

doric

FluoPulse™

User Manual

Version 1.0.3

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Safety Information

1.1 Laser Safety Information

If you are not familiar with laser light sources, seek advice from qualified personnel **BEFORE FIRST USE** and **READ CAREFULLY Important Laser Safety Information** in Application Note provided on the USB key. A copy of the Application Note is available from Doric Lenses on the website or by contacting (sales@doriclenses.com).



WARNING!
The FluoPulse Cube contains Class 1 lasers
Read Important Laser Safety Information BEFORE FIRST USE.



The *FluoPulse Cube* contains embedded lasers that are a Class 1 products. Although a Class 1 laser is considered safe during normal operation and does not pose a hazard to the eyes or skin, it is important to handle the device responsibly. Operators should also ensure that the device is used in a controlled environment and that all individuals nearby are aware of proper laser safety practices.

Avoid Direct and Reflected Exposure.:

- Avoid directly into the optical beam exiting the output FC connector or any optical fiber connected to it.
- Remove or cover reflective surfaces (e.g., mirrors, metal tools) from the beam area.
- Avoid directly at specular or diffuse reflections of the output beam.
- Consider wearing safety glasses (goggles) certified for the wavelength and power level of the light source.
- Even when wearing laser safety glasses, avoid directly into the optical beam exiting from the *FluoPulse Cube* output FC connector, an optical fiber connected to it, or its specular reflections.
- Follow all safety procedures to protect anyone working in the area.

1.2 Laser Labels

The laser class labels are provided with the system and the laser aperture is clearly identified by laser warning label and/or the text *LASER APERTURE*.



(a) Laser Classification Label Example



(b) Laser Warning Label

LASER APERTURE

(c) Laser Aperture Identification

Figure 1.1: Safety Labels

Device Overview

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 2.1) and a FluoPulse™ Console (Section 2.2), as shown in Figure 2.1. 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 3. Also, a short step-by-step instruction on how to calibrate the unit and make first measurements on samples is given there. Finally, Chapter 4 briefly presents the low-power waveform sampling method used by the FluoPulse™ system.



Figure 2.1: FluoPulse™ System

2.1 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 2.2.



Figure 2.2: *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. 2.3 & 2.4), 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. 3.1).
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% and 100 % (empty drawer) 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.

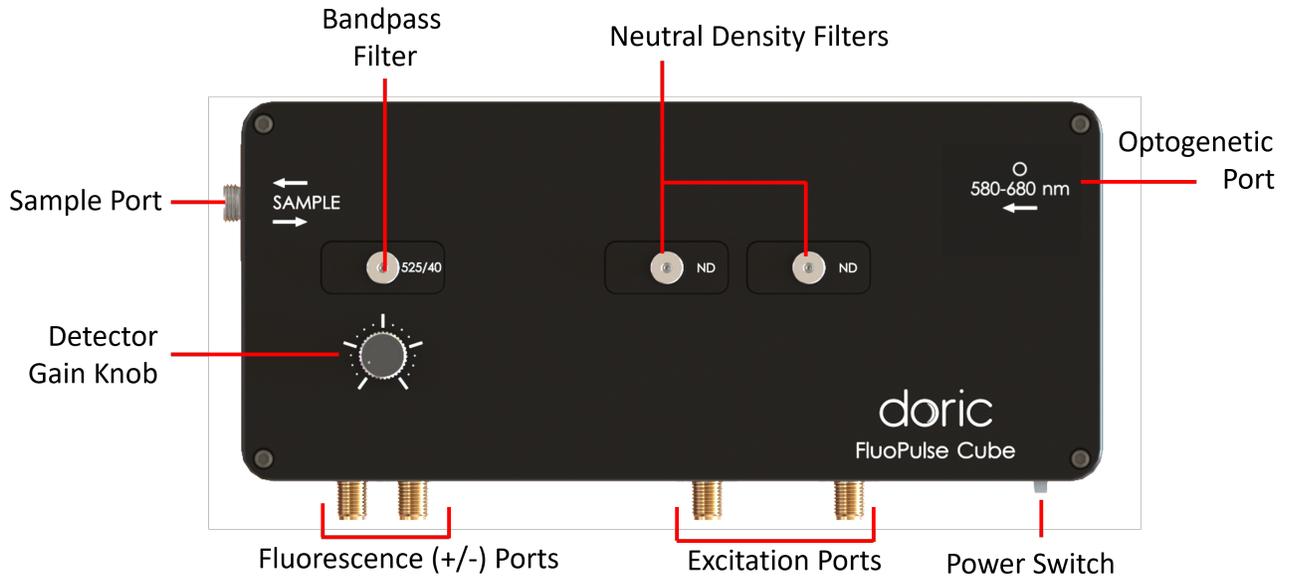


Figure 2.3: Components of *FluoPulse™ Cubes*



Figure 2.4: Components of *FluoPulse™ Cubes Back*

2.2 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 2.5.



Figure 2.5: *FluoPulse™ Console*.

The *FluoPulse™ Console* is composed of the following components (Fig. 2.6):

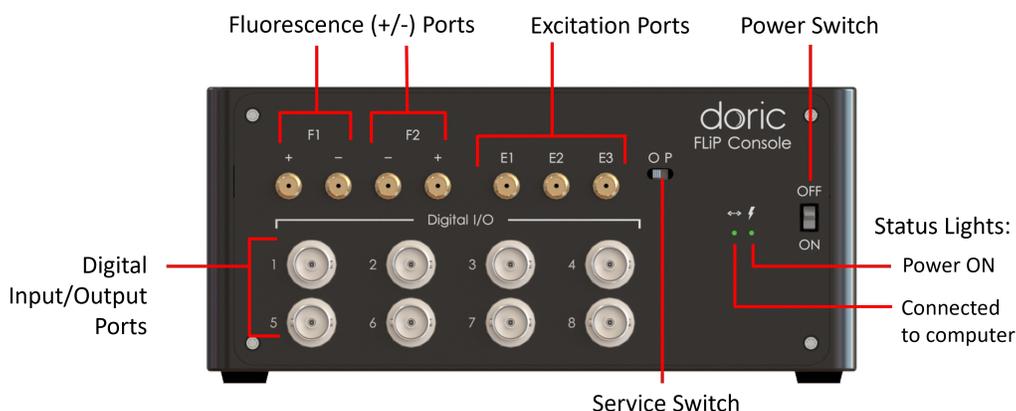


Figure 2.6: *FluoPulse™ Console Components*

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. 3.1).
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 2.7. 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. 2.7). 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.

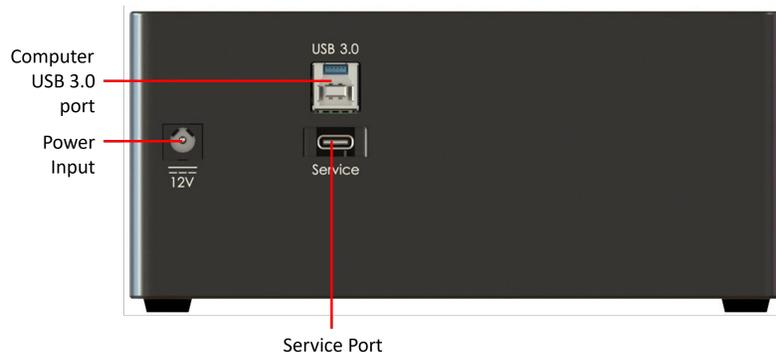


Figure 2.7: *FluoPulse™ Console back*

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.

Operations Guide

3.1 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 μ m-core, 0.37 NA, FCA-FC connectors),
- FC-FC Adaptor,
- Sample Mono-fiber optic patch cord (400 μ m-core, 0.37 NA, FC-ZF1.25 connectors),
- Mono-Fiber Optic Cannulas,
- Sleeve¹ (if applicable),
- FluoPulse™ Calibration Sample.

Optional Optogenetic Components:

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

NOTE: Fiber may vary in type and length. For optimal results we recommend using fiber with a core diameter ranging from 200 μ m to 400 μ m (400 ideal) and 0.37 NA (Low Autofluorescent (LAF)).

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

3.2 Hardware Installation

This section details the step-by-step instructions to set-up a complete *FluoPulse™* system, as in the schematic in Fig. 3.1:

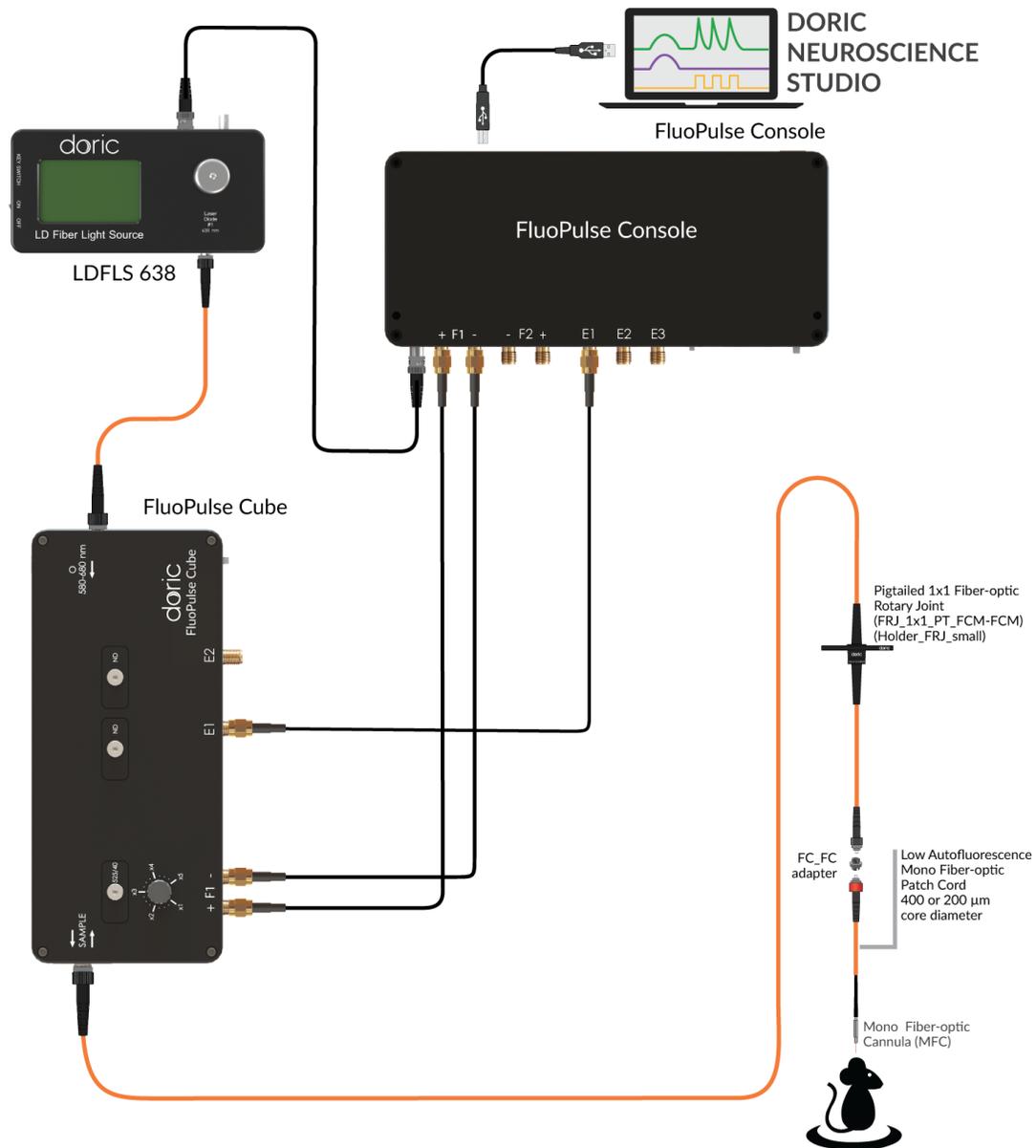


Figure 3.1: *FluoPulse™* System Schematic

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.

- 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.
- 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. 2.4, *Console*: Fig. 2.7).

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

- 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*.
- Connect the optical fibers as follows:
 - Connect the **FCA connector** (green) of the Rotary Joint to the **Sample Port** of the *FluoPulse™ Cube* (Fig. 2.3).
 - 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.

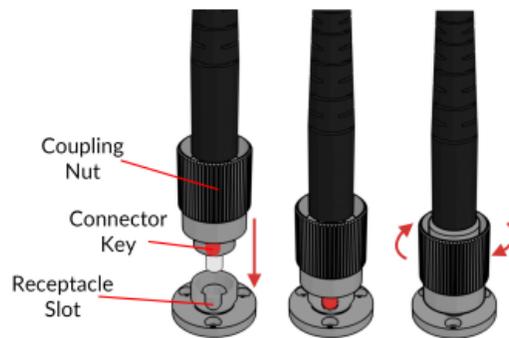


Figure 3.2: Proper Optical Connection

- 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. 2.3). When not in use, keep the port closed either with cap or a fiber patch cord.
- 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.
- 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](#).
- 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*.
- 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 3.3.

3.3 Getting Started

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. This calibration is an *Instrumental Response Function (IRF)* that characterizes the detector, electronic, and environmental delay expected during a measurement. A live deconvolution algorithm removes the system impulse response from the collected waveform, providing a more precise measure. The principle of the operation is described in Chapter 4.



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, turn on and **preheat** *FluoPulse™* system (both cube and console) using the dedicated *Preheating Options*. The system must preheat for **at least 60 minutes** to thermally stabilize immediately before acquisition. We generally recommend activating the lasers, as in Fig. 3.3, but see laser warning below.

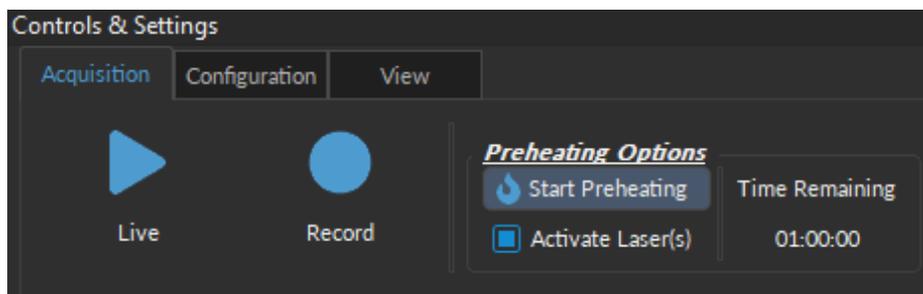


Figure 3.3: Preheat system and Activate Laser



WARNING:

During preheating remember to **cover the end of the optic fiber** with a black cap, black tape, or other secure covering.



2. Add and configure a measurement channel according to [Doric Neuroscience Studio](#) manual, Chapter 17. Briefly, select the *Add Channel* button (Fig. 3.4). 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. We recommend starting with a low or moderate sampling rate (10 Hz).

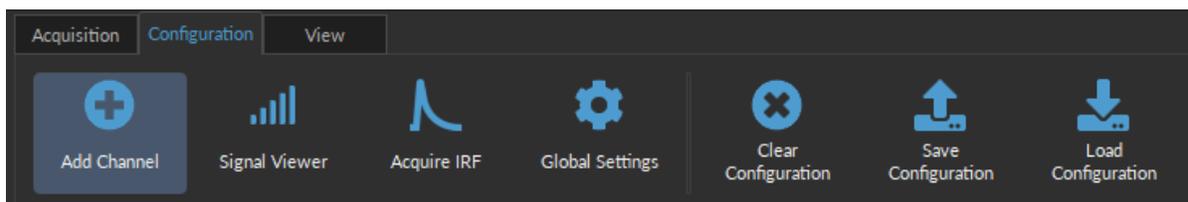


Figure 3.4: Add Channel to configure the *FluoPulse* system

3. Connect the fiber to the animal. Use isopropyl alcohol to clean the cannula and optic fiber connectors if dirty. If using MF1.25, MF1.25, ZF1.25 or ZF2.5 ferrule cannulas, make sure there is no gap between the cannula and patch cord within the sleeve.
4. Select the *Signal Viewer* button (Fig. 3.7). This view allows quick adjustment to optimize the laser attenuation and detector gain without having to take an IRF. It is very important to properly set the gain/laser power on the *FluoPulse™ cube* such that the amplitude waveform signal is within the optimal detector range.

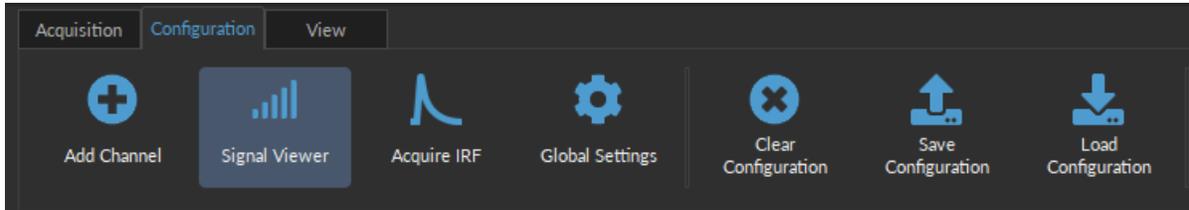


Figure 3.5: Use Signal Viewer to choose the right gain/filter

- **If this is the first time setting up and testing the system:**
 - Check what *Neutral Density (ND) filters* are used on the filter slot of the cube (see Fig 2.3). We recommend starting with the 100 % transmission (empty filter drawer) and only reducing the excitation laser transmission to 25 % and further to 10 % when indicated in the instructions below.
 - Set the detector gain to the lowest value. You will need to slowly increase it later on once the animal is connected. Generally, good biosensor expression results in gain settings within the second quarter gain range, as shown in Fig. 3.6. We recommend avoiding extreme ranges of the gain (first and last quarters).
 - Take a 'dummy' IRF, as described in Step 10-11. This will ensure you obtain a nice waveform in Step 5. You will redo the IRF properly later on.
- **If you're already established optimal cube settings:** keep the ND filter and detector gain as is. Only update the filter and detector gain when indicated in the instructions below.



Figure 3.6: Common detector gain range used in biological samples

5. Select the *Play* button (Fig. 3.7) to view the amplitude of the current signal arising from the animal.

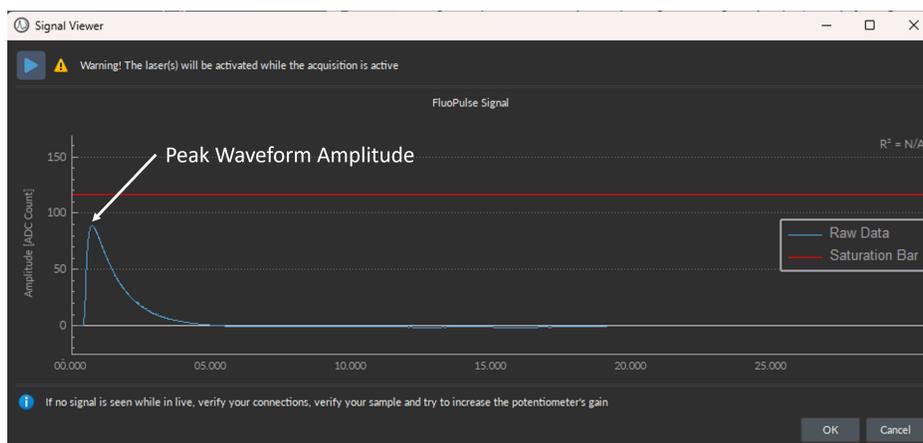


Figure 3.7: Signal Viewer - load current animal signal

6. Read the maximum value (i.e. amplitude) of the *Waveform Amplitude* graph. Fig. 3.7 display an example of optimal fluorescence lifetime waveform amplitude. **Values between 80-87 units are ideal** (Fig. 3.13), while values between 60-100 units are acceptable (Fig. 3.9). Picking the right amplitude for your experiment will depend on how you expect the amplitude (i.e. intensity) of the signal to change over time. **To start, choose a central value within the optimal range as a target amplitude**, such as 83 units.

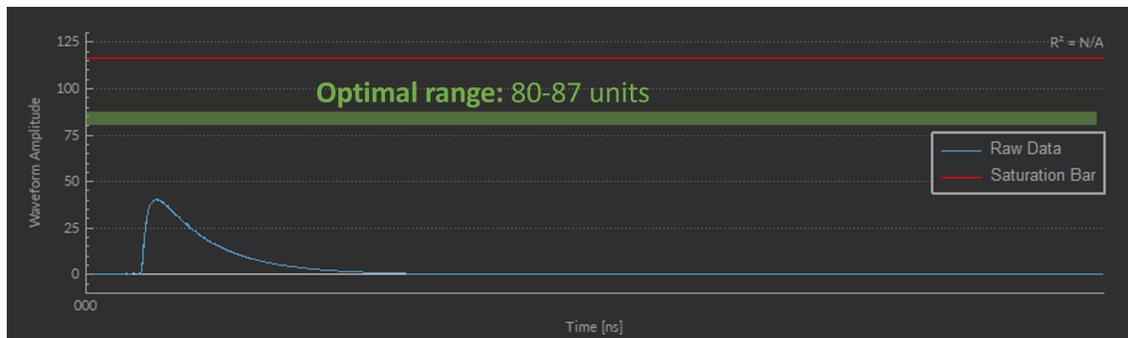
If the amplitude of the signal is expected to diminish (i.e. photobleaching was observed), higher amplitude values (87 - 100) are acceptable and will maintain the signal in the acceptable range for the entire recording.

If the biosensor intensity is expected to increase (as many lifetime biosensors also show increases in intensity), set the amplitude between 60 - 80 units to prevent detector saturation during the experiment.

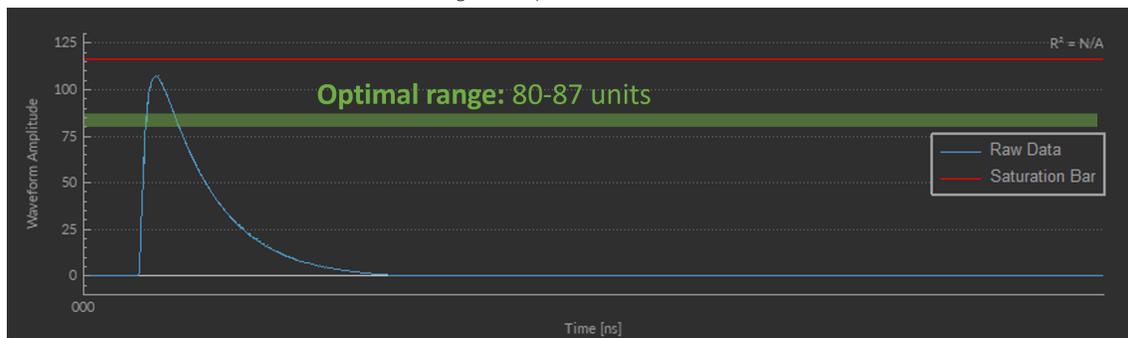
*****IMPORTANT!***** If you struggle to obtain a good waveform and/or you are getting error messages, check out **Chapter 5 for a list of troubleshooting strategies**.

7. Adjust the gain/filter settings to obtain you ideal/acceptable amplitude.

- If the waveform amplitude is **TOO LOW** (Fig. 3.8a), gently increase the gain using the *Detector Gain Knob* on the *FluoPulse™* cube (Fig 2.3), or change the ND filter to one with a higher transmission (25 % or 100 %), and take your measurement once more. Do not increase the gain above the middle of the scale before changing the ND filter – in other words, if the sample allows, it is better to generate more signal with a more powerful laser pulse than to just increase the gain. If the gain is already at the maximum value and the filter is already at 100%, check the fiber-optic patch cord / rotary joint / cannula connections, as poor connections reduce the transmission of the signal (see Fig. 3.2). If there are no connection issues, check the expression level of the biosensor and the location of the fiber in the brain ².
- If the waveform amplitude is **TOO HIGH** (Fig. 3.8b), lower the gain using the *Detector Gain Knob* on the *FluoPulse™* cube (Fig 2.3), and take your measurement once more. If the gain is already near the minimum, exchange the ND filter for one with a lower transmission to attenuate the laser power. If you are still saturating even with the 10 % transmission filter, this means that the biosensor expression level is too high and you should reduce the virus titer.



(a) Signal Amplitude is TOO LOW



(b) Signal Amplitude is TOO HIGH

Figure 3.8: Examples signal that require further adjustments

²The fiber tip should be extremely close to the labeled neurons, AT MOST 20-100 μm away

Repeat these steps until you reach a waveform with an amplitude in the Optimal/Acceptable Range (Fig 3.9).

Waveforms with amplitudes BELOW 60 units or ABOVE 100 units will provide INACCURATE lifetime measures.

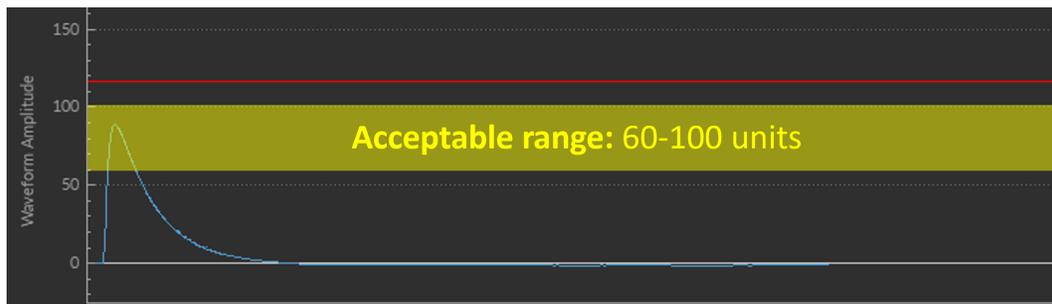


Figure 3.9: Acceptable Waveform Amplitude - animal signal

8. **Record the exact animal signal peak amplitude value**, then close *Signal Viewer*. This value will be important during the calibration steps coming up next. Note that if you are doing multi-day/multi-trial experiments with the same animal, it is important to use the same amplitude value from trial to trial. This means that it's okay if the gain and/or filter settings are different from day to day, so long as the amplitude signal is identical.

*****IMPORTANT***.** For valid cross-trial and cross-animal comparisons, keep the target animal signal waveform amplitude CONSISTENT between day/animal. *i.e. if Animal1-Amp-Day1 = 83 units, then Animal1-Amp-Day2 = ~ 83 units.* This ensures a consistent reference point.

9. Remove the fiber from the animal and without changing the laser and gain settings, and connect the fiber to the *FluoPulse Calibration Sample* (Fig. 3.10, step A). Lock the fiber in place using the screw (Fig. 3.10, step B). If it's the first time using the system, start with the *Knob* (Fig. 3.10, step D) at 5mm. When adjusting the *Knob*, remember to first loosen the knut (Fig. 3.10, step C). The 5mm position sets the fluorescent calibration sample in the middle of the range (with 0mm setting the sample as close to the patch cord as possible).

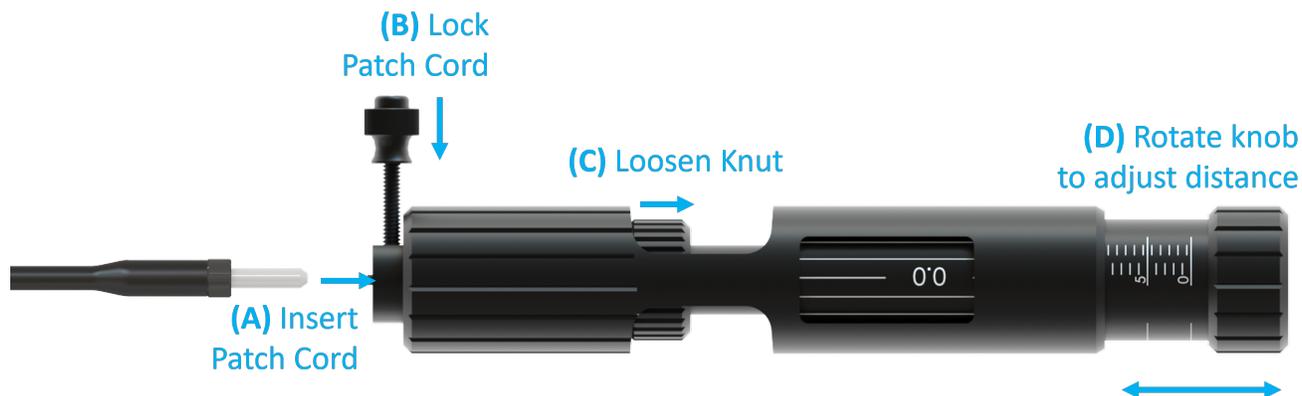


Figure 3.10: Use Calibration Sample as Reference for IRF

10. Select *Acquire IRF* button from the *Configuration Tab*, as in Fig. 3.11.
11. From the pop-up window, verify that *sample lifetime* is set to 4 ns (the known fluorescence lifetime of the calibration sample). This will serve as the reference point for all our measures.
12. Select the *Acquire IRF from device* button (Fig. 3.12) to begin calibrating the system (Fig. 5.4).

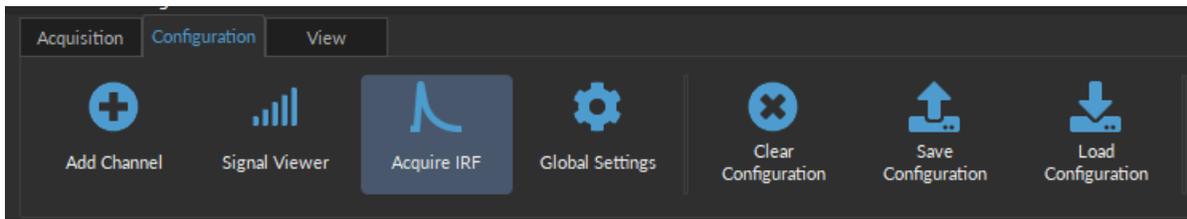


Figure 3.11: Acquire IRF button

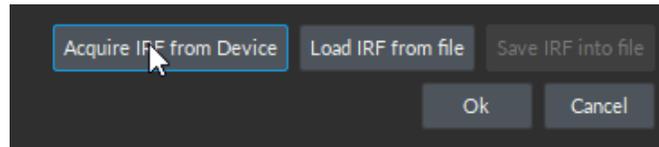


Figure 3.12: Acquire IRF from device

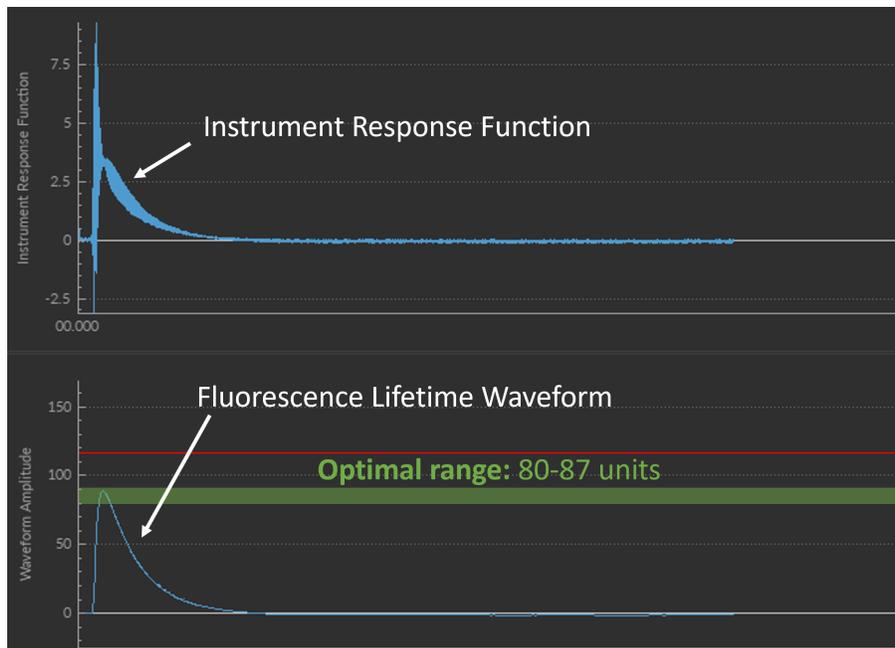
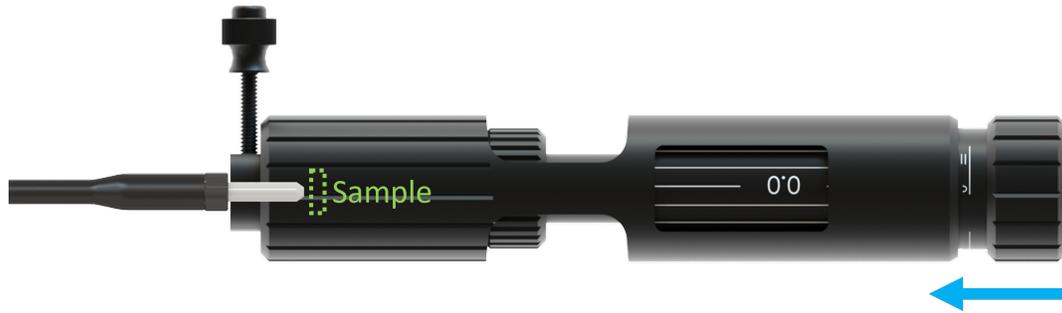


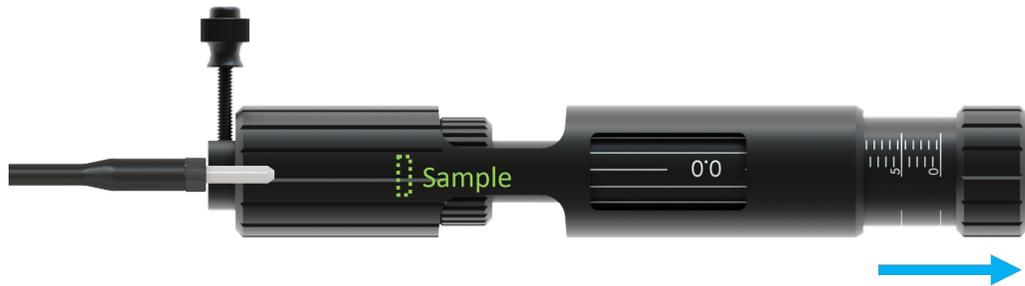
Figure 3.13: Optimal Waveform Amplitude

- **IMPORTANT:** The closer the amplitude of the calibration sample is to the amplitude of the animal signal, the more accurate the lifetime measure.
13. Adjust the Calibration sample by rotating the *Knob* (Figure 3.10, D) so that amplitude matches exactly the animal signal amplitude in Step 8. DO NOT change the gain and laser settings (already optimized for the experimental animal).
- If the standard sample waveform is too low (Fig. 3.8a), decrease the distance in mm, as in Fig. 3.14a.
 - If the standard sample waveform is too high (Fig. 3.8b), increase the distance in mm, as in Fig. 3.14b.

If you are having trouble acquiring a good IRF / Waveform amplitude and/or you are getting error messages, check out the **Chapter 5 for a list of common errors and troubleshooting strategies.**



(a) Increase amplitude: towards 0 mm



(b) Decrease amplitude: towards 10 mm

Figure 3.14: Adjust calibration sample to change signal amplitude during IRF

- Once you've obtained a waveform of an appropriate range then select *Ok* (Fig. 3.15). 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.

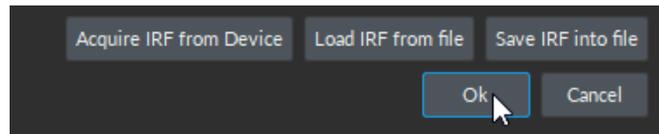


Figure 3.15: Select OK to save IRF

- We recommend recording the exact distance in mm and number of partial rotations *locking Knut*, to facilitate the next IRF at a later time.

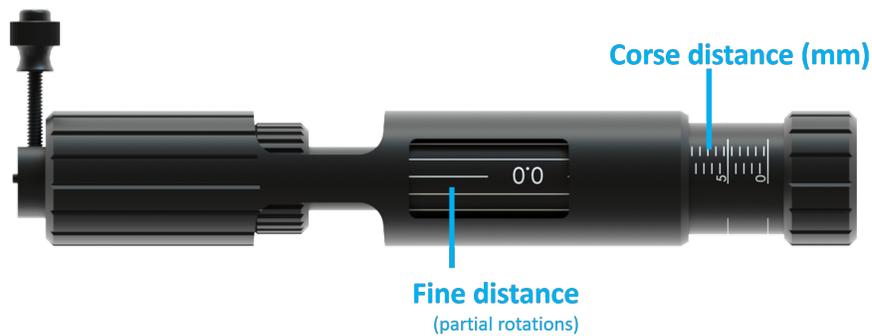


Figure 3.16: Record coarse and fine distance

*****IMPORTANT***:** Before each new recording, ALWAYS take a new IRF. Do NOT use an IRF saved on a previous day/animal, as it was likely obtained with different detector gain/filter settings.

16. With the established and saved IRF, reconnect it to the experimental animal.

DO NOT changing the gain and laser settings. You can now begin data acquisition on the animal using *Live* or *Record* buttons. Don't forget to specify the path where the data will be saved using the *Saving Options* button (under the *Acquisition Tab*).

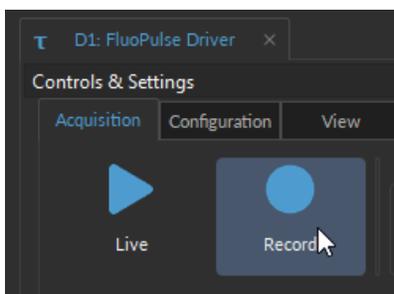


Figure 3.17: Start an experiment using Record button

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 4.1 depicts the signal processing from raw data to aligned and averaged fluorescence response.

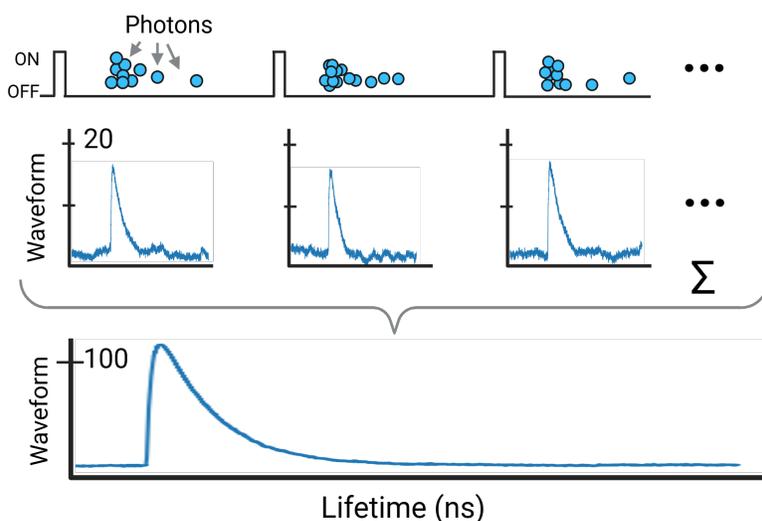


Figure 4.1: *Waveform Sampling process.*

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.

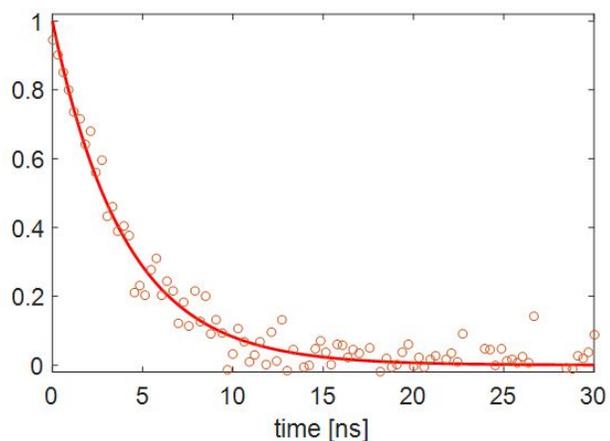


Figure 4.2: The waveform is deconvolved with the IRF. Least squares exponential fit can estimate the decay rate or lifetime.

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.

Troubleshooting and FAQ

This chapter describes common errors obtained with the FluoPulse™ system and recommend troubleshooting steps to resolve them.

5.1 ERROR: Unable to Acquire IRF

This message occurs when the detector is not receiving a strong enough signal:

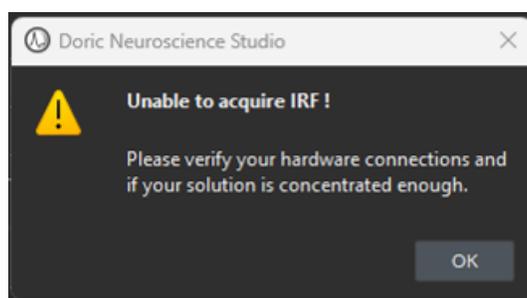


Figure 5.1: ERROR: Unable to Acquire IRF

SOLUTIONS:

For Standard Fluorescence Sample:

1. If you obtain this error when taking the IRF with a standard sample, change to a sample with a higher concentration. **DO NOT CHANGE THE DETECTOR GAIN OR FILTER**¹ (especially if you already set them for the animal signal).

For experimental animal:

1. Amplify the signal by gently increasing the detector gain. Rotate the knob by a small amount, then take a new measurement (Acquire IRF). Repeat this pattern (change detector and measure) until the error disappears. The gain detector knob is extremely sensitive, so small changes in the gain can have dramatic effects on the amplification.
 - Do not increase the gain above the middle of the scale before changing the ND filter – in other words, if the sample allows, it is better to generate more signal with a more powerful laser pulse than to just increase the gain. If the gain is already at the maximum value and the filter is already at 100%, check the expression level of the biosensor and the location of the fiber in the brain.
2. If the detector gain knob is at its maximum, increase the excitation power by changing the ND filter (either to 25 % or 100 % transmittance and return the detector to its minimum value. Repeat *Step 1* until you no longer receive the message.

¹You can TEMPORARILY change the filter to help estimate the necessary concentration change needed for the new standard solution, but you MUST return to the original filter when taking the IRF with the standard with new concentration.

5.2 IRF and/or Waveform with unusual shapes

When the detector is saturated, weird IRF and shapes can occur, as in Fig. 5.2:

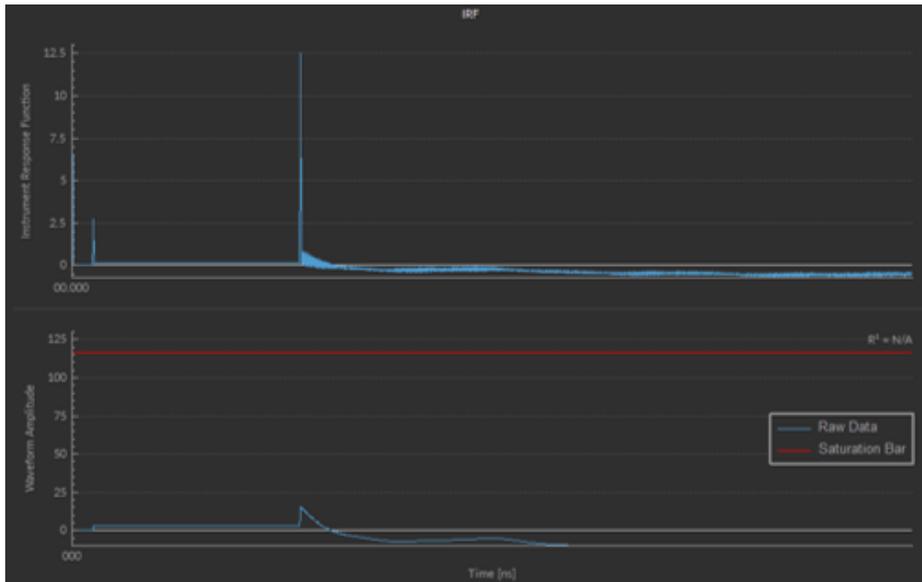


Figure 5.2: Saturated IRF / Waveform

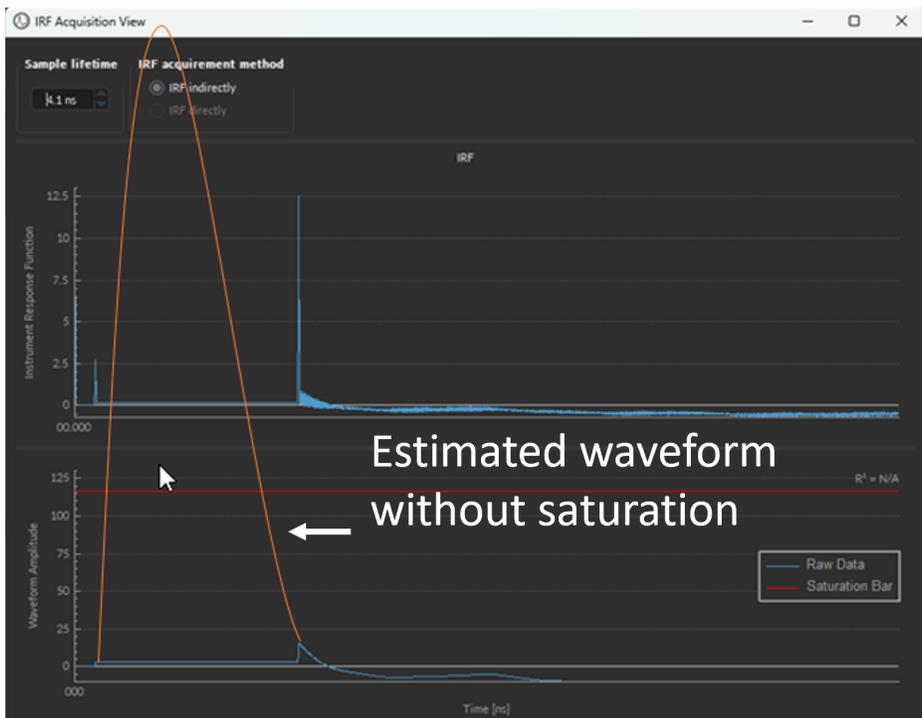
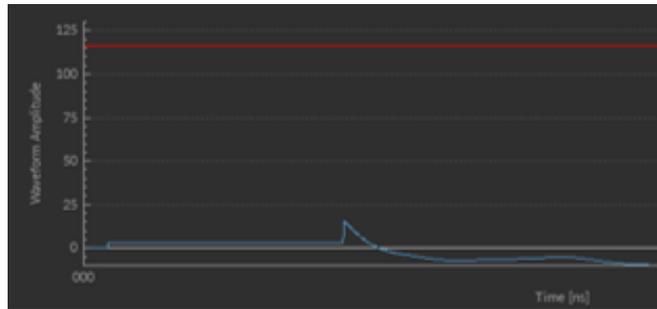


Figure 5.3: Saturated Waveform - with anotation

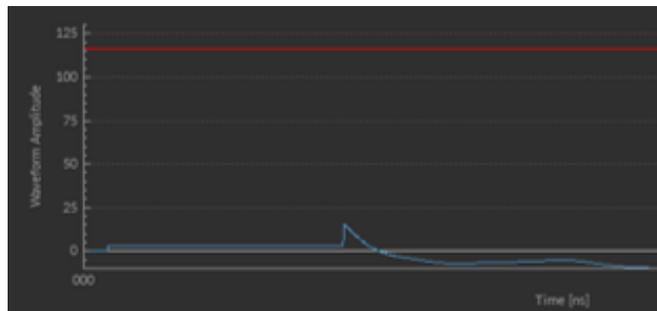
SOLUTION: See solution for detector saturation in Section 5.5.

5.3 Changing the Gain does not affect Waveform Amplitude

No matter whether the gain is at the minimum value or at its maximum, the amplitude of the waveform does not update. This issue is actually a **Detector Saturation** issue. Since the detector is already at maximum, any increase or decrease in the gain while it remains saturated will always result in a similar saturated curve.



(a) Low Gain



(b) High Gain

Figure 5.4: Waveform Amplitude

SOLUTION: See solution for detector saturation in Section 5.5.

5.4 Changes in Signal Amplitude do not match changes in Standard Concentration

When using a batch of standard samples at different concentrations to calibrate the system and the changes in waveform amplitude do not match changes in solution concentration. For example, the amplitude increases when moving to a lower concentration (likely the standard solution has dried on the tip of the fiber, biasing the measures).

SOLUTION:

1. Forgot to clean the fiber when switching to a new fluorescence sample. Use Isopropanol and a clean tissue to wipe the tip of the fiber. Make sure there is no residue still stuck on the fiber. Also, mix a fresh new batch of standard solutions, as the uncleaned fiber may have contaminated the solutions and changed their concentrations.
 - TIP: The best way to confirm that your fiber is clean is to take an IRF in air. If the IRF throws an error (as in Fig. 5.1), then that means that there is correctly no signal (i.e. no fluorescent residue) on the tip of the fiber and it is clean.
2. The detector is saturated: see Section 5.5.

5.5 Detector Saturation

The *FluoPulse*[™] detector is extremely sensitive, and the amplitude signal (intensity) is saturated at 116 ADC units. Lifetime measures will be inaccurate if the detector is saturated. This issue explains several different common issues when taking the IRF:

- IRF and/or Waveforms with unusual shapes (Section 5.2);
- Changing Gain does not affect Waveform Amplitude (Section 5.3);
- Changes in amplitude do not match changes in Sample concentration (Section 5.4)
- Changes in Tau Signal are much larger than the what is reported in litterature (Section 5.6)
- Lifetime Biosensor has large intensity changes (Section 5.8)

SOLUTIONS:

1. The detector is saturated: see Section 5.5.

For Standard Fluorescence Sample:

1. If you obtain this error when taking the IRF with a standard sample, change to a sample with a lower concentration. **DO NOT CHANGE THE DETECTOR GAIN OR FILTER** (especially if you already set them for the animal signal).

For experimental animal:

1. Reduce the signal amplification by gently decreasing the detector gain. Rotate the knob by a small amount, then take a new measurement (IRF). Repeat this pattern until the error disappears. The gain detector knob is extremely sensitive, so small changes in the gain can have dramatic effects on the amplification.
2. If the detector gain knob is at its minimum, decrease the excitation power by changing the ND filter (either to 25 % or 10 % transmittance. Repeat *Step 1* until you no longer receive the message.

5.6 Changes in Tau Signal are too large

Most lifetime biosensors show a change of lifetime between 100 ps - 1 ns. If changes in the tau signal greatly surpass what is reported in literature (as in Fig. 5.5), it is likely an artifact resulting from detector saturation. This can often occur for biosensors that also show a large change in intensity (signal amplitude).

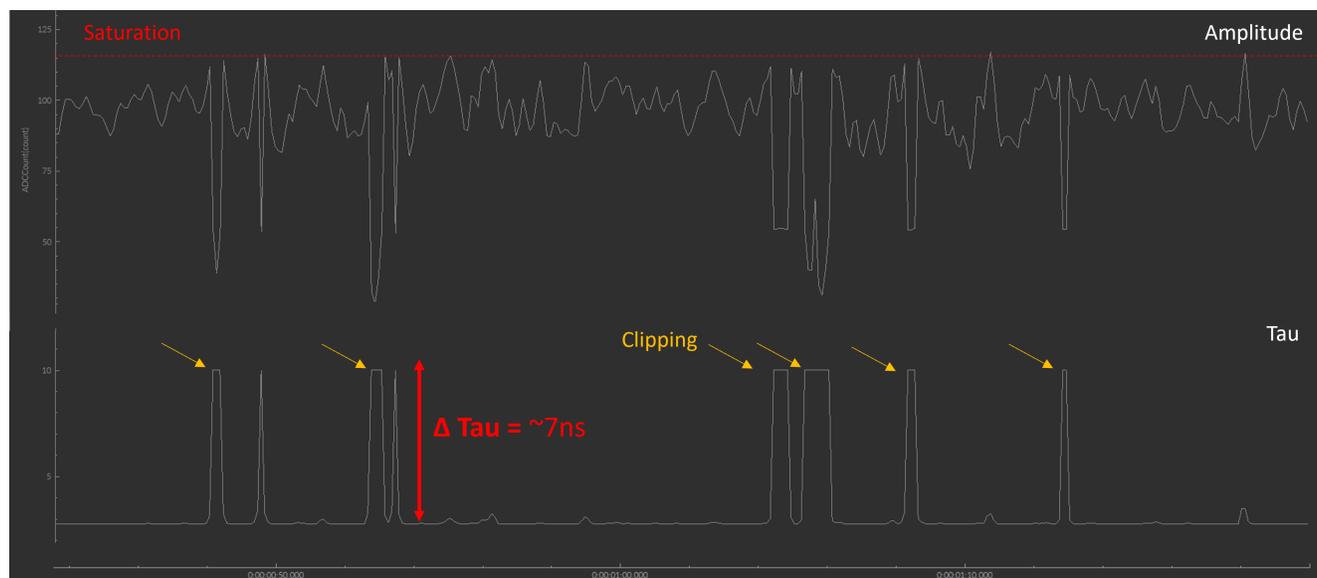


Figure 5.5: Example: Tau Signal is too large

SOLUTIONS:

1. The biosensor has a large amplitude change. See Section 5.8.
2. See solution for detector saturation in Section 5.5.

5.7 Tau Signal Clipping

The Tau signal will get clipped if the amplitude signal is too low or reaches saturation (116 ADC units). This type of effect also explains why changes in tau can be unrealistically large (as detailed in Section 5.6).

Note that if the signal exceeds 116 by a large amount, the amplitude will drop artificially and result in **negative spikes** that correspond to tau clipping.

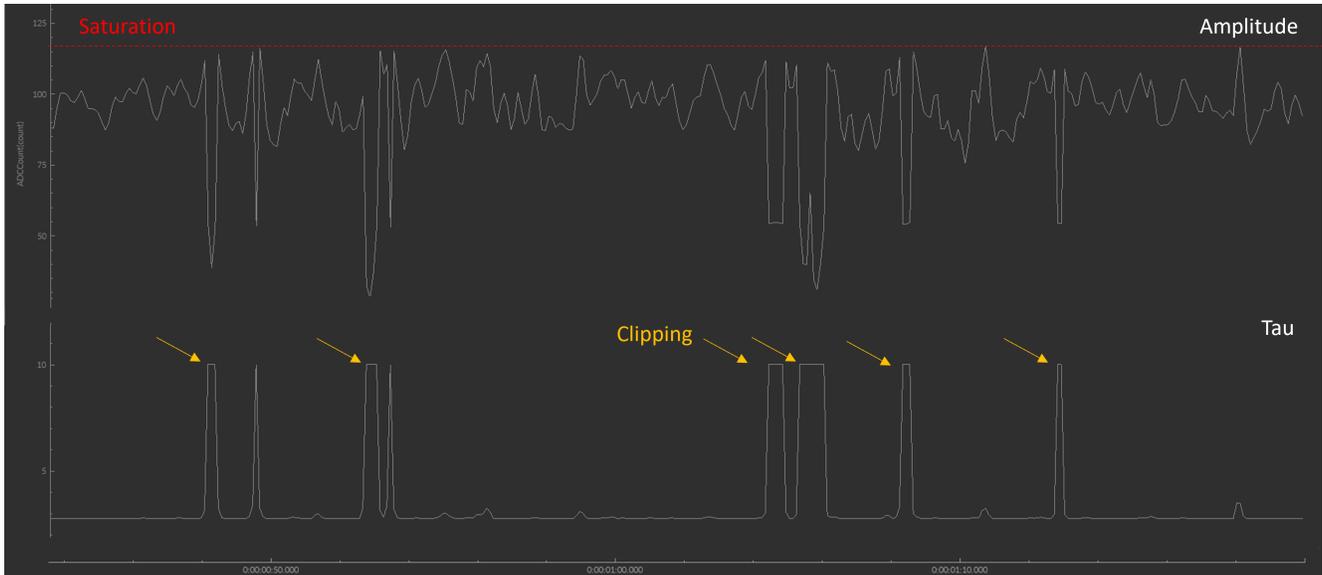


Figure 5.6: Example: Tau Signal Clipped

Sustained Tau signal clipping can remain even when the amplitude signal drops back under 116 ADC units. This type of artifact is the result of the saturation propagating artificially due to the live software algorithm that processes the data in real-time. This type of algorithm is extremely computationally intensive and is the primary bottleneck limiting the data acquisition sampling rate.

To accommodate high sampling rates, the real-time algorithm uses the previous waveform fit data to inform the current waveform fit, iteratively. If the previous waveform was saturated, the tau fit of that waveform will be inaccurate, and can 'contaminate' the following data points in the Tau Signal.

This sustained clipping does NOT affect the raw waveforms (Detector01) collected when the Amplitude Signal was no longer saturated.

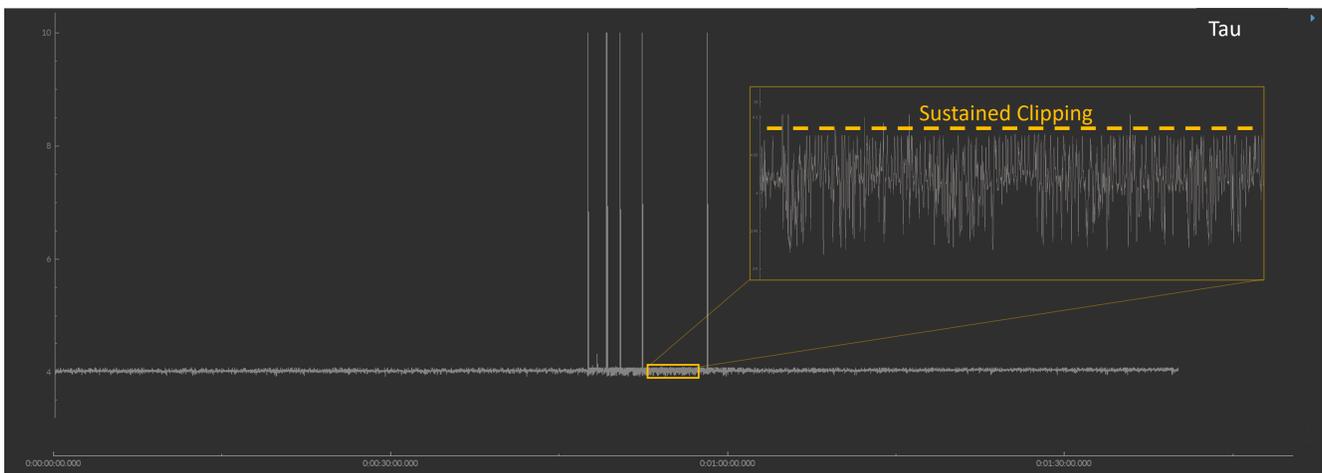


Figure 5.7: Example: Sustained Tau Signal Clipped

SOLUTION:Instantaneous & sustained clipping:

1. The biosensor has a large amplitude change. See Section 5.8
2. See solution for detector saturation in Section 5.5.

Sustained clipping only:

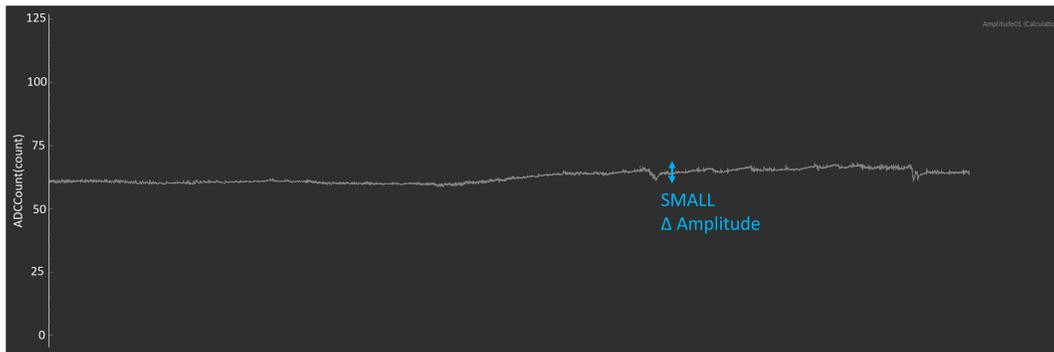
3. Re-process the data with a more thorough algorithm that calculates the lifetime of each waveform independently.
Note that re-processing will NOT recover data when the amplitude signal was truly saturated.

5.8 Biosensors has large amplitude changes

For optimal results, we recommend using biosensors with fluorescence intensity changes < factor 2. See below (Fig. 5.8 comparison between two sensors (dLight3.8 and glucose lifetime sensors) which show very different changes in intensity (i.e amplitude signal) over time.

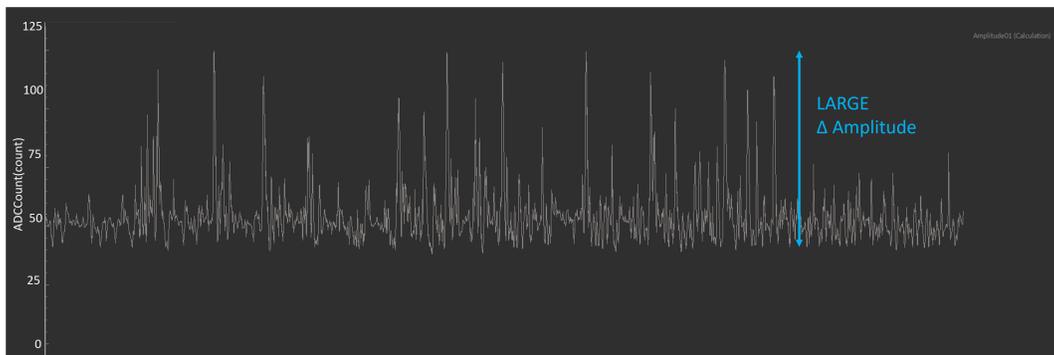
Large changes in intensity lead to inaccurate tau measures, which can either:

- Saturate the detector in response to stimuli/behavior (ACDCount > 116 units):
 - Results as *Tau Signal Clipping* artifacts (Section 5.7)
- Fall below the dynamic range of the detector (ACDCount < 50 units)
- Result in baseline signal too weak for a measurement (obtain error like Fig. 5.1)



(a) Unpublished glucose biosensor

Figure 5.8: Biosensor Comparison: Change in Amplitude Signal



(a) dLight3.8

SOLUTIONS:

1. Run some tests to get an idea of the minimum and maximum amplitude. Try to reduce the gain and adjust filter settings in the animal (as detailed in Section 5.5) to leave room for the expected large change in amplitude. Aim for setting the animal baseline amplitude around 60-80 units. Do NOT use values below 50 units.
IMPORTANT: Don't forget to calibrate with your standard sample once you've found ideal detector and filter settings.
2. Modify the experimental paradigm to give stimuli that result in a weaker signal response, or try using a lower titre of the virus.
3. If possible, switch to a lifetime biosensor with a smaller change in intensity.

5.9 Decreasing Tau Signals overtime

While the Amplitude signal will photobleach over time which can lead to exponential decay in the signal for long recordings, the tau signal should be much more stable over time. However, BOTH the tau and amplitude signals steadily decrease (as in Fig. 5.10) over time if the laser has NOT thermally stabilized.

Turning ON the *FluoPulse™* console and *FluoPulse™* cube is NOT SUFFICIENT to warm-up the system (and especially the laser).

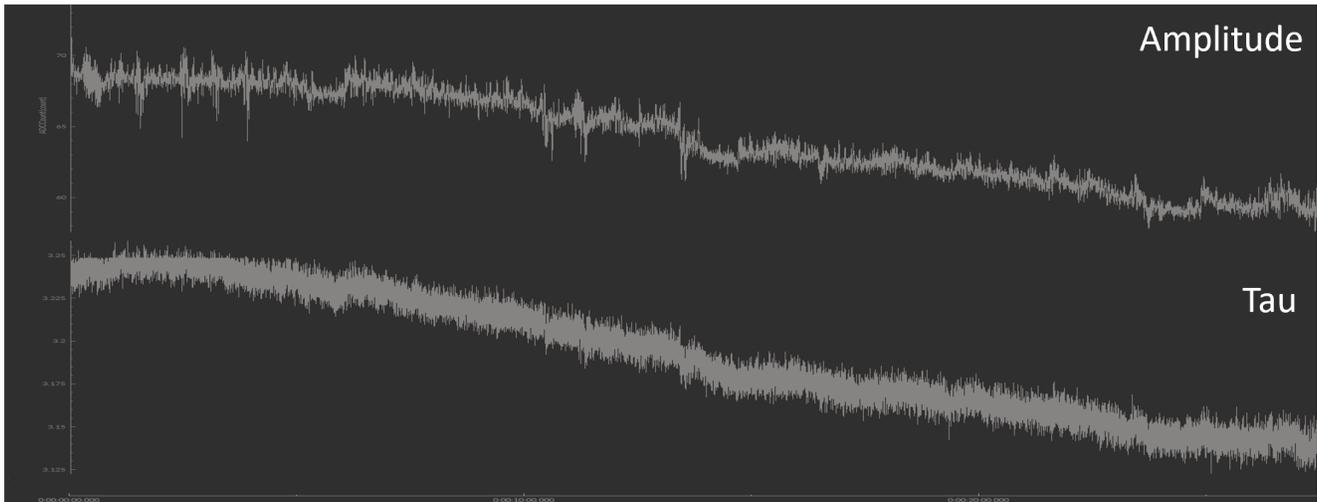


Figure 5.10: *Tau & Amplitude Signals BOTH show decreasing trend*

SOLUTION:

One hour before an experiment session, it is imperative to launch a mock acquisition using the *Live* mode (1 hour duration). Make sure to use the laser channel of the corresponding wavelength that will be used during the actual experiment (when creating your configuration).

FOR SAFETY: Cover the end of the fiber optic patch cord with a black cap, black tape, or other secure covering that cannot be easily removed.

5.10 Warning: Possible bad fit

Chi square is a measure of how well the exponential function fits the waveform trace. Any bad fit triggers a warning signal.

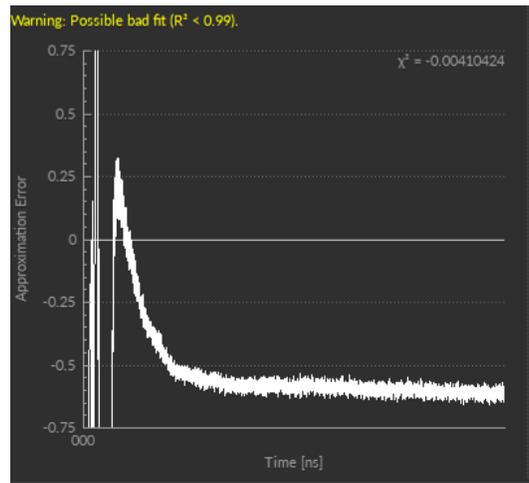


Figure 5.11: WARNING: Potential Bad Fit

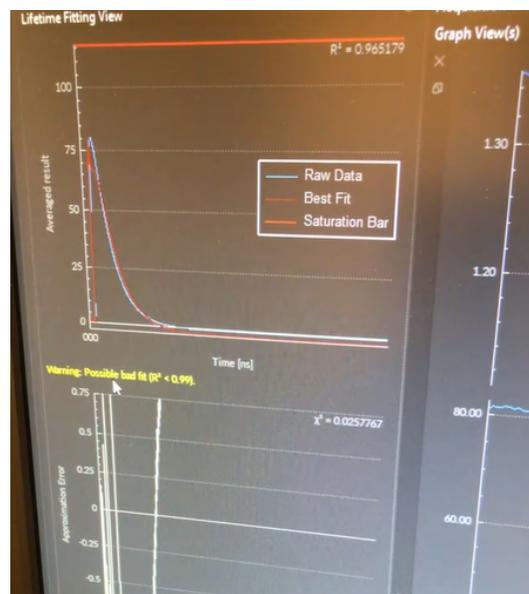


Figure 5.12: WARNING: Potential Bad Fit - Fitted and raw waveform do not match

SOLUTION:

1. If this occurs when setting us the parameters for the recording, simply take a new IRF to refresh the current condition of the system. If that does not solve the issue, check whether the signal amplitude is:
 - too low (amplitude under 60 units): Section 5.1;
 - too high/saturating (amplitude over 100 units): Section 5.5.
2. If you notice mid-recording, either:
 - Stop the recording, take a new IRF. This usually corrects the bad fit between the raw data and exponential decay fitting curve. Then restart experiment/recording.

- If the experiment cannot be restarted. Finish the recording. Then retake the IRF and save it in a different file using the *Save IRF into file* button (Fig. 5.13). If you are already mid-recording, don't panic! Since the software also saves the raw data containing all waveforms, we can **recalculate the lifetime using the new IRF during post-processing analysis**.

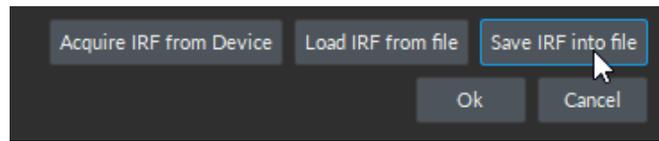


Figure 5.13: *Save IRF to file for post-processing*

NOTE: This only works if the amplitude signal was still within the acceptable range. If the signal is too low or saturating, re-analyzing with a new IRF will not be useful.

Specifications

Table 6.1: 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 Average Power (405nm/450nm/488nm)	40 / 30 / 20	μW
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 6.2: *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)

Support

7.1 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.

7.2 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](#).

7.3 Disposition



Figure 7.1: 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.

7.4 Contact us

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

Phone 1-418-877-5600

Email sales@doriclenses.com

doric

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