# Bundle-imaging Fluorescence Mini Cubes

User Manual

Version 1.1.1

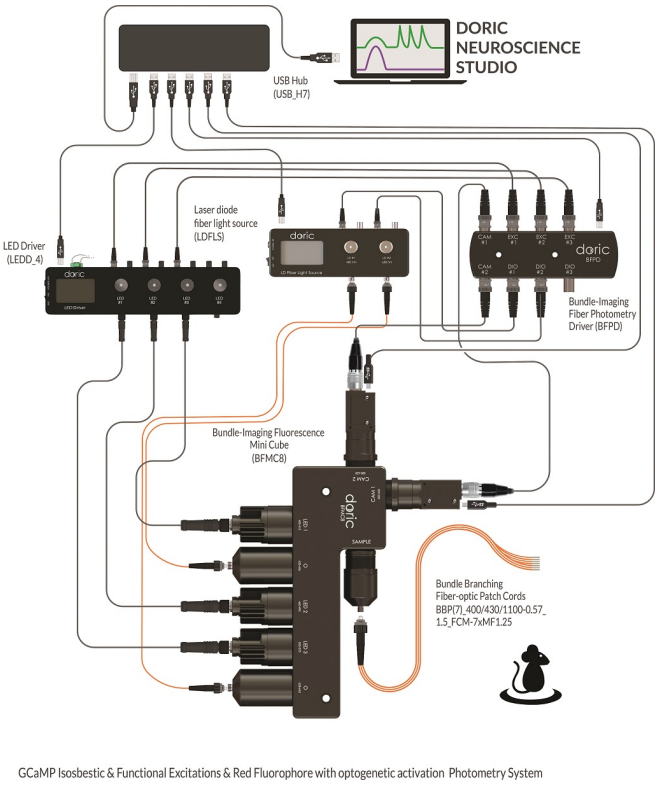
# Contents

# 1

# Introduction

The ***Bundle-imaging Fiber Photometry System (BFPS)*** is an elegant alternative for multiple site measurements. By bundling individual fiber together in a SMA connector, separate experiment sites are imaged onto a CMOS detector simultaneously which greatly simplified parallel fiber photometry measurements. The overall fluorescence signal from each site is recorded from pixel intensity variations within Doric Neuroscience Studio 1.1b.

The system is available for single and dual color measurements with isosbestic reference excitation as well as optogenetical synchronized experiments 1.1a.




(a) *GCaMP, RFP, Optogenetics 450 and 638 nm configuration*

(b) *Doric Neuroscience Studio BFPS interface*

Figure 1.1: *Bundle-Imaging Fiber Photometry System overview*

# 2

# System Overview

## 2.1 Bundle-imaging Fluorescence Cube (BFMC) : Port type and description

The *Bundle-imaging Fluorescence Cube* have four types of optical port : Sample, Excitation, Camera and Optogenetic. According to the experiment, the number of ports and their design are modified to achieve the desired purpose (Fig 2.1).

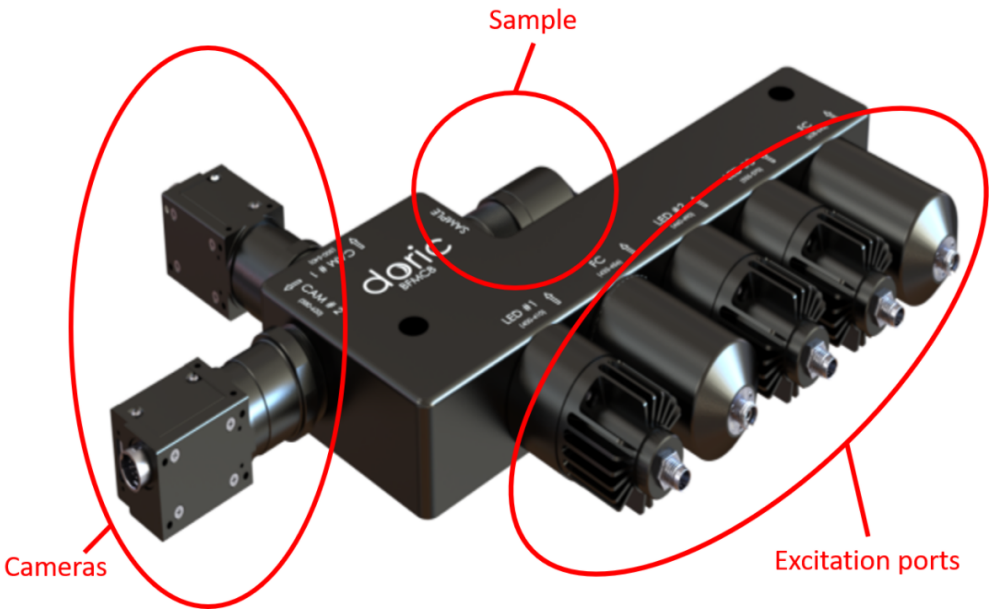



Figure 2.1: *BFMC 8 overview. Note that the cube are classified by their number of ports. In this case the cube has 8 ports : 5 excitation ports, 2 cameras and 1 port sample.*

### 2.1.1 Sample port

Each mini fluorescence cube has a single sample port. This is the only port without any spectral filtering, all wavelengths can pass freely through it. The sample port consists of a microscope lens and a fiber adapter to image and focus the fiber bundle onto the cameras. To accommodate larger fiber bundles (up to 2.5 mm), an SMA receptacle is used on the sample port.

A fiber bundle has two or more optical fibers bundled together in an SMA optical connector at one end. The other end consists of loose optical fibers with individual connectors. Low autofluorescence materials and black epoxy are used to reduce background fluorescence and prevent cross-talk between each fiber.

### 2.1.2 Excitation ports

To obtain a stable and uniform illumination, LEDs are favored. Excitation ports are designated as LED on the top engraving. If there is more than one excitation port, they are labelled as LED1, LED2 and potentially LED3. Each excitation port contains a filter chosen to correspond to the excitation peaks of the fluorescent protein the BFMC is designed to measure.

### 2.1.3 Camera ports

Camera ports are designated as CAM on the top engraving. If there is more than one camera port, they are labelled as CAM1 and CAM2. Each detector port contains a very wide filter to maximize the detection of the fluorescence.

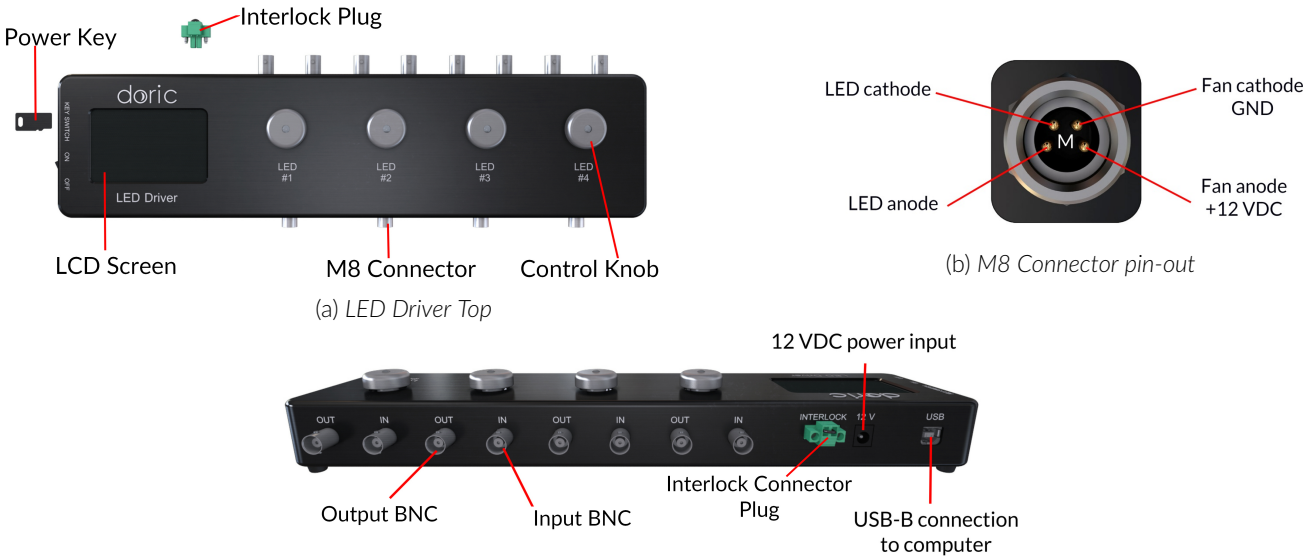
### 2.1.4 Opsin ports

If required, BFMC with Optogenetics excitation ports are available. Opsin ports are designated as O on the top engraving and contains a filter chosen to match the excitation spectrum of an opsin.

## 2.2 Bundle-imaging Fiber Photometry Subsystems

### 2.2.1 LED driver

LEDs are connected to the LED driver (Fig.2.2), which deliver the excitation current, with a M8 cable(Fig.2.2b). The LED number designated on the BFCM top engraving should be the same as the channel number of the LED driver to which it is connected. For more information on the LED driver, see the related User Manual.




(a) *LED Driver Top*

(b) *M8 Connector pin-out*

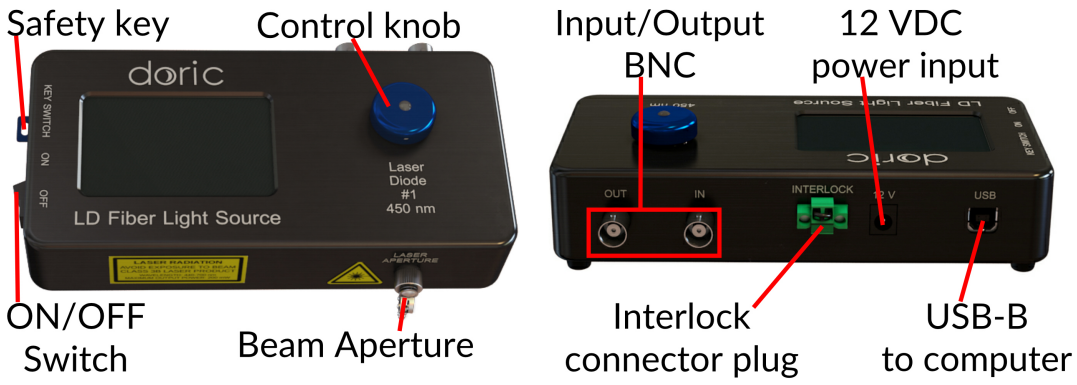
(c) *LED Driver Rear*

Figure 2.2: *LED Driver*

### 2.2.2 Laser Diode Fiber Light source

The Doric Laser Diode Fiber Light Source is a compact multiple-source laser system, available with 1, 2 or 4 channels (Fig.2.3). Laser diode outputs are FC/APC optical fiber receptacle. Optogenetic excitation is injected into the cube via a mono fiber-optic patch cord.

For more information on the Laser Diode Fiber Light source, see the related driver User Manual.




(a) *Fiber Light Source Top* (b) *Fiber Light Source Rear*

Figure 2.3: *Laser Diode Fiber Light Source Views*

### 2.2.3 Bundle imaging Fiber Photometry Driver (BFPD)

The Bundle-imaging Fiber Photometry Driver coordinates the BFMC system with Doric Neuroscience Studio (Fig.2.4). The BFPD synchronizes the LED and Laser drivers as well as the CMOS cameras to allow interleaved acquisitions.

LEDs must be connected to the EXC entries, Cameras must be connected to the CAM entries and the Optogenetic ports to the DIO entries of the BFPD. Available digital inputs or outputs (DIO) can also be used to synchronize other equipments.

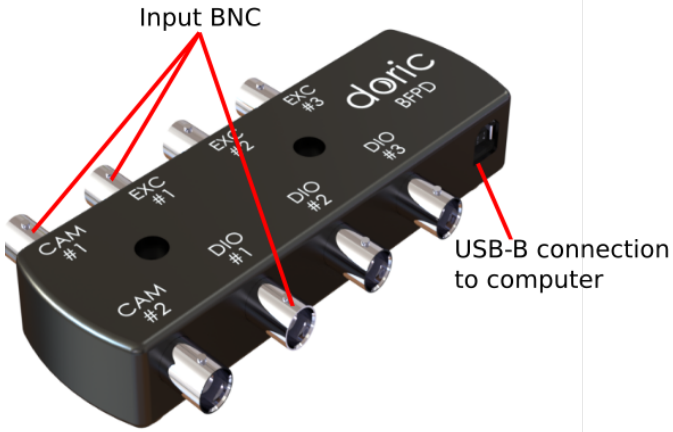



Figure 2.4: *BFP Driver*

# 3

# Getting Started : General Setup Guidelines

## 3.1 Connecting the Bundle-Imaging Fiber Photometry System

*All cables, power supply splitters as well as the USB hub are included with the Bundle-imaging Fiber Photometry System. Figure 3.1 illustrates connections between all subsystems.*

*If the Bundle-Imaging Fiber Photometry system has been ordered with a rack, must connections are already done. Skip to step 9.*

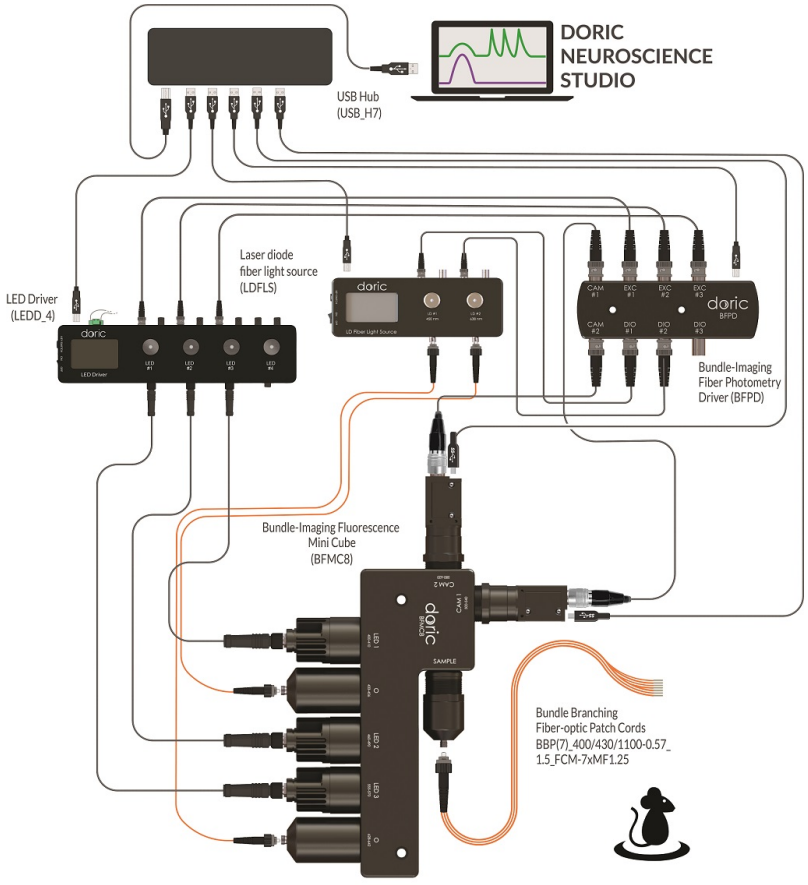



Figure 3.1: *Bundle-imaging Fiber Photometry System : Connections between subsystems.*

1. **Connect** a USB 3.0 cable between the cameras and the USB hub ports 1 and potentially 2, according to the number of detector ports.
2. **Connect** a USB 2.0 cable between the LED driver and the USB hub port 3
3. **Connect** a USB 2.0 cable between the BFPD and the USB hub port 4.
4. If the BFMC cube has Opsin ports, **connect** a USB 2.0 cable between the laser driver and the USB hub port 5.
5. **Connect** the integrated LEDs to the corresponding channel number of the LED driver with M8 cables.
6. If the BFMC cube has Opsin ports, **connect** the appropriate optical fiber between the laser source output and the optogenetic port. **FC/APC connector is identified by a green strain relief and should be connected to the laser diode light source.**
7. **Connect** the LED driver and Laser driver digital inputs as well as the camera to the BFPD with BNC cable. To ease experiment configuration in Doric Neuroscience Studio, we recommend connecting CAM1 with CAM1, LED1 with EXC1, Laser1 with DIO1 and so on.
8. **Connect** a USB 3.0 cable from the USB Hub to the PC.
9. **Connect** the LED driver, the Laser driver and the USB Hub to the 12 V AC/DC and 60W power supply with the power supply splitters.
10. **Open** Doric Neuroscience Studio. To set up an experiment refer to chapter 4.

## 3.2 Optical fiber patch cord

- **Clean** the optical fiber connector before insertion. Use isopropanol and a lint-free wipe.
- With an FC connector (Opsin ports), the **connector key must be oriented to enter within the receptacle slot** to ensure proper connection (Fig. 3.2).

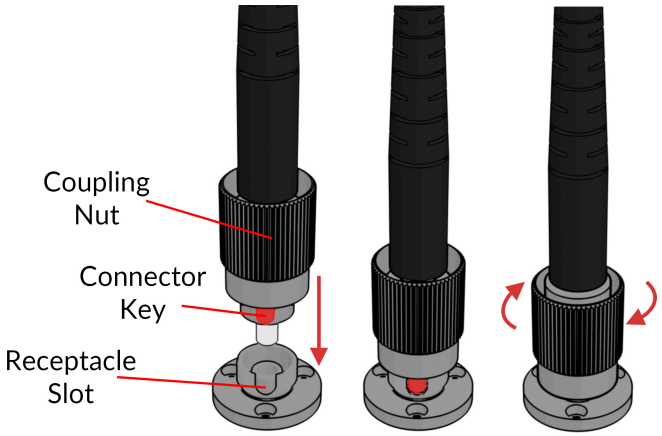



Figure 3.2: *FC connector, Fiber Installation*

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

### 3.2.1 Focus Adjustement

*The BFMC fiber adapter allow to adjust the focus of the fiber bundle image on the camera (Fig.3.3).* Cube are adjusted at factory but manual adjustment may be required over time.

1. **Connect** the optical fiber bundle to the fiber adapter and start an acquisition.
2. **Loosen** the counter-nut and **rotate** the fiber adapter until you get a clear image of the fiber bundle. It may be necessary to rotate the fiber adapter several times to adjust focus. **To avoid twisting the cable, disconnect and reconnect optical fiber cable during this alignment process.**

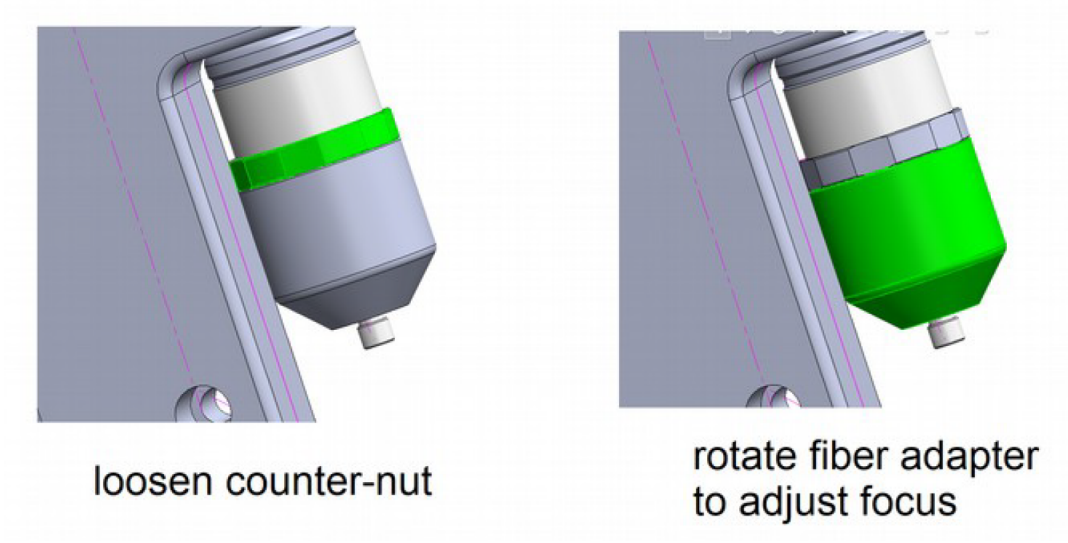



Figure 3.3: *Fiber adapter components to adjust focus*

# 4

# Specifications

## 4.1 General specifications

Table 4.1: *General specifications for BFMC, connectorized LEDs and cameras*

| Specification | Value | Unit |
|---|---|---|
| **Bundle-imaging Fluorescence Mini Cube** | | |
| Wavelength range | 350 to 1100 | nm |
| Field of view | 2.5 | mm |
| Optical Fiber connector | SMA | - |
| **Connectorized LEDs** | | |
| Input Current | Min : 0 Max : 1000 | mA |
| Cable connector | M8-4pins Male | - |
| Output NA | 0.55 | - |
| Maximum Output Power | See Table 5.2 | - |
| **Cameras** | | |
| CMOS image sensor | Sony IMX249LLJ | - |
| Pixel Size | 5.86 x 5.86 | $\mu$m |
| Quantum Efficiency | 82% at 520nm | |
| Computer interface | USB3.0 | - |
| I/O Connector | Hirose | - |
| | HR10A-10P-125(73) | - |
| Power consumption (supplied by USB) | 200 | mA |

## 4.2 Optical specifications

Table 4.2: *Typical Connectorized LED Output Power vs Optical Fiber Core Diameter*

| LED | | TYPICAL OUTPUT POWER @1000 mA (mW)[1] | |
|---|---|---|---|
| Central Wavelength (nm) | Bandwidth FWHM (nm) | Core 200 µm 0.37 NA | Core 400 µm 0.57 NA |
| 405 | 10 | ~0.100 | ~0.470 |
| 415 | 10 | ~0.130 | ~0.500 |
| 474 | 23 | ~0.180 | ~0.700 |
| 563 | 9 | ~0.020 | ~0.090 |

Table 5.3: *Typical filter configuration of BFMC*

| Fluorescence Mini Cubes | Excitation (nm) | Fluorescence (nm) | Opsin (nm) |
|---|---|---|---|
| **BFMC4** | | | |
| GCAMP Isosbestic and Functional | 400-410[2]<br>460-490 | 500-550 | |
| **BFMC5/BFMC6**[3] | | | |
| GCaMP Isos. + Func. and RFP | 400-410[2]<br>460-490<br>555-570 | 500-550<br><br>580-680 | |
| GCAMP Isos. + Func. and Opsin | 400-410[2]<br>460-490 | 500-550 | 580-650 |
| GFP + RFP and Opsin | 460-490<br>555-570 | 500-550<br>580-680 | 628-642 |
| **BFMC7/BFMC8**[3] | | | |
| Three-fluorophore Fluorescence and Opsin | 400-410[2]<br>460-490<br>555-570 | 500-540<br><br>580-620 | 433-456<br>628-642 |

[1] All power values taken at a maximum current of 1000 mA, except for 405 and 415 nm LEDs (500 mA).
[2] GCAMP Isosbestic excitation can be modified to 410-420 nm.
[3] These configurations are offer with one or two cameras to detect the fluorescence bands.

Table 4.4: *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 |

## 4.3 Mechanical specifications

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

Table 4.5: *Overall size*

| **Model** | **Depth (mm)** | **Width (mm)** |
|---|---|---|
| BFMC4, 1 Camera 2 Port | 154.6 | 189.4 |
| BFMC5, 1 Camera 3 Port | 159.6 | 188.4 |
| BFMC6, 2 Camera 3 Port | 238.6 | 246.6 |
| BFMC7, 1 Camera 5 Port | 159.6 | 293.0 |
| BFMC8, 2 Camera 5 Port | 238.6 | 327.0 |

# 5

# Support

## 5.1 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.

## 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 Contact us

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

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