doric

Integrated Fluorescence Mini Cubes

Gen2

User Manual

Version 1.0.1

Contents

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Quick Start

The basics of the *Integrated Fluorescence Mini Cubes* operations.

Figure 1.1: *An example of a typical cube with all type of ports present shown as an iFMC and an iIFMC*

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)**
    a) **iFMC** (FC receptacle): Connects to a LED with either a *Mono Fiber-optic Patch Cord* or a *Attenuating Mono Fiber-optic Patch Cord*. Check the table 2.1 for the correct LED and patch cord for each illumination port.
    b) **iIFMC** (M8 connector): Connects to a LED driver with the provided M8 cable. The silver ring controls a variable attenuator in front of the integrated LED, turn clockwise for more power and counter-clockwise for less.
3. **Opsin port (O)** (FC receptacle): Connects to the source used for optogenetic stimulation. Check the table 2.2 for the most common sources for each port.
4. **Fluorescence Detector Emission port (F)** (BNC female connector): Connects to an analog input of the *Fiber Photometry Console* or another *Data Acquisition* box. This is the output fluorescence signal.
5. **Amplifier controls**: Adjusts the amplifier mode (AC or DC) and gain (1x, 10x or 100x) for each channel.
6. **Power supply (2.1 mm jack)**: Connect to the provided 12V power supply. A splitter is sometime provided to connect multiple cubes on the same power supply.
7. **Power switch**: Turns on the photodetectors and amplifiers for all the channels. You are ready to go.

2

Operation Guide

2.1 The difference between iFMC & iIFMC

This user manual covers both versions of the *Integated Fluorescence Mini Cubes*: the *iFMC* and the *iIFMC*. The added "I" in iIFMC stands for LED. It means this type of cube also integrate the light sources of the excitation ports directly in the cube.

(a) *iFMC4* (b) *iIFMC4*

Figure 2.1: *The same 4 port cube in both versions*

Some sections of the manual will specifically refer to a certain type (like section 2.5 that refers only to iIFMC). When there is no mention of a difference, the information applies to both versions. To quickly refer to both version, the abbreviation *i(I)FMC* is sometime used.

2.2 Ports type and description

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

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 or through a rotary joint. It is recommended to use a low auto-fluorescence patch cord between the cannula and the i(I)FMC 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, they are labelled as E1, E2 and E3. Isosbestic excitation ports are labeled IE. When excitation and fluorescence ports are designed in pair, they are labeled with the same number, E1 goes with F1, E2 with F2 and so on.

Excitation ports contains a filter chosen to correspond to the excitation peaks of the fluorescent protein the i(l)FMC is designed to measure. Figure 2.2 shows the spectral filter of an excitation port designed for GFP.

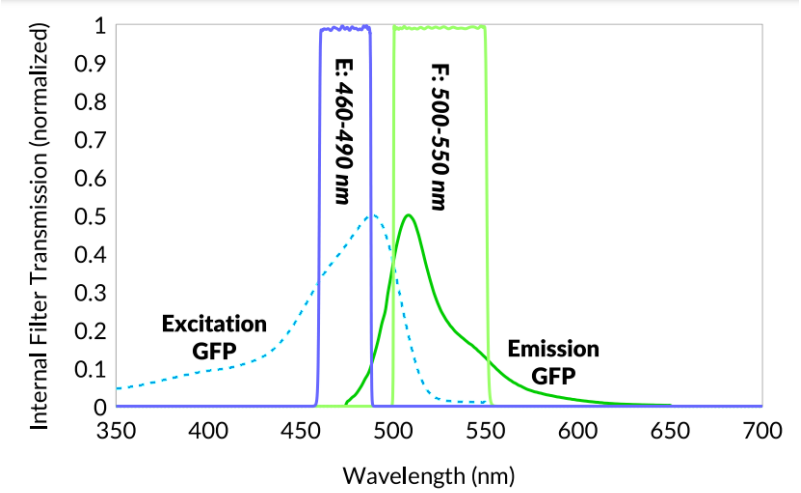


Figure 2.2: *Example of a pair of excitation and fluorescence port designed for GFP*

To obtain a stable and uniform illumination in the measured brain region, LEDs are used to inject light into the excitation port of the i(l)FMC. 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 mode of a fiber, preventing power output variation when the fiber is bent.

The drawback to filling all the transmission mode 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 are connected to the iFMC with attenuating patch cords to reduce their power output (see table 2.1). The iIFMC cubes are also internally attenuated (see section 2.5 for more details).

Table 2.1: *LED and patch cord to use for each illumination port*

Port wavelength range	LED	Recommended patch cord
400-410	405nm	Attenuating T=5%
420-445	420nm	Attenuating T=5%
460-490	470nm	Attenuating T=5%
555-570	560nm	Standard

It is possible to use a FC connectorized excitation port as an opsin port as there is no functionnal difference between the two except the filter choice. A typical example of this is the usage of the E(460-490) port for either GCaMP excitation or ChR2 activation. Please contact Doric technical support to know more about this option.

2.2.3 Fluorescence ports

Fluorescence detector ports are designated by F on the top engraving. If there is more than one fluorescence port, they are labelled as F1, F2 and F3. There is always two labels for each fluorescence port, one for the position of the photodetector and amplifier control and one for the output BNC. When excitation and fluorescence ports are designed in pair, they are labeled with the same number, E1 goes with F1, E2 with F2 and so on.

Fluorescence ports contains a filter chosen to correspond to the emission peaks of the protein the i(I)FMC is designed to measure. This filter is usually very wide to collect as much fluorescence as possible. Figure 2.2 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

Opsin ports are designated as O on the top engraving. Not all i(I)FMC have dedicated opsin ports. Their ports contains a filter chosen to correspond to the excitation spectrum of an opsin.

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

Port wavelength range	Light source	Opsins
450-490	470nm LED, 450nm & 473nm Laser	Channelrhodopsin
580-650	Ce:YAG, 590nm Laser, 638nm Laser	Halorhodopsin, Chrimson
628-642	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 receptacle slot to ensure good connection (Fig. 2.3). Always screw the barrel tightly to prevent connection instability.

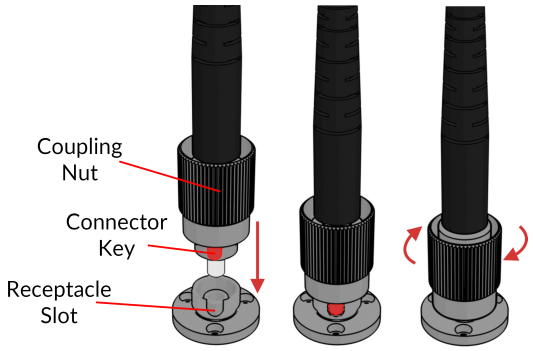


Figure 2.3: *FC connector, Fiber Installation*

2.4 Integrated photodetectors & amplifiers

Detector port of the i(I)FMC contain an integrated photodiode and amplifier. If there are several fluorescence ports, the amplification circuit of each port is completely separated. They only share the same power supply.

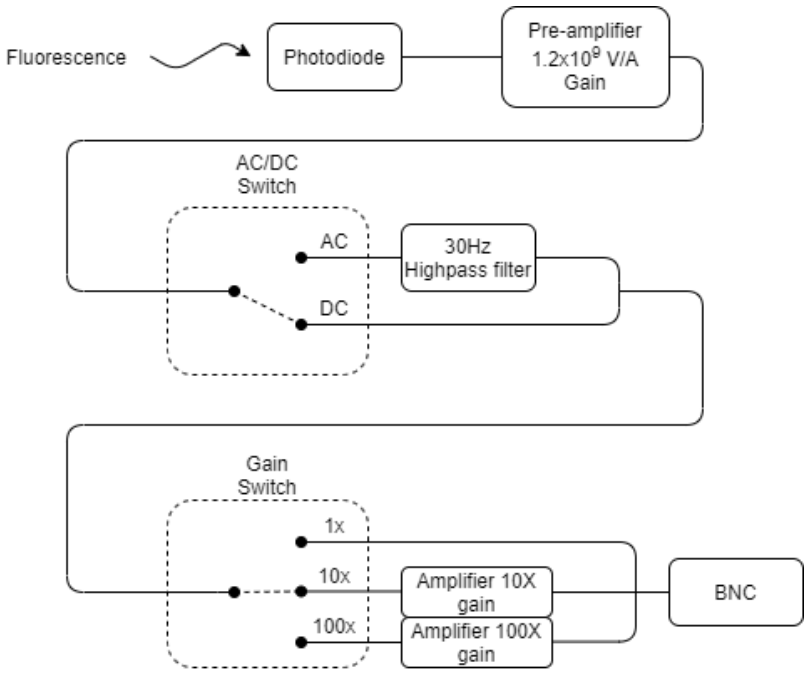


Figure 2.4: *Amplification chain of a i(I)FMC fluorescence channel*

Each port has 2 selector switch and an output BNC connector. The first switch is for the amplification mode (AC/DC) selection and the second for amplification level.

2.4.1 Amplification mode

The amplification mode switch selects between DC (direct current) and AC (alternating current) mode.

When in **DC mode**, the signal from the photodiode is directly passed along to the amplifier without alteration. The output range at the BNC connector is 0V to 5V. This mode is recommended for photometry experiments and should be used by default unless specified otherwise.

When in **AC mode**, the signal from the photodiode is passed through a 30Hz high-pass filter before going to the amplifier. Any variation slower that 30 oscillation per second as well as the average value of the signal is removed. The output range at the BNC connector is -5V to 5V. This mode offers twice the dynamic range but can hide a state of saturation of the pre-amplifier and should be used with care.

2.4.2 Gain selection

The gain switch selects between three output level for the same input fluorescence light. The 1x gain passes the signal from the pre-amplifier directly to the BNC while the 10x and 100x add 2 other amplification levels.

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 Integrated LEDs (iIFMC only)

The iIFMC has integrated LED directly in the excitation ports. These LEDs connects to the LED driver with a M8 cable. Because they are passively cooled, all integrated LEDs are limited to a maximum current of 500mA.

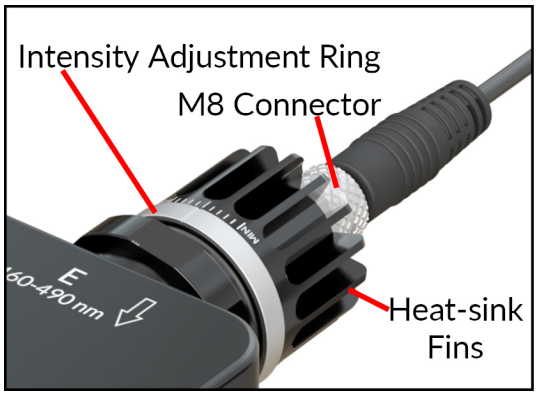


Figure 2.5: *Integrated LED with an intensity adjustment ring*

Integrated LEDs (except the 555-570 nm) have adjustment ring that controls a variable attenuator. A clicking mechanism stops the ring at 11 discreet position describe in table 2.3. Adjusting power this way should be considered a coarse adjustment and fine tuning should be done by varying LED current.

Integrated LEDs in the 555-570 do not have variable attenuator because the integrated 560nm LED used are already strongly attenuated by the narrow spectral filter.

Table 2.3: *Attenuation function of the ring position for the integrated LEDs*

Position		Transmission (%)
1	(MAX)	100
2		95
3		90
4		80
5		70
6	(CENTER)	55
7		40
8		25
9		10
10		2-3
11	(MIN)	1-2

2.6 Power supply

Each *Integrated Fluorescence Mini Cubes* is provided with a 12V wall plug power supply unit. An additionnal battery pack can be purchased if needed. We suggest using the wall plug power supply unless you run into noise or grounding problems.

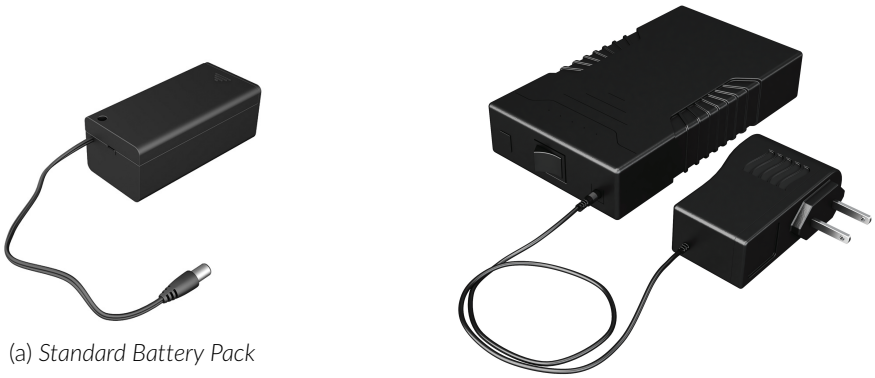
To turn the *Integrated Fluorescence Mini Cube* on, lift the power switch up. The lights on the amplifier controls will light up.

2.6.1 Wall plug power supply

Always use the provided power supply that came with either the *Fluorescence Mini Cubes* itself or a complete Doric system. Each *Fluorescence Mini Cubes* consumes less than 50mA so multiple cubes can be connected on the same

power supply with a power splitter. Such 1 to 2 or 1 to 4 power splitters are often provided with Doric systems.

2.6.2 Battery pack



(a) *Standard Battery Pack*

(b) *Rechargeable Battery Pack*

Figure 2.6: *Doric Fluorescence Detector Battery Packs*

There are two available battery packs in option.

- A *9V Battery Pack* (Fig. 2.6a) using standard 9V/E type battery. These allow optimal usage of the detector when wall plugs are unavailable or when the current from a wall plug introduces significant noise in the signal. The pack can be connected directly to the **Power Supply** port and a single battery will last an average of 20 hours for a single fluorescence port (10h for 2 and 6h for 3).
- A *Rechargeable Battery Pack* (Fig. 2.6b) can be provided when requested. These 12V 6000 mAH battery packs can keep a single detector powered for approximately 1 week without interruption. These power supplies are ideal for those who want to use the detectors solely using battery power.

3

Specifications

3.1 Optical specifications

Table 3.1: *General optical specifications*

Specification	Value	Unit
Maximum transmitted numerical aperture	0.50	-
Excitation port typical transmission	70	%
Opsin port typical transmission	70	%
Optical filter attenuation outside the bandwidth of interest	$< 10^{-5}$	-
Integrated LED		
Illumination size	400	μm
Illumination numerical apperture	0.22	-
Detector		
Material	Si	-
Size	1.1 x 1.1	mm
Wavelength range	320-1100	nm
Responsivity	0.6	A/W

Table 3.2: *Integrated LED typical optical power for each excitation port at 500mA. Minimum and maximum power is controlled by the integrated attenuator. 540-570 and 555-570 are not adjustable.*

Port	Minimum power μW	Maximum power μW
400-410	9.25	175
420-445	8	250
460-490	3.25	300
540-570	-	780
555-570	-	312.5

Table 3.3: *Typical filter transmission bandwidth for both standard configuration of iFMC and ilFMC*

Fluorescence Mini Cubes	Excitation (nm)	Fluorescence (nm)	Opsin (nm)
FMC3			
GFP	460-490	500-550	
RFP	540-570	580-680	
FMC4			
GFP and Opsin	460-490	500-550	580-650
GCAMP Isosbestic and Functional	400-410	500-550	
	460-490		
FRET	420-445	460-500	
		528-556	
FMC5			
GFP + RFP	460-490	500-540	
	555-570	580-680	
GCAMP Isos. + Func. and Opsin	400-410	500-550	580-650
	460-490		
FMC6			
GCaMP Isos. + Func. and RFP	400-410	500-540	
	460-490	500-540	
	555-570		
FMC7			
Three-fluorophore Fluorescence	400-410	420-450	
	460-490	500-540	
	555-570	580-680	

Table 3.4: *Filter transmission and blocking band for standard filters (in nanometer)*

Port	Blocking bands 1 ($T < 10^{-5}$)	Transmission band ($T > 0.9$)	Blocking bands 2 ($T < 10^{-5}$)
400-410	350-393	398-411	415-850
420-445	200-415	418-444	449-1000
460-490	291-451	463-498	497-710
460-500	279-459	463-498	507-962
500-540	268-493	499-539	549-935
500-550	298-496	500-549	559-953
528-556	268-521	526-555	566-938
540-570	274-536	540-567	570-885
555-570	303-552	556-569	572-784
580-650	269-568	576-549	670-1000
580-680	320-575	582-678	688-975

3.2 Electrical specifications

Table 3.5: *General electrical specifications*

Specification	Value	Unit
Minimum Input voltage	6	V
Recommended Input voltage	9 to 12	V
Maximum Input voltage	32	V
Minimum Input voltage before irreversible damage	-0.3	V
Maximum Input voltage before irreversible damage	36	V
Current consumption per channel	25	mA
Electrical output interface	BNC	-
Output impedance	50	Ω

Table 3.6: *Detector electrical specifications*

Specification	Value	Unit
Transimpedance gain	$2 \cdot 10^9$	V/A
Noise equivalent power (NEP)	12	fW$\sqrt{Hz}$
DC bandwidth	0 - 1000	Hz
AC bandwidth	30 - 1000	Hz
Detector sensitivity		
550nm	0.38	A/W
960 nm (peak)	0.60	A/W
Conversion gain at 550 nm		
1x	$0.76 \cdot 10^9$	V/W
10x	$7.60 \cdot 10^9$	V/W
100x	$76.0 \cdot 10^9$	V/W
Conversion gain at 960 nm	$1.2 \cdot 10^9$	V/W
Saturation level at 550 nm		
1x	7.2	nW
10x	0.72	nW
100x	72	pW

3.3 Mechanical specifications

Please consult the customer drawing of each iFMC and iIFMC for more detailed dimension of the products. They are available for download on the corresponding product page on the website.

Table 3.7: *Overall size*

Model	Depth (mm)	Width (mm)
iFMC3	57	81
iFMC4	82	76
iFMC5	92	93
iFMC6	92	108
iFMC7	92	127
iIFMC 3 1 DET 1 LED	95	82
iIFMC 4 1 DET 2 LED	111	114
iIFMC 4 1 DET 1 LED 1 FC	111	76
iIFMC 4 2 DET 1 LED	92	131
iIFMC 5 1 DET 2 LED 1 FC	151	90
iIFMC 5 1 DET 2 LED	122	131
iIFMC 6 2 DET 2 LED 1 FC	151	108
iIFMC 6 2 DET 3 LED	151	146
iIFMC 7 3 DET 3 LED	151	165

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. • Battery pack is completely discharged. Change the 9V battery or recharge the rechargeable battery pack.
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 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 connnected to the i(l)FMC.

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.	• Turn adjustment ring clockwise to increase transmitted power through the variable attenuator. • Turn adjustment ring counter-clockwise to decrease transmitted power through the variable attenuator. • Use a power meter and a 400um NA0.57 patch cord with the current set to 200mA to reproduce the minimum and maximum power figures given on the iIFMC datasheet. If they differ more than 10%, contact Doric support to ensure the iIFMC is working properly.
There is no adjustment ring on my integrated LED.	Some LEDs are too faint to be further attenuated without compromising the experiment. These are shipped without variable attenuator and thus they do not possess an adjustment ring.
Wavelength and current limit are not correctly read when connected to the LED driver.	Contact Doric support.

4.2 Maintenance

The product does not require any specific maintenance. 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 Contact us

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

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