doric

FluoPulse™

User Manual

Version 1.0.1

Contents

1

Introduction

FluoPulse™ is a system designed for fluorescence lifetime fiber photometry measurements in freely-moving animals. The system is intended for sensors exhibiting lifetimes in the range 1ns to 10ns. This document explains the main principles of operation of the FluoPulse™ system and its main components. It provides a list of equipment, demonstrating how to connect, install, set up, and use the FluoPulse™ system.

The FluoPulse™ system consists mainly of a FluoPulse™ Cube (Section 1.1) and a FluoPulse™ Console (Section 1.2), as shown in Figure 1.1. The FluoPulse™ Cube generates and receives optical signals. FluoPulse™ Cube is configured by the user when ordered, and contains the chosen lasers, detectors, filters and fiber ports. The FluoPulse™ Console triggers the lasers, collects, synchronizes and processes the data from the FluoPulse™ Cube, and sends the data to a computer with *Doric Neuroscience Studio* (DNS) software via a USB link. The lifetime calculations are performed in DNS and displayed in a user-friendly environment.

For use of DNS in context of fluorescence lifetime monitoring, please consult the latest *Doric Neuroscience Studio* user manual, under the *Support* tab of the webpage.

Connecting and powering the FluoPulse™ system and basic functions in DNS are explained in Chapter 2. Also, a short step-by-step instruction on how to calibrate the unit and make first measurements on samples is given there. Finally, Chapter 3 briefly presents the low-power waveform sampling method used by the FluoPulse™ system.

Figure 1.1: *FluoPulse™ System*

1.1 FluoPulse™ Cube

FluoPulse™ Cube is a configurable modular unit of the FluoPulse™ system containing all the optics-specific components. It contains the excitation lasers, drivers, detectors, dichroics, mirrors, filters and fiber ports with collimators. The Cube is shown in Figure 1.2.

Figure 1.2: *FluoPulse™ Cube*

While the cubes can come in different configurations (FLPC4, FLPC5, FLPC6, etc.), differing in terms of the number of excitations and detectors, each cube is composed of common ports (Fig. 1.3 & 1.4), including:

1. **Sample port (S)** (FC/PC receptacle): Connects to a *Low-Autofluorescence Patch Cord* or a *Pigtailed Rotary Joint* going to the animal. The sample fiber port is an FC/PC optical fiber port where the fiber accessing the sample is inserted. We recommend using a 400 µm core diameter fiber to maximize signal collection and with 0.37 NA to minimize autofluorescence.

2. **Laser excitation ports (E)** (SMA connector): Excitation ports E1, E2 and E3 connect to the corresponding E ports of the *FluoPulse™* Console. These ports receive digital trigger signals that initiate laser pulses. *FluoPulse™ Cube* has slots for up to three lasers. Depending on the configuration, the slots can be populated with diode lasers emitting at 405 nm, 450 nm, or 488 nm.

3. **Optogenetics port (O)** (FC/PC receptacle): Connects the cube to a light source using a patchcord to allow for simultaneous optogenetic stimulation of the recorded site in the animal. This is an optional port placed opposite to the sample port. It is intended for 560-570 nm or 630-640 nm light sources. The optogenetic light source is only possible if there is no spectral overlap with the second detection window.

4. **Fluorescence Detector Emission ports (F)** (dual SMA connectors): Two coaxial cables connect to the corresponding F ports of FluoPulse™ Console. These ports send the lifetime waveform signal from the detectors to the console. It is important to match the polarity of the detector outputs and inputs on the *FluoPulse™ Cube* and *Console*. In some configurations, the order of polarity is different and the coaxial cables will cross to match "-" with "-" and "+" with "+" (as in Fig. 2.1).

5. **Power switch**: Turns on the photodetectors and amplifiers for all the channels. An indication LED shows the Cube is turned on.

6. **12 V Power input**: Connects to the provided 12V power supply. Note that a single power supply can support both *FluoPulse™ Cube* & *Console* using a Y-branching power connector cable.

7. **Filter drawers**:

    - **Bandpass Filter**: Slots for chromatic filters in front of every detector. The filters limit further the wavelength band transmitted to the detectors. *E.g.* In the 500-550 nm window, a 520/40 nm filter is typically inserted.
    - **Neutral Density Filter**: Every laser has a filter slot for a Neutral Density (ND) filter. In case the laser excitation is too high for a specific sample, it is possible to attenuate a particular laser by inserting ND filters in the respective slot. The following ND filters are provided with the *FluoPulse™ Cube*: 10% & 25% transmission filters. Note that the power at the sample depends on the fiber type and other components inserted in the optical path as well.

8. **Detector Adjustment Knob**: Tunes the sensitivity of each detector. To increase the gain, turn clockwise and to decrease the gain turn counter-clockwise.

NOTE: It is strongly recommended to keep the fiber port and all filter slots closed all the time to prevent dust from entering the *FluoPulse™ Cube* and degrading the optics.

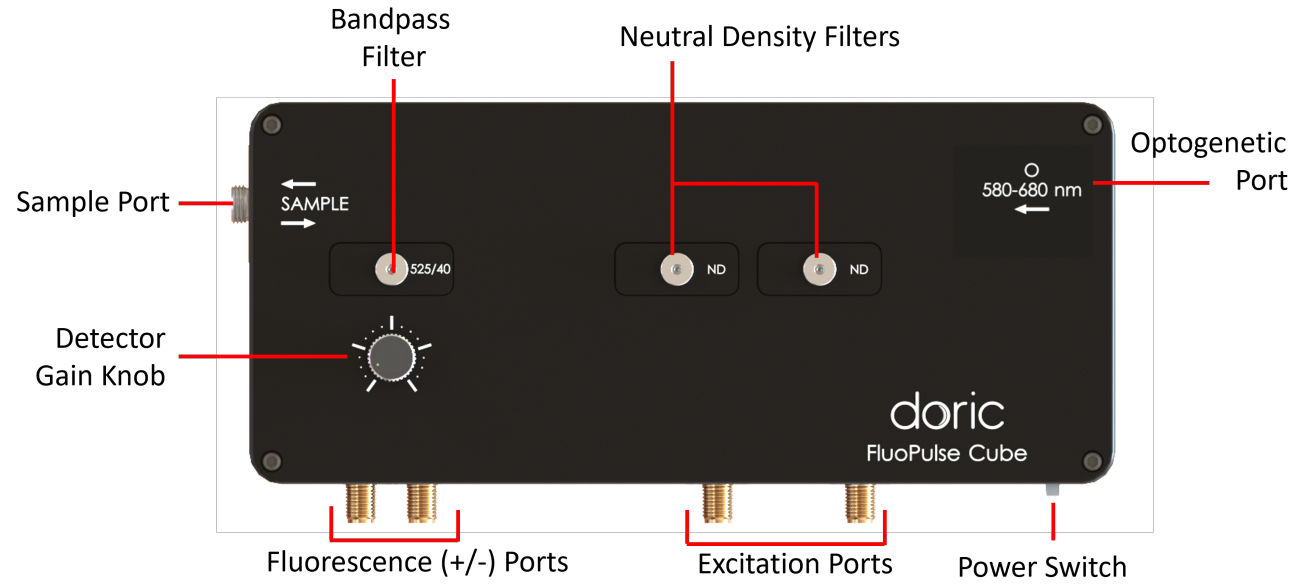


Figure 1.3: *Components of FluoPulse™ Cubes*

Figure 1.4: *Components of FluoPulse™ Cubes Back*

1.2 FluoPulse™ Console

FluoPulse™ Console is an FPGA-based electronics unit that triggers the lasers and processes fluorescence signals. It contains a fast signal sampler, it controls the eight digital inputs and outputs (DIOs), and has means of communication with the governing computer application (DNS) via USB cable. The *FluoPulse™ Console* is compatible with all configurations of *FluoPulse™ Cubes* (FLPC4, FLPC5, FLPC6, etc.). The Console is shown in Figure 1.5.

Figure 1.5: *FluoPulse™ Console*.

The *FluoPulse™ Console* is composed of the following components (Fig. 1.6):

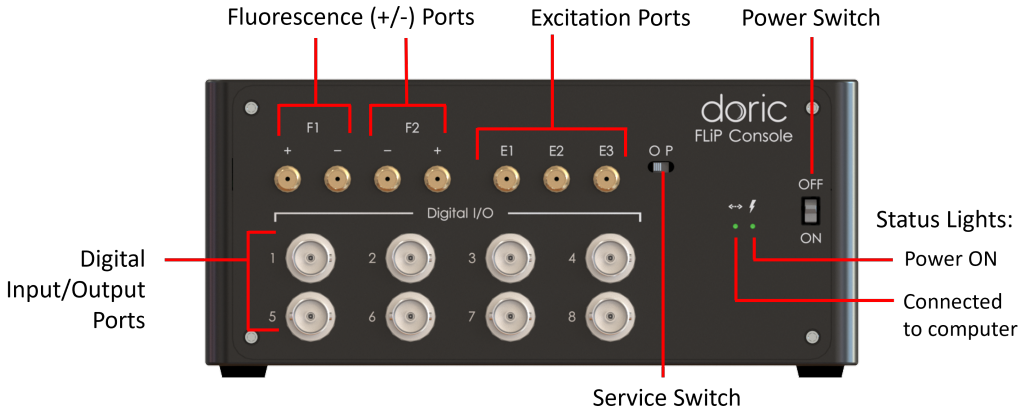


Figure 1.6: *FluoPulse™ Console Components*

1. **Excitation ports (E)** (SMA connector): Excitation ports E1, E2 and E3 connect to the corresponding E ports of the *FluoPulse™ Cube*. These ports send a digital trigger signal that triggers the corresponding laser.
2. **Fluorescence Detector Emission ports (F)** (dual SMA connectors): Twin coaxial cables connect to the corresponding F ports of *FluoPulse™ Cube*. The Console can connect up to two detectors on the FluoPulse™ Cube (F1 and F2). It is important to match the polarity between the *FluoPulse™ Cube* and *Console*. In some configurations (FLP6), the order of polarity is different, and the coaxial cables will cross to match "-" with "-" and "+" with "+" (as in Fig. 2.1).
3. **Digital Input/Output (DIO) Ports** (BNC): Eight BNC-type connectors are compatible with 5V and 3.3V TTL logic. These ports can either receive or output TTL pulses, which is useful when synchronizing the lifetime experiment with other devices.
4. **Power switch**: Turns ON/OFF the console. The right white indication LED is on when the console is powered.
5. **Status LED**: Is the left white LED and indicates the status of the Console. When communication with a computer is established, this LED is on.

6. **Service Switch**: Is used to reprogram the firmware of the device. The “P” stands for “programming”, while “O” stands for “operating”. During normal usage, the switch should always be kept in the “O” position. Refrain from switching to “P” position unless instructed by the manufacturer.
7. **12 V Power input**: Connects to the provided 12V, 3A power supply. Note that a single power supply can support both *FluoPulse™ Cube* & *Console* using a Y-branching power connector cable.
8. **USB 3.0 Port**: The console connects to the computer by a **USB 3** cable. At the back of the console, there are two USB connectors as seen in Figure 1.7. The upper, type B **USB 3.0** connector must be used.
9. **Service USB Port**: This USB-C port will only be used when the firmware is reprogrammed (Fig. 1.7). For proper reprogramming, the **Service Switch** should also be set to “P” when using this port. During regular usage no cable should be connected to the USB-C port.

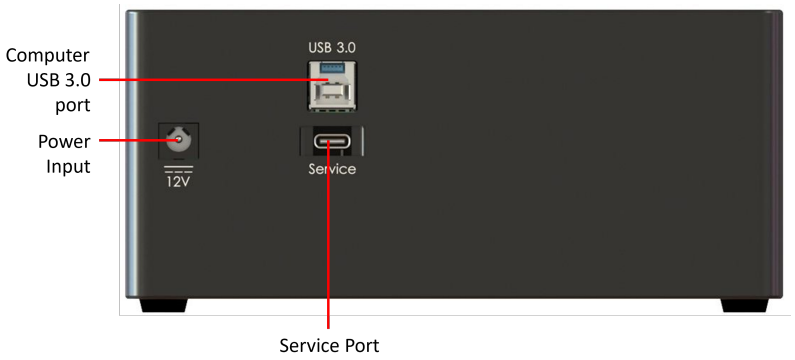


Figure 1.7: *FluoPulse™ Console back*

NOTE:

- Electronic components in the FluoPulse™ Console dissipate heat in normal operation. The housing and in particular the SMA connectors tend to get warm. This is normal.
- Air intake and exhaust must not be blocked at any time the Console is ON. Allocate at least 25 cm of free space on both left and right side of the console to allow unrestricted air circulation.

2

Operations Guide

2.1 FluoPulse™ System Item List

Before setting up, check for the presence of all the listed components.

Components of a typical FluoPulse™ system are:

- FluoPulse™ Console,
- FluoPulse™ Cube,
- 12V, 3A power supply,
- Y-branching power supply connector cable,
- USB 3.0 cable with type A and type B connectors,
- Coaxial Cables (SMA-SMA), 6 inch, 6GHz, (3 to 7 pcs.),
- Pigtailed 1x1 Rotary Joint (400 µm-core, 0.37 NA, FCA-FC connectors),
- FC-FC Adaptor,
- Sample Mono-fiber optic patch cord (400 µm-core, 0.37 NA, FC-ZF1.25 connectors),
- Mono-Fiber Optic Cannulas,
- Sleeve[1] (if applicable),

Optional Optogenetic Components:

- Laser Diode Fiber Light Source (LDFLS),
- LDFLS 12V, 3A power supply,
- Optogenetic Mono-Fiber Optic Patch Cord: 200 µm-core fiber, 0.22 NA, FCA-FC connectors,

NOTE: Fiber may vary in type and length. It is possible to use fiber with a core diameter ranging from 100 µm to 400 µm and NA larger than 0.2 (Low Autofluorescent (LAF), 0.37 NA recommended). Note that fibers with smaller core and NA collect less signal making the resulting calculated lifetime potentially less precise.

[1] For ZF1.25, ZF2.5, MF.125 and MF2.5 type cannulas

2.2 Hardware Setup Instructions

This section details the ste-by-step instructions to set-up a complete *FluoPulse™* system, as in the schematic in Fig. 2.1:

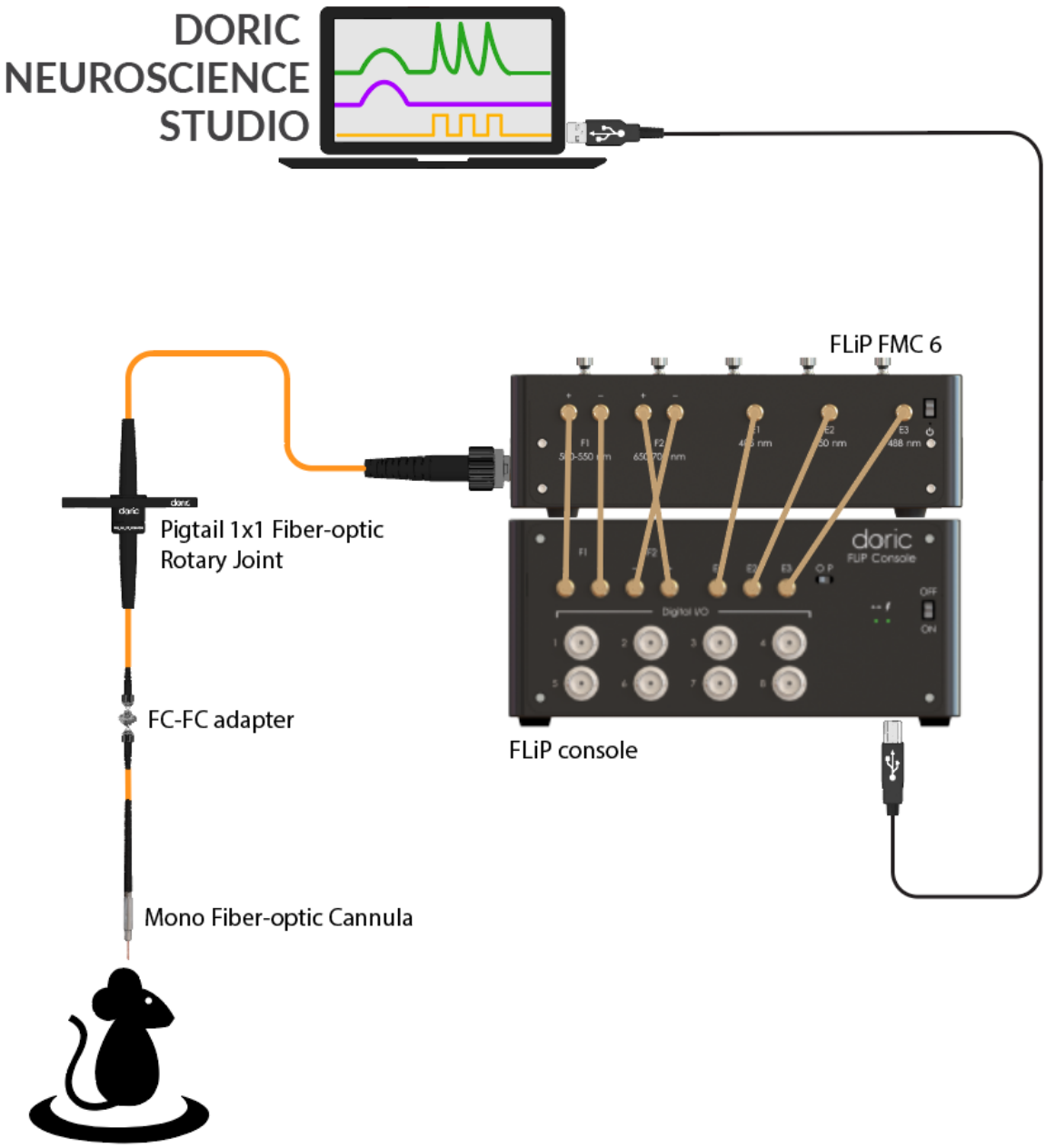


Figure 2.1: *FluoPulse™ System Schematic*

1. Place the *FluoPulse™ Cube* on top of the *FluoPulse™ Console*. Make sure there is open space to the left and right of the console and nothing blocks air flow for cooling the unit.
2. With all power supplies disconnected and taking precautions to avoid electrostatic discharge, connect the **F1** (and **F2** if present) port(s) of the *FluoPulse™ Cube* to the **F1** (and **F2**) port(s) of the *FluoPulse™ Console* using short **Coaxial Cables** with male **SMA-SMA connectors**. It is important to match the polarity between the *FluoPulse™ Cube* and *Console*, such that **"-"** should connect with **"-"** and **"+"** with **"+"**.

   NOTE: The **F2** port of the *Console* has an inverted polarity so the **coaxial cables** will cross.

3. Using the remaining **Coaxial Cables** (SMA-SMA), connect all laser triggers **E1**, **E2**, and **E3** (if present) of the *FluoPulse™ Cube* to the corresponding **E1**, **E2**, and **E3** of the *FluoPulse™ Console*. Connect all the cables firmly.

4. The *Console* and the *Cube* share a single 12V, 3A **Power Supply** using the **Y-branching cable**. Before connecting, confirm that the power switches on the *Cube* and *Console* are in the OFF position, then plug each connector of the branching Y-cable to the **12V power input** at the back of each device (*Cube*: Fig. 1.4, *Console*: Fig. 1.7).

   For the optogenetic configuration: plug in the dedicated **Power Supply** to the LDFSL **12V power input**.

5. Connect the *Console* **USB 3.0 type B** port to the computer's **USB 3.0 port** with the provided **USB cable**. Avoid using a USB hub between the computer and the *Console*.

6. Connect the optical fibers as follows:

   - Connect the **FCA connector** (green) of the Rotary Joint to the **Sample Port** of the *FluoPulse™ Cube* (Fig. 1.3).
   - Connect the **FC connector** (black) of the Rotary Joint to the **FC-FC Adaptor**.
   - Connect the other side of the **FC connector** to the **FC Connector** of the **Sample Patch Cord**.
   - Connect the **MF**, **ZF** or **M3** connector of the patch cord to the cannula (implant on the animal's head). Use a **Sleeve** for **ZF**/**MF** connectors.

   NOTE: When inserting the FC connector or FCA connector, make sure the **Connector Key** is well aligned in the **Receptacle Slot**, especially when screwing the **Coupling Nut**. Improper connection will lead to both excitation power and signal loss. Do not lose the protective cap for the port.

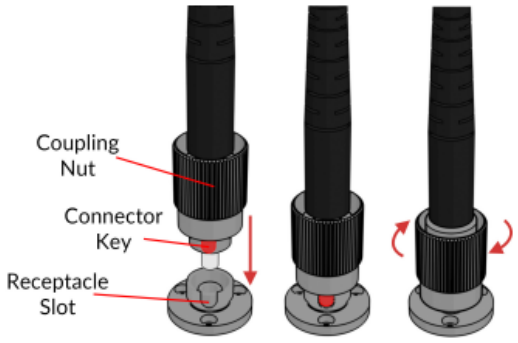


Figure 2.2: *Proper Optical Connection*

7. If applicable, connect the fiber-coupled light source to the **Optogenetic port** of the *FluoPulse™ Cube* with a **Mono Fiber-optic patch cord (FCA-FC)**. Make sure the green side (FCA) of the patch cord is connected to the laser and the black side (FC) to the **Optogenetic port** (Fig. 1.3). When not in use, keep the port closed either with cap or a fiber patch cord.

8. Check that all **Bandpass Filter** and **Neutral Density Filter** slots on the top of the *FluoPulse™ Cube* are filled to avoid ambient light reaching the detectors.

9. Download and install *Doric Neuroscience Studio (DNS) software* from the website: HERE . The software installation instructions are in the dedicated DNS User Manual: Chapter 2.

10. Switch **ON** the *Console* and the *Cube* (and the *LDFLS* if applicable), and launch *DNS* software on the computer. DNS should automatically recognize the presence of the *FluoPulse™ Console*.

11. Adjust the signal level at the beginning of the experiment. Signal level adjustment is typically done by adjusting excitation power and/or detector sensitivity. For more details, see Section 2.3.

2.3 Typical measurement procedure

This section describes the procedure of a typical measurement using the *FluoPulse™* system. Knowing how to set up the equipment and the principle of measurement makes it easier to understand the different steps that the user needs to take to obtain valid results.

One major difference between classic fiber photometry and lifetime fiber photometry with the *Doric Lenses* system, is that a calibration must be done before a recording. The *Instrumental Response Function (IRF)* represents the combined effects of the detector, electronic, and environmental noise occurring during each laser pulse. By removing the system impulse response from the collected waveform using a live deconvolution algorithm, the relative fluorophore lifetime can be obtained. The principle of the operation is described in Chapter 3.

WARNING:

Before every measurement (new animals and/or new day), **always calibrate** with IDENTICAL settings (detector gain and laser power optimized for the CURRENT animal). Proper calibration is required for cross-animal and cross-trial comparisons.

An experiment will typically include the following steps:

1. For high precision and repeatable results, it is advisable to turn on the *FluoPulse™* system (both cube and console) and let it run for at least 60 minutes to thermally stabilize.
2. Add and configure a measurement channel according to Doric Neuroscience Studio manual, Chapter 17. Briefly, select the *Add Channel* button (Fig. 2.3). This opens the *FluoPulse Configuration* pop-up window, where you can set the detector, triggering, and fitting options. In particular, make sure to specify the correct laser channel (E1, E2, or E3) that you will need for your experiment.

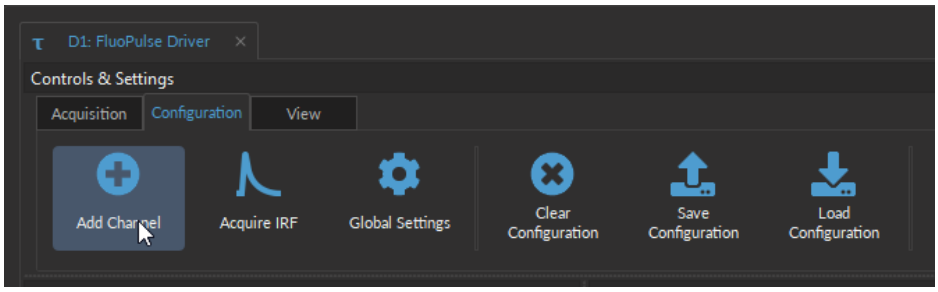


Figure 2.3: *Add Channel to configure the FluoPulse system*

3. Connect the fiber to the animal and select the *Acquire IRF* to measure the signal magnitude (as in Fig. 2.4).

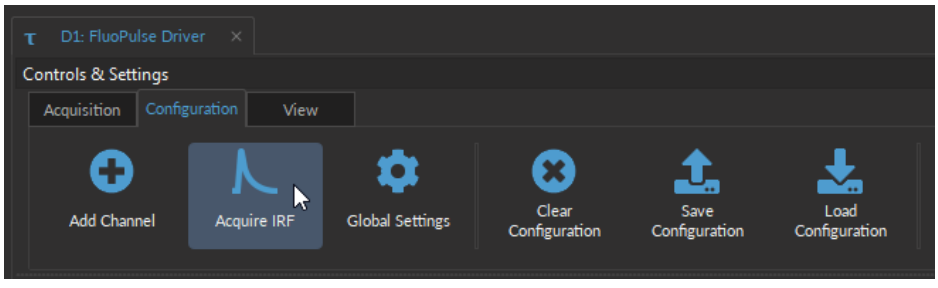


Figure 2.4: *Acquire IRF button*

4. From the pop-up window, select the *Acquire IRF from device* button (Fig. 2.5) to display the waveform (Fig. 2.6).

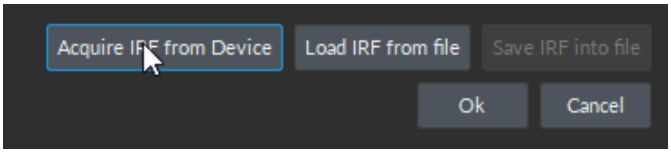
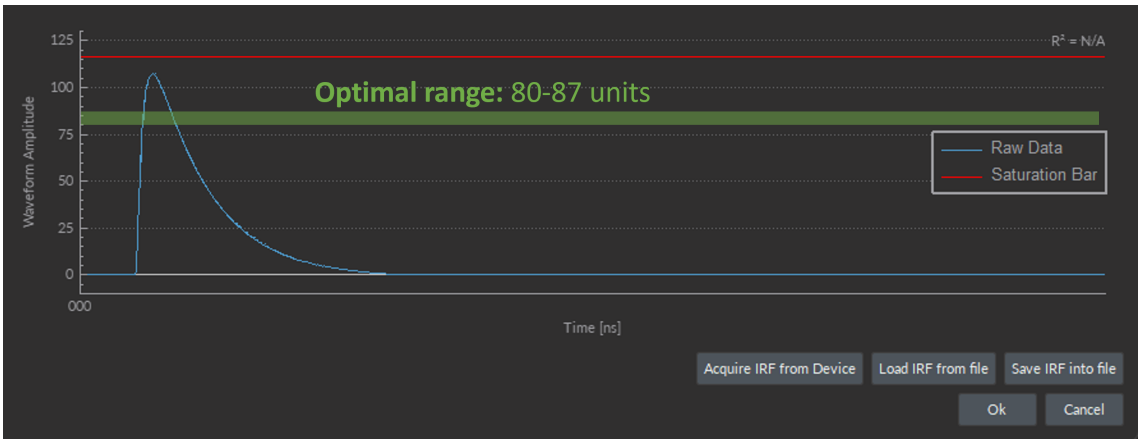


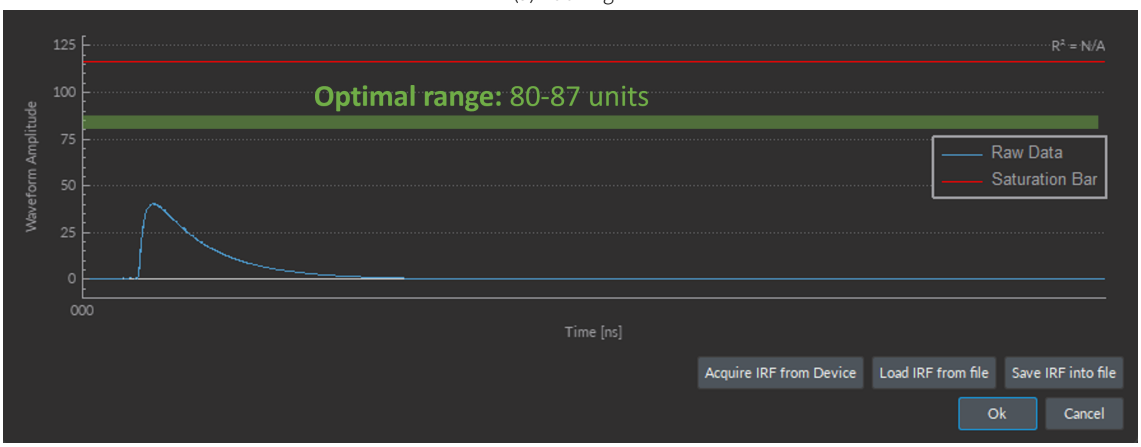
Figure 2.5: *Acquire IRF from device*

5. Read the maximum value of the *Waveform Amplitude* graph. **Values between 80-87 units are ideal**, but if the amplitude of the signal is expected to diminish over time (i.e. significant photobleaching was previously observed), higher values (below 100) are acceptable.

    - If the signal is too high (Fig. 2.6a) and **saturates the detector** (more than 116 units), lower the gain using the *Detector Gain Knob* on the *FluoPulse™* cube (Fig 1.3). If the signal is still too high even with minimal gain, insert an *Neutral Density Filter* in the correct excitation laser slot on the cube.
    - If the signal is initially too low (Fig. 2.6b), check whether there is an *ND filter* inside the excitation laser slot. Remove the ND filter (or choose a filter with lower attenuation) then measure again. If the signal is still too low, increase the gain using the *Detector Gain Knob* on the *FluoPulse™* cube (Fig 1.3).



(a) *Too High*



(b) *Too Low*

Figure 2.6: *Waveform Amplitude Examples*

6. Remove the fiber from the animal and without changing the laser and gain settings, place it in a glass cuvette containing a standard fluorescent sample with a known fluorescence lifetime. Hold the fiber firmly in the solution and minimize the exposure to the ambient light.

NOTE: We recommend preparing in advance several small solutions of fluorescein at different concentrations. The standard sample should have a similar emission spectrum and lifetime range (by 2 - 5 ns) to the biosensor of interest. Minimize the presence of any other fluorescence using pure and non-fluorescent solvents.

7. Enter the sample fluorescence lifetime value into the window (as in Fig. 2.7). (e.g. Fluorescein Fluorescence lifetime = 4 ns[2]).

Figure 2.7: *Enter Sample Lifetime*

8. Select the *Acquire IRF from Device* button (Fig. 2.5). The amplitude of the waveform taken with the standard sample **should also be within 80-100 units**, WITHOUT changing the gain and laser settings (optimized for the experimental animal).
    - If the standard sample saturates the detector (Fig. 2.6a), use a standard with a lower concentration.
    - If the standard sample waveform is too low (Fig. 2.6b), use a standard with a higher concentration.
9. Once you've obtained a waveform of an appropriate range, select *Ok* (Fig. 2.8). This saves the IRF, which will be used in the live deconvolution algorithm. Each time you change the detector gain or laser power, you MUST retake the IRF before (or after) the recording.

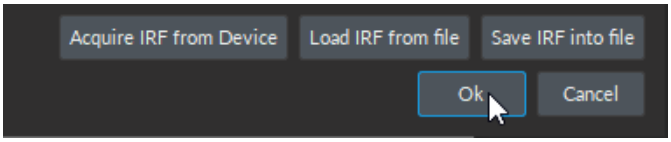


Figure 2.8: *Select OK to save IRF*

10. With the established and saved IRF, clean the fiber and reconnect it to the experimental animal. You can now begin data acquisition on the animal using *Live* or *Record* buttons.

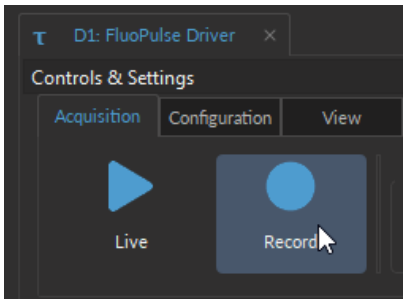


Figure 2.9: *Start an experiment using Record button*

NOTES: Changing the gain or a significant change in laser excitation power may slightly alter the IRF. Large variations in signal strength during the experiment may cause results to deviate from the optimal window of signal values (50-100 units). Therefore, **setting the initial gain and power values and keeping them constant in one experiment is very important**. The dynamic range of the device in a single experiment is 5 dB (approximately a factor of 2.3). Tuning the gain by the knob can change the signal by up to 20 dB (a factor of approximately 70 [3]). The ND filters are used

[2] This value can change if the concentration of the sample is very low.
[3] Depends on the ND filter used.

to attenuate the laser by up to 10 dB (a factor of 10). **Between experiments**, **we recommend readjusting the gain and/or laser power when necessary to retain the signal at an optimal level and retake the IRF**.

In addition to the lifetime, the quality of the fit parameters χ^2 and R^2 are displayed to be aware of the fit credibility. Any bad fit triggers a warning signal.

During a recording, if users had to adjust detector gain mid-experiment, we recommend retaking the IRF at the end of the recording and using the *Save IRF into file* button (Fig. 2.10). Since the software also saves the raw data containing all waveforms, we can **recalculate the lifetime using the new IRF during post-processing analysis.**

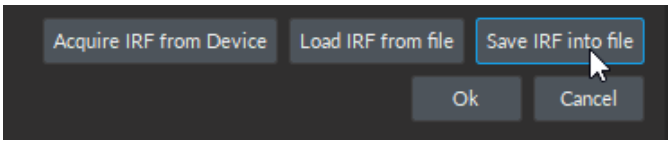


Figure 2.10: *Save IRF to file for post-processing*

3

Waveform Sampling

Waveform sampling, also called pulse sampling, is a method for estimation of fluorescence lifetime based on direct analog sampling of the fluorescence decay signal excited by a short light pulse.

FluoPulse™ utilizes low-power waveform sampling, that is, pulses are only a few tens of milliwatts in peak power, and where the average power irradiating the sample is expressed in microwatts - typically in the range 5-20 µW.

When a measurement is initiated, the FluoPulse™ Console sends a trigger signal to the lasers within the Cube. In turn, the laser emits a short pulse of light (450-500 ps) that travels through the optic fibers (rotary joint, patch cord, and cannula) to excite the fluorophores expressed in the animal tissue. The detectors collect fluorescence scattering. Typically, the back-scattered fluorescence from a single pulse contains a hundred to a few hundred photons. The received waveforms are aligned, averaged, and sent to the computer application. Every waveform signal from the FluoPulse™ Console is a result of averaging thousands of responses to excitation pulses. The number of averaged waveforms influences the signal-to-noise ratio (SNR) and thereby the precision of the instrument, but also affects the time it takes to make a single measurement. Figure 3.1 depicts the signal processing from raw data to aligned and averaged fluorescence response.

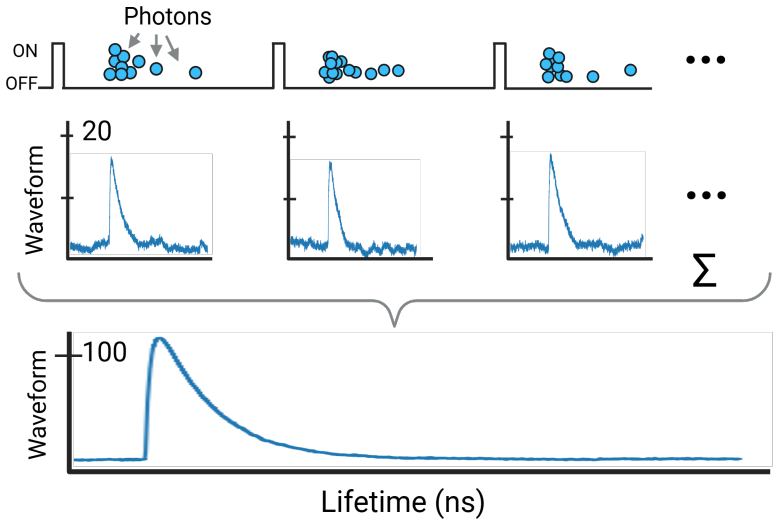


Figure 3.1: *Waveform Sampling process.*

The conditioned signal with a high SNR is not pure fluorescence decay, as the response characteristics of the system components strongly influence it. To compensate for its influence on the recorded waveform, we use an Instrument Response Function (IRF) to describe the intrinsic system behavior.

The algorithm for lifetime extraction uses the conditioned signal and the system IRF as inputs. Deconvolution of the signal with the IRF gives the actual fluorescence decay as a single or a sum of multiple exponentially decaying functions.

The resulting smooth waveform, if de-convolved with previously determined instrument response function (IRF) of the system, gives (a sum of) exponentially decaying functions.

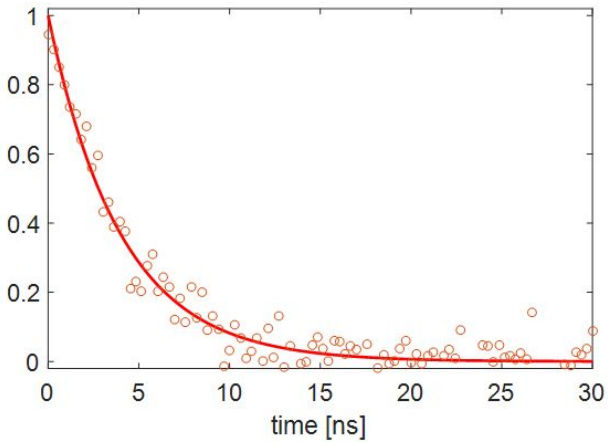


Figure 3.2: *The waveform is deconvolved with the IRF. Least squares exponential fit can estimate the decay rate or lifetime.*

In this technique, it is important to adjust the signal level at the beginning of the experiment. Signal level adjustment is typically done either by adjusting excitation power or detector sensitivity. Average power depends on the pulse repetition rate and peak power. In this version of FluoPulse™, only the peak power is variable by inserting ND filters in the excitation laser filter slots.

4

Specifications

Table 4.1: *General specifications of FluoPulse™ system*

SPECIFICATION	VALUE	UNIT
FluoPulse™ Cube		
Built-in detectors		
Wavelength detection range F1	500 to 550	nm
Wavelength detection range F2	580 to 680	nm
Optical isolation	> OD 10	
Built-in laser diodes		
Excitation wavelength options	405/450/488	nm
Max Current (405nm/450nm/488nm)	120 / 150 / 60	mA
Maximum Output Power (405nm/450nm/488nm)	100 / 150 / 50	mW
Pulse repetition rate	400	kHz
Optical ND filter attenuation	0-90	%
Fiber connection		
Optical fiber core diameter	100, 200 or 400	µm
Numerical Aperture (NA)	0.37	
Optical fiber port connector	FC/PC	-
Fiber collimation port NA	0.50	-
FluoPulse™ Console		
Fluorescence Lifetime		
Lifetime range	1-10	ns
Sample rate with 1 excitation	5 to 40	Hz
Measurement precision	50	ps
Dynamic range	5	dB
Digital Inputs / Outputs		
DIO count	8	
Voltage level	5 / 3.3 / TTL	V
DIO Sampling rate	10 - 100k	Hz
Computer interface	USB 3.0	-
Physical properties		
Dimensions Cube (width x depth x height) without knobs, connectors	165 x 76 x 51	mm
Dimensions Console (width x depth x height) without connectors	165 x 76 x 75	mm
Mass (Cube / Console)	950 / 750	g
Power supply Cube and Console		
Voltage	110 - 240	VAC
DC power supply	12	VDC
Power	36	W
Output current	3	A

Table 4.2: *Computer requirements*

Operating system	Microsoft 10, 11, 64 bit
Memory	Minimum 16 GB (32 GB recommended)
Processor speed	3 GHz with 12 cores
Hard drive	1 GB of free hard disk space (SSD recommended)
Data link	USB3.0 (cable included)

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Support

5.1 Maintenance

The product does not require any maintenance. Do not open the enclosure. Contact Doric Lenses for return instructions if the unit does not work properly and needs to be repaired.

5.2 Warranty

This product is under warranty for a period of 12 months. Contact Doric Lenses for return instructions. This warranty will not be applicable if the unit is damaged or needs to be repaired as a result of improper use or operation outside the conditions stated in this manual. For more information, see our Website.

5.3 Disposition

Figure 5.1: *WEEE directive logo*

According with the directive 2012/19/EU of the European Parliament and the Council of the European Union regarding Waste Electrical and Electronic Equipment (WEEE), when the product will reach its end-of-life phase, it must not be disposed with regular waste. Make sure to dispose of it with regards of your local regulations. For more information about how and where to dispose of the product, please contact Doric Lenses.

5.4 Contact us

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

Phone 1-418-877-5600

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