gain, Maximum (V/W)	$1 \times 10^{11}$
Responsivity (Peak)	0.5 A/W
Transimpedance Gain (V/A)	$2 \times 10^{10} \& 2 \times 10^{11}$
Output Impedance ( $\Omega$ )	100
NEP $(W/\sqrt{Hz})$	16 f
Saturation Power CW	0.5 nW
Output Connector	Male BNC
Detector Material	Si
Detector Diameter (mm)	1.0
Power Requirements	Internal 9 V battery
PRODUCT	Ordering Code
Newport Photoreceiver Module + Doric FC Adapter Doric FC Adapter only	NPM_2151_FOA_FC FOA_2151_FC

Table 104: Newport Visible Femtowatt Photoreceiver Module Specifications

## Photosensor Module H10722-20

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 a focusing lens, the port for this sensor has a lens that adjusts the beam size to fit the size of the PMT and instead of a receptacle, it has a thread that matches the thread on the Doric adapter for the photosensor. The photomultiplier tube (PMT) is highly sensitive and can be easily damaged if



Hamamatsu H10722-20 Photosensor Module with a FC connector d if exposed to high optical

tube (PMT) is highly sensitive and can be easily damaged if exposed to high optical power. The photosensor module requires a power supply model C10709.

	Minimum optical power detected (W)		
Photodetector	in CW	with lock-in	
Newport 2151 Hamamatsu H10722	$\begin{array}{c} 0.4 \times 10^{-12} \\ 6.0 \times 10^{-15} \end{array}$	$4.2 \times 10^{-15}$ not tested in lock-in	

Table 105: Limit of Detection for each Photodetectors



Hamamatsu H10722-20 Photosensor modules directly attached to the mini cube

## **ORDERING:**

To get PMT ready cube, replace fluorescence port code from the Fluorescence Mini Cube, F, F1 or F2 with PMT (*e.g.* FMC3\_E(460-490)\_**PMT**(500-550)\_S).

#### Power Supply for PMT Module C10709

This Power Supply unit can drive photomultiplier tube modules. Both drive voltages and control voltages can be supplied from this one unit.



Power Supply for PMT Module C10709

## **ORDERING CODE: PS\_PMT**

## Fiber Photometry Accessories

#### Fiber Photometry Rack for FMC5

This small *Fiber Photometry Rack* can fit the Fiber Photometry Console, the 2-channel LED driver, 2 LEDs, 2 photodetectors (Doric or Newport) and up to 2 FMC5. It is suitable for use with the following systems: all the 1-site Fiber Photometry Systems and the 2-site Fiber Photometry Systems for GCaMP recording with two excitation wavelengths.



Fiber Photometry Rack for FMC5

## **ORDERING CODE: PR\_5**

#### Fiber Photometry Rack for FMC7

#### Fiber Photometry

This rack-mount (19") *Fiber Photometry Rack* allows for the housing of more complex systems requiring the bigger 4-channel LED driver. It is suitable for use with systems using FMC6 or FMC7, or for multi-site fiber photometry systems as the 2-site Fiber Photometry Systems for GCaMP Isosbestic and Functional Excitations.



Fiber Photometry Rack for FMC7

## **ORDERING CODE: PR\_7**

## **Bundle-imaging Fiber Photometry Driver**

The Bundle-imaging Fiber Photometry Driver is used to coordinate the many devices used by a Bundle-imaging Fiber Photometry System. These include LED light sources and cameras. The driver is connected to a computer or the HUB using a USB cable, provided with the system.

## **ORDERING CODE: BFPD**

## 4-port USB3.0 Hub

The 4-port USB3.0 Hub is used to transfer the high-density data throughput involved in using the Bundle-imaging Fiber Photometry System. The hub is provided with all necessary electrical cables.

## **ORDERING CODE: USB\_H\_4**

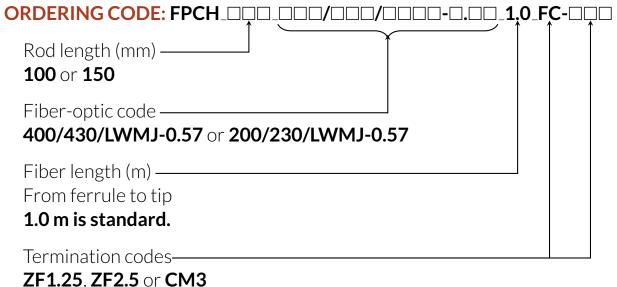
## Fiber Photometry Cannula Holders

The Fiber Photometry Cannula Holder is designed to enable the recording of the fluorescence during the implantation of the cannula. It is a stainless steel rod having an FC receptacle on one end that allows a light delivery patch cord to be plugged in and at the other end a receptacle where a cannula can be screwed on. The two receptacles are mutually connected with an internal optical fiber housed within



Fiber Photometry Cannula Holder

the 6.35 mm diameter rod that fits most popular micro-manipulators. To avoid unnecessary optical losses, the selected optical fiber parameters such as the core diameter and NA match those used in the fiber photometry system.



(see Table 48)

## Notes:

- The Fiber Photometry Cannula Holder is compatible with Mono Fiber-optic Cannulas.
- A holder compatible with Dual Fiber-optic Cannulas is available on request.
- An optional clamp (**SCL**) can be used to fix the Fiber Photometry Cannula Holder on stereotaxic apparatus.

# **Behavioral Tracking**

Technically speaking, the behavior study of freely-moving animals resembles the filmmaking or film production process involving scriptwritting, choreography, recordings, editing etc. From the neuroscientist stand point it requires:

- A) the recording of neuronal activity of the specific brain region using calcium imaging, fiber photometry or electrophysiology,
- B) the behavioral tracking or simply filming of the animal activity in a given situation synchronized with the recordings of neural activity,
- C) the behavior triggers that can be external in the form of the stage event or internal in the form of light or electric signals directed to specific brain region.

Optogenetics enable precise triggering or silencing of the brain cells with light. The electrophysiology can be used to deliver electrical trigger signals and to record the neuronal activity. The chronically implanted fluorescence microscopes and fiber photometry probes can monitor the neuronal activity. The filming of the animal is complementary information needed to establish correlation between the neuronal activity of the specific brain region and the animal behavior. The Doric Neuroscience Studio software seamlessly integrates neuronal imaging, fiber photometry, electrophysiological recording, optogenetics stimulation and behavioral tracking of the freely-moving animals. Another first from Doric.

# Behavior Tracking Cameras

## **USB 3.0 Behavior Tracking Camera**

These Doric Color and B&W cameras use an USB 3.0 interface standard typical of highperformance industrial cameras. This interface provides a framework for streaming high-speed video and related control data. The camera control and the image acquisition are done through the Doric Neuroscience Studio software. The system includes a Trigger cable to synchronize with external devices. The power is feed directly to the camera by the USB cable. An articulated holder is also included with the Behavior Tracking



USB 3.0 Behavior Tracking Camera with Wide-angle Lens

holder is also included with the Behavior Tracking Camera.

The purchase of the USB 3.0 Behavior Tracking Camera includes:

- Camera (B&W or Color)
- C-Mount camera lens for 1/2 sensor, 1.5MP
- Articulated holder
- Trigger cable

Table 106: USB 3.0 Behavior T	racking Camera Specifications
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SPECIFICATION	VALUE
Video formats	B&W 1920 x 1080 Y800
	Color 1920 x 1080 RGB32
Frame rate @ full resolution	60
Resolution	H: 1920, V: 1080
Format	1/2.8 "
Pixel size	2.9 μm x 2.9 μm
Lens mount	C/CS
Interface	USB 3.0
Exposure	20 µs to 30 s
Gain	0 to 72 dB

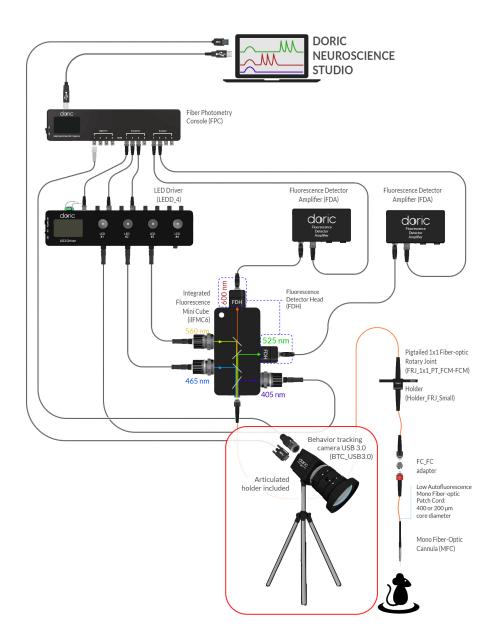
<sup>\*</sup>Minimum object distance

Table 107: Behavior Tracking Camera Lens Specifications

Focal Length (mm)	Iris Range	MOD <sup>*</sup> (m)	FOV @ 1 m
5	F1.4 - 16C	0.10	1.0 × 1.0

Table 108: Behavior Tracking Camera Ordering Codes

CHROMA	Ordering Code
Color (RGB32)	BTC_USB3.0_CO
B&W (Y800)	BTC_USB3.0_BW



USB3 Behavioral Tracking Synchronized within a GCaMP Isosbestic and Functional Excitations and Red Fluorophore Fluorescence System

## **GigE Behavior Tracking Camera**

The Doric Color and B&W cameras use a GigE Vision interface standard typical of highperformance industrial cameras. This interface provides a framework for streaming high-speed video and related control data over Ethernet networks. The camera control and the image acquisition are done through the Doric Neuro-science Studio software. The system includes a Power/Trigger cable to synchronize with exter-



GigE Behavior Tracking Camera with Wide-angle Lens

nal devices. If the system is coupled with a fluorescence microscope driver, a Gigabit PoE+ Switch is included to the system. This switch allows the connection of multiple Ethernet devices to a single Ethernet port on the computer and feeds the camera power directly through the Ethernet cable. An articulated holder is also included with the Behavior Tracking Camera.

The purchase of the GigE Behavior Tracking Camera includes:

- Camera (B&W or Color)
- C-Mount camera lens for 1/2 sensor, 1.5MP
- Articulated holder
- Power/trigger cable
- PoE+ Switch (when coupled with a microscope driver)

SPECIFICATION	VALUE
Video formats	B&W 1920 x 1200 Y800
	Color 1920 x 1200 RGB32
Frame rate @ full resolution	50
Resolution	H: 1920, V: 1200
Format	1/2.8 "
Pixel size	2.8 μm x 2.8 μm
Lens mount	C/CS
Interface	GigE
Exposure	20 µs to 30 s
Gain	0 to 48 dB

Table 109: GigE Behavior Tracking Camera Specifications

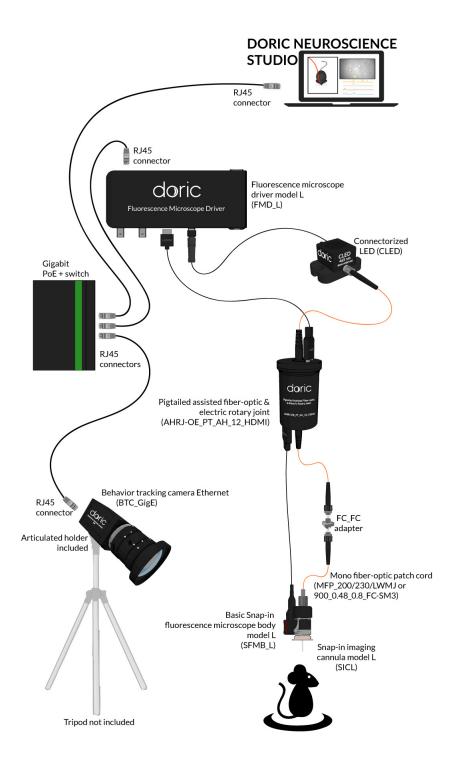
Table 110: Behavior Tracking Camera Lens Specifications

Focal Length (mm)	Iris Range	MOD <sup>*</sup> (m)	FOV@1m
5	F1.4 - 16C	0.10	1.0 × 1.0

Table 111: Behavior Tracking Camera Ordering Codes

CHROMA	Ordering Code
Color (RGB32)	BTC_GigE_CO
B&W (Y800)	BTC_GigE_BW

<sup>\*</sup>Minimum object distance



Ethernet Behavioral Tracking Synchronized to the Basic Miniature Fluorescence Microscopy System

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