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Integrated Fluorescence Mini Cubes

Gen3

User Manual

Version 1.0.0

Contents

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Quick Start

This user manual presents the LED integrated version of the Integrated Fluorescence Mini Cubes: the iIFMC (Fig.1.1). It means this type of cube integrates the light sources of the excitation ports directly in the cube as compared to the regular version of the Integrated Fluorescence Mini Cubes or iFMC. Compared to Fluorescence Mini Cubes with only FC connectors on all ports, the ones with integrated photodetectors provide a 100% better signal-to-noise ratio (30% signal increase and a 40% noise reduction). Moreover, in this version of Mini Cubes, LEDs and LED drivers are integrated as well to reduce the number of connections and the system's overall footprint.

Figure 1.1: *An example of a typical iIFMC cube with all types of ports present shown*

1. **Sample port** (FC receptacle): Connects to a *Low-Autofluorescence Patch Cord* or a *Pigtailed Rotary Joint* going to the animal.
2. **Excitation port (E)** (BNC connector): Connects to the analog output of a Fiber Photometry Console, or another device with analog output capabilities with a BNC/BNC cable.
3. **Isosbestic Excitation port (IE)** (BNC connector): Connects to the analog output of a Fiber Photometry Console, or another device with analog output capabilities, with a BNC/BNC cable.
4. **Fluorescence Detector Emission port (F)** (BNC connector): Connects to an analog input port of the Fiber Photometry Console or another Data Acquisition box with a BNC/BNC cable. This is the output of the fluorescence signal.
5. **Amplifier controls**: Adjusts gain (1x or 10x) for each channel.
6. **Power switch**: Turns on the photodetectors and amplifiers for all the channels.

7. **Optogenetics port** (FC receptacle): Connects the cube to a light source using a patchcord to allow for a simultaneous optogenetic stimulation of the recorded site in the animal.
8. **12 V Power input (2.1 mm jack)**: Connects to the provided 12V power supply. A splitter is sometimes provided to connect multiple cubes or the acquisition console and the cube on the same power supply.
9. **Filter drawers**: Filters drawers are located at the bottom of the cube. There is one filter drawer per light source and they contain the spectral filter specific for the port need. Some ports contains removable neutral density filters.

2

Operation Guide

2.1 The different versions of Doric Fiber Photometry Minicubes

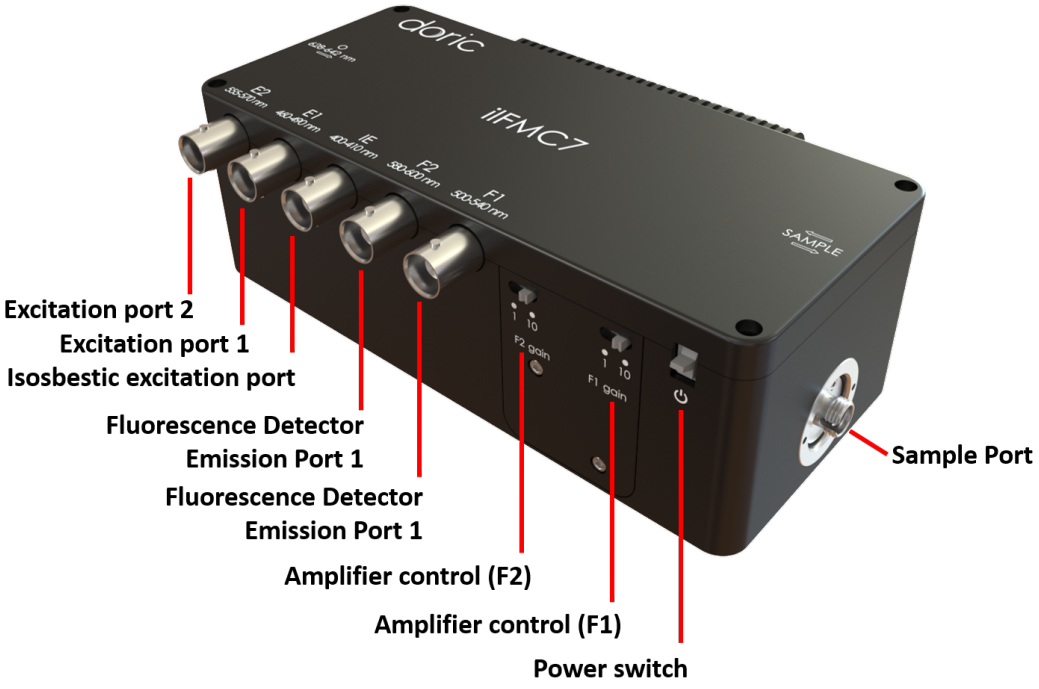
Compared to Fluorescence Mini Cubes with only FC connectors on all ports (**FMC**, Fig. 2.1), minicubes with integrated photodetectors provide a 100% better signal-to-noise ratio, with 30% signal increase and a 40% noise reduction (**iFMC**, Fig. 2.1). In the **ilFMC** version of fiber photometry minicube designed by Doric Lenses, both the photodetector and LED light source are integrated in the minicube (**ilFMC Gen1**, Fig. 2.1). In a vision of further integrating the system to simplify and optimize it, iIFMC Gen2 minicubes also integrated the signal amplifier. In the latest generation of iIFMC Mini Cubes, **iIFMC Gen3** on which this user manual will focus, both LEDs and LED drivers are integrated as well to reduce the number of connections and the system's overall footprint. In this generation, it is also possible to replace the excitation filter and decrease the excitation power by adding attenuating filters (for low power photometry).

	FMC	iFMC	iIFMC		
	GEN 1 2015	GEN 2 2020	GEN 1 2018	GEN 2 2020	GEN 3 2022
					
High-quality optics & Spectral filtering	✓	✓	✓	✓	✓
Integrated detector for higher sensitivity		✓	✓	✓	✓
Integrated amplifier to simplify system		✓		✓	✓
Integrated LED with adjustable power			✓	✓	✓
Integrated LED & driver to simplify the system					✓

Figure 2.1: *Overview of the different fiber photometry minicubes*

2.2 Ports type and description

Integrated Fluorescence Mini Cubes Gen3 have multiple types of optical ports (Fig. 2.2). The design of each port is made to achieve a certain purpose.



(a) *iIFMC7 Gen3 front view*

(b) *iIFMC7 Gen3 back view*

Figure 2.2: *Overview of the iIFMC7 Gen3 minicube*

2.2.1 Sample port

There is a sample port on every *Fluorescence Mini Cubes*. It is the only port without any spectral filtering, that is, all wavelengths can pass freely through it.

The sample port is connected to the animal either directly via a fiber optic patchcord or through a rotary joint. It is recommended to use a low auto-fluorescence patch cord between the cannula and the iIFMC to reduce background fluorescence that may occur in standard patch cords. We also recommend using pigtailed rotary joints as they reduce the amount of transmission variation.

2.2.2 Excitation ports

Excitation ports are designated by E on the top engraving. If there is more than one excitation port, such as in the 6 or 7 ports versions of the Integrated Fluorescence Minicubes, the iIFMC6 and iIFMC7 respectively, they are labeled as E1, and E2. Isosbestic excitation ports are labeled IE. When excitation and fluorescence ports are designed in pairs, they are labeled with the same number, E1 goes with F1, E2 with F2, and so on. Excitation ports contain a filter chosen to correspond to the excitation peaks of the fluorescent protein the iIFMC is designed to measure. More details about the spectral characteristics of the different iIFMC minicubes can be found at section 3.

To obtain a stable and uniform illumination in the measured brain region, LEDs are used to inject light into the excitation port of the iIFMC. LEDs are advantageous for fluorescence illumination in two ways. First, they provide incoherent light and are free of the speckle problem that the lasers have. Second, their emitter is big and emits light in all directions. This allows LED to completely fill all the available transmission modes of a fiber, preventing power output variation when the fiber is bent.

The drawback to filling all the transmission modes of a fiber is that it emits too much power for most fiber photometry applications, even at the lowest electrical driving current. For this reason, most LEDs have a neutral density filter installed in their path to reduce their output power (see table 2.1).

Table 2.1: *LED and neutral density filter to use for each illumination port*

Port wavelength range	LED	Neutral density filter
400-410 nm	405nm	Attenuating T=10%
410-420 nm	415nm	Attenuating T=10%
460-490 nm	470nm	Attenuating T=10%
555-570 nm	560nm	No neutral density filter

2.2.3 Fluorescence ports

Fluorescence detector ports are designated by F on the top engraving. If there is more than one fluorescence port, such as in the 6 ports version of the Integrated Fluorescence Minicube or iIFMC6, they are labeled as F1, and F2. When excitation and fluorescence ports are designed in pairs, they are labeled with the same number, E1 goes with F1, E2 with F2, and so on.

Fluorescence ports contain a filter chosen to correspond to the emission peaks of the protein the iIFMC is designed to measure. This filter is usually very wide to collect as much fluorescence as possible. Figure 3.1 shows the spectral filter of a fluorescence port designed for GFP.

In each fluorescence port, an integrated photodetector and amplifier convert the fluorescence response of the sample to a measurable voltage. The output signal of each detector is sent to the corresponding output BNC. See section 2.4 for more details on the integrated photodetectors and amplifiers.

2.2.4 Opsin ports (optional)

Opsin ports are designated as O on the top engraving. Only the 5 and 7-ports versions of the iIFMC (i.e the iIFMC5 and iIFMC7 minicubes) have dedicated opsin ports. Their ports contain a filter chosen to correspond to the excitation

spectrum of an opsin (see table 2.2).

Table 2.2: *Typical opsins and recommended light source for each standard opsin port*

Port wavelength range	Light source	Opsins
580-650 nm	LISER™, Ce:YAG, 590nm Laser, 638nm Laser	Halorhodopsin, Chrimson
628-642 nm	638nm Laser	Chrimson

2.3 Fiber optics & FC connectors

The *Fluorescence Mini Cubes* are designed to be used with FC connectorized patch cords.

Clean the connector end tips of the patch cords before connecting them to *Fluorescence Mini Cube*. Use isopropyl alcohol or a similar cleansing solution. When not in use, place the plastic cap on the connector for protection and cleanliness.

To reduce the risk of eye injury, **it is sound practice to avoid looking directly at the fiber or connector openings** when the light source is turned on

When connecting an FC connector, the key must be oriented to enter within the receptacle slot to ensure a good connection (Fig. 2.3). Always screw the barrel tightly to prevent connection instability.

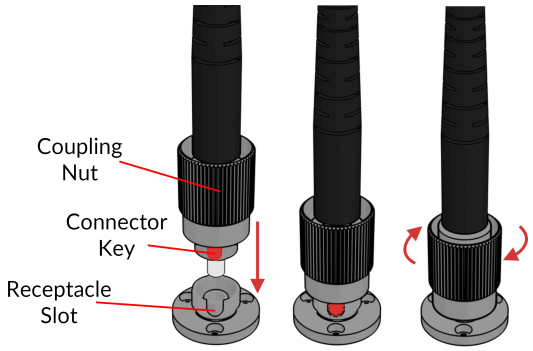


Figure 2.3: *FC connector, Fiber Installation*

2.4 Built-in photodetectors & amplifiers

The detector port of the iIFMC contains an integrated photodiode and amplifier (Fig. 2.4). If there are several fluorescence ports, the amplification circuit of each port is completely separated. They only share the same power supply.

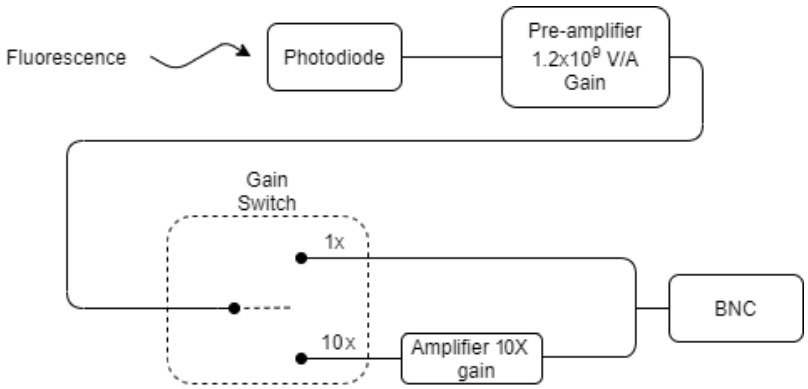


Figure 2.4: *Amplification chain of an iIFMC fluorescence channel*

Each port has 1 selector switch and an output BNC connector. The switch allows to select the gain level (x1 or x10). The gain switch selects between two output levels for the same input fluorescence light. The 1x gain passes the signal from the pre-amplifier directly to the BNC while the 10x adds another amplification level. Gain selection should be done only for very coarse signal adjustment. Gain should be lowered if the amplifier is saturated (flat line around 5V) and increased if is lower than 0.5V.

2.5 Built-in LEDs

The iIFMC has integrated LED and LED drivers directly in the excitation ports. Because they are passively cooled, all integrated LEDs are limited to a maximum current of 500 mA. Fine adjustment of LED output power is performed through the BNC ports, the voltage sent to these ports corresponding to the current of the LED according to the ratio 100 mA/V (ex: 2.5V = 250 mA). The tuning of this voltage can be performed in Doric Neuroscience Studio software by controling an acquisition console with an analog output port (i.e a fiber photometry console for instance) to send a variable voltage to the iIFMC minicube.

2.6 Power supply

Each *Integrated Fluorescence Mini Cubes* is provided with a 12V wall plug power supply unit. To turn the *Integrated Fluorescence Mini Cube* on, lift the power switch up. The lights on the amplifier controls will light up. Always use the provided power supply that came with either the *Fluorescence Mini Cube* itself or a complete Doric Photometry system.

3

Specifications

Table 3.1: *Specifications of ilFMC systems*

Specification	Value	Unit
Minicube		
Optical fiber connector	FC	-
Optical fiber compatibility	- Core Ø 200 or 400 - NA 0.37 to 0.57	µm
Optical filter attenuation	≥ OD 5 outside band	-
Built-in LEDs		
Illumination size	400	µm
Illumination NA	0.22	-
Maximum current	500	mA
Maximum output power (at 200 mA, CW)		
- LED 400-410 nm	60 [1]	µW
- LED 410-420 nm	60 [1]	µW
- LED 460-490 nm	130 [1]	µW
- LED 540-570 nm	130	µW
Built-in LED Driver		
Signal Conversion Ratio	100	mA/V
Input Voltage range	0 - 5	V
Output Current range	0 - 500	mA
Built-in detectors		
Sensor type	Si photodiode	-
Sensor area	1 x 1	mm
Wavelength range	350 to 1000	nm
Sensitivity	- 0.38 at 550nm	A/W
	- 0.60 at 960 nm (peak)	A/W
Amplification levels	1x - 10x	-
Transimpedance gain	- 2.0 x 10^9 (1x)	V/A
	- 2.0 x 10^{10} (10x)	V/A
Conversion gain (at 550 nm)	- 0.76 (1x)	V/nW
	- 7.60 V (10x)	V/nW
Noise equivalent power (NEP)	≤ 12	fW/$\sqrt{Hz}$
Bandwidth	0 - 1500	Hz
Electrical interface	BNC (Output)	-
Output voltage range	0.0 to 5.5	V
Output impedance	50	Ω

[1] *with the ND filter T=10% included*

Minimum current (Imin)	7 (@ Vin = 0.07V)	mA
Max modulation frequency (FCmax)	10	kHz
Rise / Fall time	≤ 10	µsec
Zero leak current for Vin	≤ 0.01	V
Electrical interface	BNC (Input)	-
General		
Power supply voltage	12	VDC
Current consumption	- max 0.8 (3 LEDs variant)	A
	- max 0.5 (2 LEDs variant)	A
Size		
- ilFMC4	107 x 109 x 52	mm
- ilFMC5	107 x 109 x 52	mm
- ilFMC6	152 x 109 x 52	mm
- ilFMC7	152 x 109 x 52	mm

Table 3.2: *Typical filter transmission bandwidth for standard configuration of ilFMC Gen3 minicubes*

ilFMC Gen3 model	**Excitation (nm)**	**Fluorescence (nm)**	**Opsin (nm)**
ilFMC4 (see Fig.3.1)			
GCaMP			
Excitation 1 (isosbestic)	400-410 or 410-420	500-550	N/A
Excitation 2 (functional)	460-490	500-550	
ilFMC5 (see Fig.3.2)			
GCaMP + Optogenetics			
Excitation 1 (isosbestic)	400-410 or 410-420	500-540	580-680
Excitation 2 (functional)	460-490	500-540	
ilFMC6 (see Fig.3.3)			
GCaMP + red fluorophore			
Excitation 1 (isosbestic)	400-410 or 410-420	500-540	N/A
Excitation 2 (functional)	460-490	500-540	
Excitation 3 (red fluorophore)	555-570	580-680	
ilFMC7 (see Fig.3.4)			
GCaMP + red fluorophore + Optogenetics			
Excitation 1 (isosbestic)	400-410 or 410-420	500-540	628-642
Excitation 2 (functional)	460-490	500-540	
Excitation 3 (red fluorophore)	555-570	580-600	

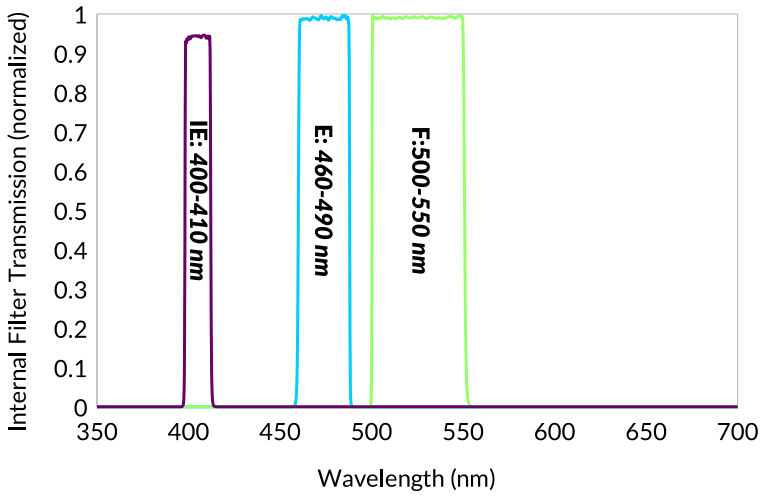


Figure 3.1: *Example of a filter configuration in the ilFMC4 (GCaMP)*

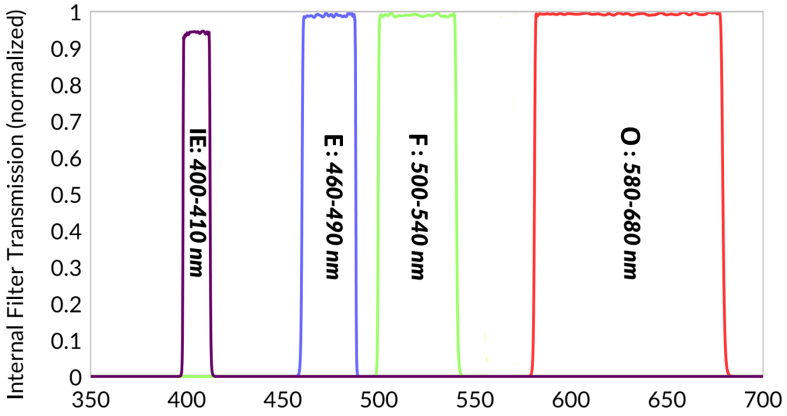


Figure 3.2: *Example of a filter configuration in the ilFMC5 (GCaMP + Optogenetics)*

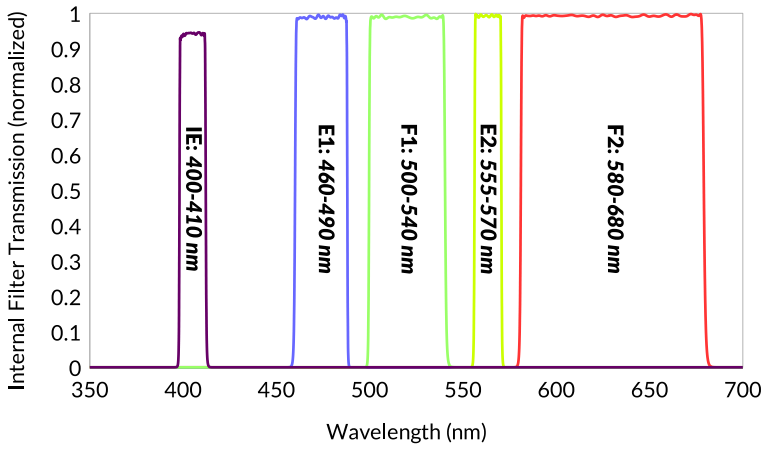


Figure 3.3: *Example of a filter configuration in the ilFMC6 (GCaMP + Red fluorophore)*

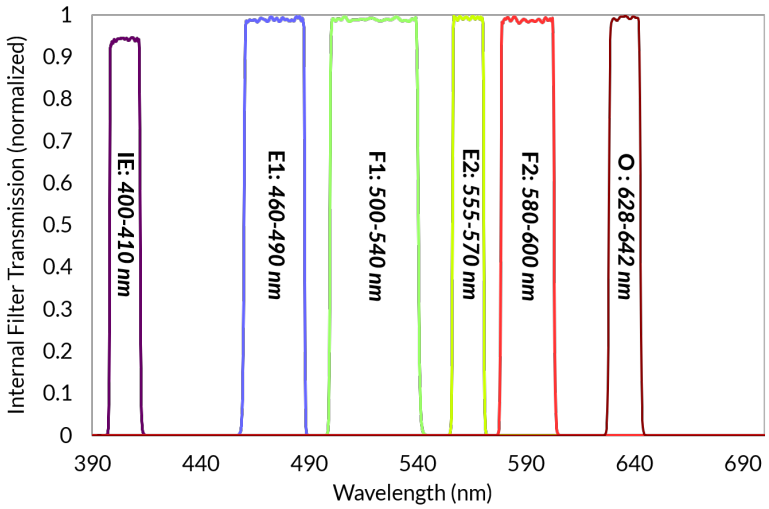


Figure 3.4: *Example of a filter configuration in the ilFMC7 (GCaMP + Red fluorophore + Optogenetics)*

4

Support

4.1 Troubleshooting

4.1.1 Integrated detector

Problem	Possible solutions
Voltage is flatlined around 0.8V and the amplification status LEDs are off.	• Power switch is off. Push the power switch upward to turn the amplifiers on. • Corded power supply is not properly connected. Check that the correct power supply is used and that the connectors are pushed in all the way.
Voltage is flatlined around 0V and the amplification status LEDs are on.	Gain is at 1X and the cap is present on the sample port. Unscrew the cap or use a higher gain to see the signal appear. If there is no signal with the sample port open in a bright room, contact Doric support.
Voltage is flatlined at 5.84V.	Amplifier is saturated. Lower gain or reduce light input in the sample port.
Voltage is flatlined at 2.47V.	BNC is not correctly connected to the iIFMC.

4.1.2 Excitation or Opsin port

Problem	Possible solutions
Light is not correctly transferred to the sample port.	Check that the wavelength of the source is within the excitation or opsin port wavelength range.

4.1.3 Integrated LED

Problem	Possible solutions
Light output is too faint or too bright.	Use a power meter and a 400um NA0.57 patch cord with the current set to 500mA to reproduce the maximum power figure given on the iIFMC datasheet. If they differ more than 10%, contact Doric support to ensure the iIFMC is working properly.

4.2 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.

4.3 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.

4.4 Disposition

Figure 4.1: *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.

4.5 Contact us

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

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