



Fiber Photometry Console

User Manual

Version 1.1.2

Contents

1 Overview	3
1.1 System Overview	3
1.2 Fiber Photometry Console	4
2 Operations Guide	6
2.1 Connecting the Fiber Photometry Console	6
3 Doric Neuroscience Studio	8
3.1 Photometry Console	8
3.2 Photometry Analyser	27
4 Specifications	31
5 Support	33
5.1 Important handling information	33
5.2 Maintenance	33
5.3 Warranty	33
5.4 Contact us	33

Overview

1.1 System Overview

In neuroscience, the fiber photometry denotes a method whereby chronically implanted optical fiber delivers excitation light to neurons tagged with a fluorescent calcium indicator(s) and collects their overall activity-induced fluorescence. Within the field of view, the fiber photometry sums up the activity-induced fluorescence of all neurons expressing the indicator(s).

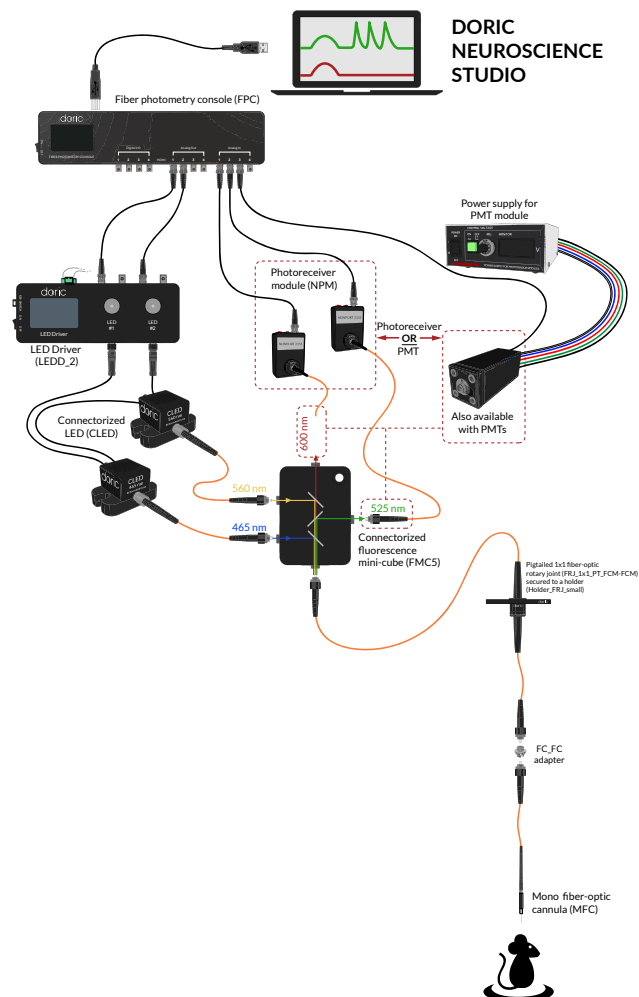


Figure 1.1: Typical Modular Fiber Photometry Measurement Setup

The typical set-up for freely behaving animals consists of the fiber photometry console, the excitation light source(s), a connectorized fluorescence mini cube with beam splitters that combine the excitation wavelengths and separate the fluorescence light, the fiber-optic rotary joint, the optical cannula with connecting fiber-optic patch cords and photodetectors capable of sensing the low level light. *In vitro* or head-fixed animal photometry setup uses a probe holder and an optical probe instead of the rotary joint and the optical cannula. The fluorescence emission can be collected with one photodiode and subsequently demodulated or, after spectral separation, collected with respective photodiodes.

1.2 Fiber Photometry Console

This FPGA based data acquisition unit synchronizes the output control and the acquisition of the input data. This device seamlessly integrates with Doric Neuroscience Studio software, which provides the user-interface for multi-channel photometry experiments.

The software interface enables control over the CW excitation light pulses, square or sinusoidal waveform of an external source (i.e. LED driver) with 4 digital input/outputs and 4 analog voltage outputs. The software interface displays real-time data of up to 4 detectors input signals and performs data acquisition. The other new functionalities are being developed and users can upload them as they become available.

The Fiber Photometry Console inputs and outputs are shown in figures 1.2 and 1.3.

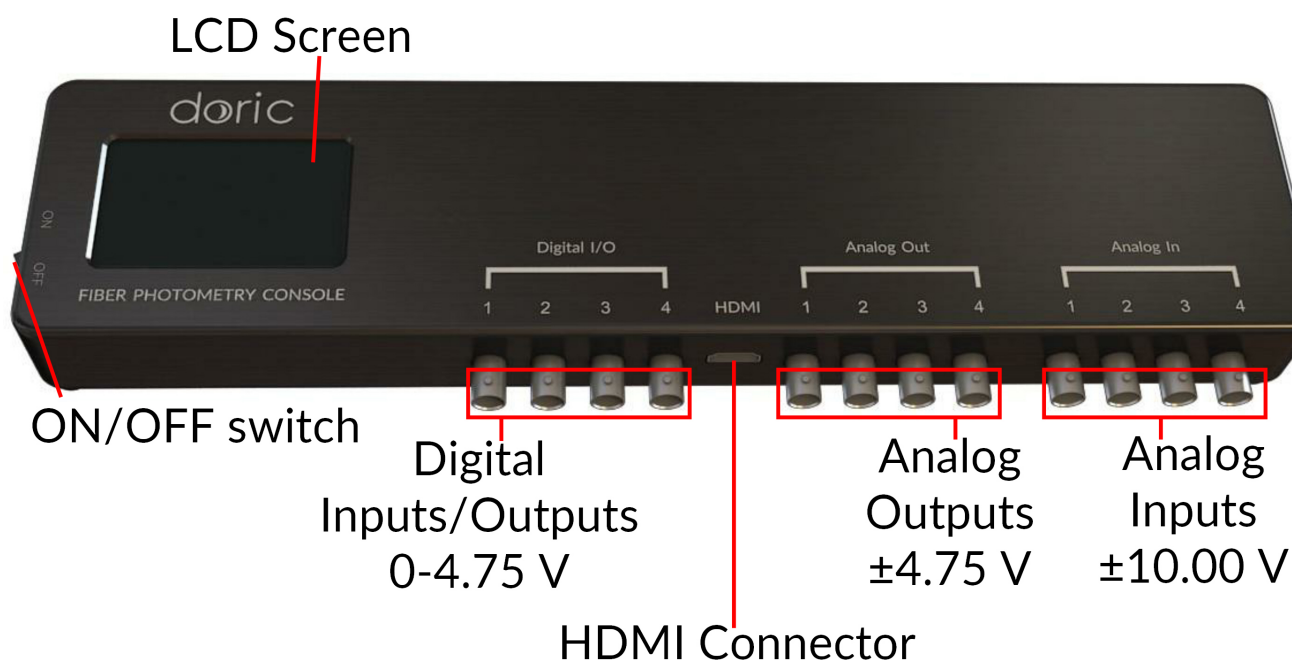


Figure 1.2: Front view of the Fiber Photometry Console: Inputs and outputs

- The **LCD Screen** displays console information.
- The **Digital/IO** ports sends 0-4.75 V TTL pulses.
- The **HDMI** port acquires digital signals and digital communication SPI and LVDS via a custom pinout HDMI connector.
- The **Analog-out** ports send a variable ± 4.75 V analog signal signal.
- The **Analog-in** ports acquire analog signals up to ± 10 V.
- The **Power On/Off** opens and closes the device.



Figure 1.3: Back view: Maintenance and Power Supply

- The **12V port** connects to the 12 VDC Power supply.
- The **USB port** allows a USB-B connection to a computer.
- The **Service** port is a USB-B port through which the firmware of the device can be updated.
- The **USB-3** charging port is currently disabled.

Operations Guide

2.1 Connecting the Fiber Photometry Console

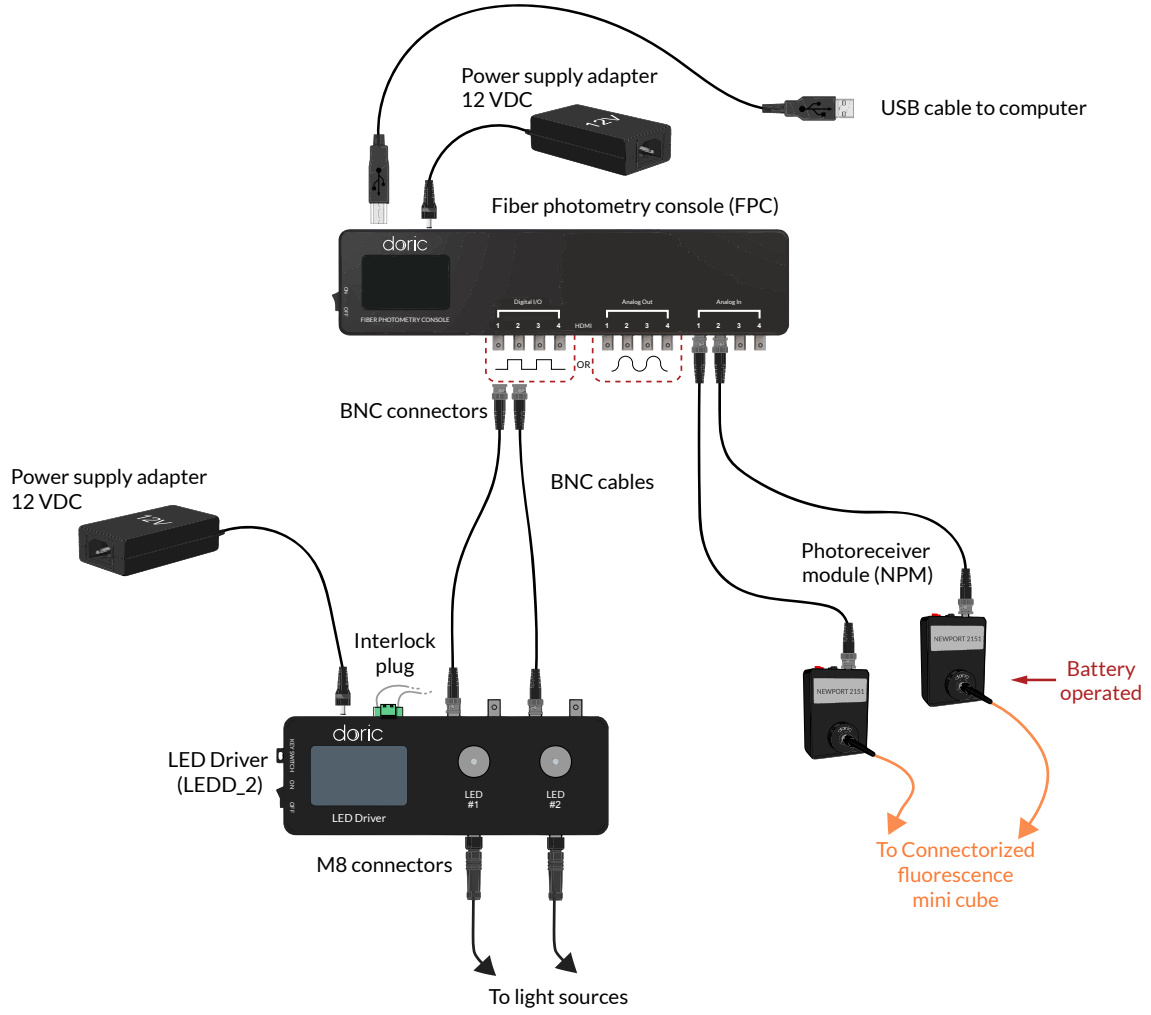


Figure 2.1: Connections to the Fiber Photometry Console

Follow this quick start procedure to install and connect the system. We recommend the following order to avoid device and driver detection problems. If you need more information about each device or the software, refer to their respective user manuals.

1. **Install Doric Neuroscience Studio Software.** Follow the on-screen instructions to install the Doric Neuroscience Studio Software on the hard drive of your computer. For more details, refer to the Doric Neuroscience Studio Software User Manual.
2. **Connect the Fiber Photometry Console.** The console unit is operated with a 12 VDC power supply adapter. It is important to disconnect all other elements from the inputs/outputs before turning on the console. When the console is powered, turn on the switch, then connect the console to the computer via a USB cable. It is important to **power on the console before connecting the USB cable**. Failure to do so can lead to an unstable state in which the console draws power from the USB cable instead of the power adapter. A Windows USB driver will be automatically installed. Open the software to complete the installation of others devices.
3. **Connect outputs.** Digital outputs can send TTL pulses (0 - 4.75 V square pulses) while analog outputs can send a sinusoidal signal. Connect devices to Digital I/O or Analog Out of the *Fiber Photometry Console* with a BNC cable (Fig. 2.1).
4. **Connect inputs.** Digital inputs receive TTL pulses (0 - 5 V square pulses) while analog inputs receive analog signal (± 10 V signals) that can be displayed and saved using the *Doric Neuroscience Studio*.

Doric Neuroscience Studio

3.1 Photometry Console

The Fiber Photometry Console module controls the *Fiber Photometry Console*, an FPGA based data acquisition unit which synchronizes the output control and the input data of the acquisition. The photometry-oriented interface provides different functionalities for multi-channel experiments. It enables control over the excitation light pulses, or the sinusoidal waveform trig of an external source (i.e. Doric LED driver) with 4 **Digital input/outputs** and 4 **Analog outputs**, which allow the creation of pulse sequences. The module interface displays real-time recordings of up to 4 input signals and performs basic signal processing. The system is controlled using 3 **Control and Settings** tabs. Separate channel windows are used to define output/input specifications.

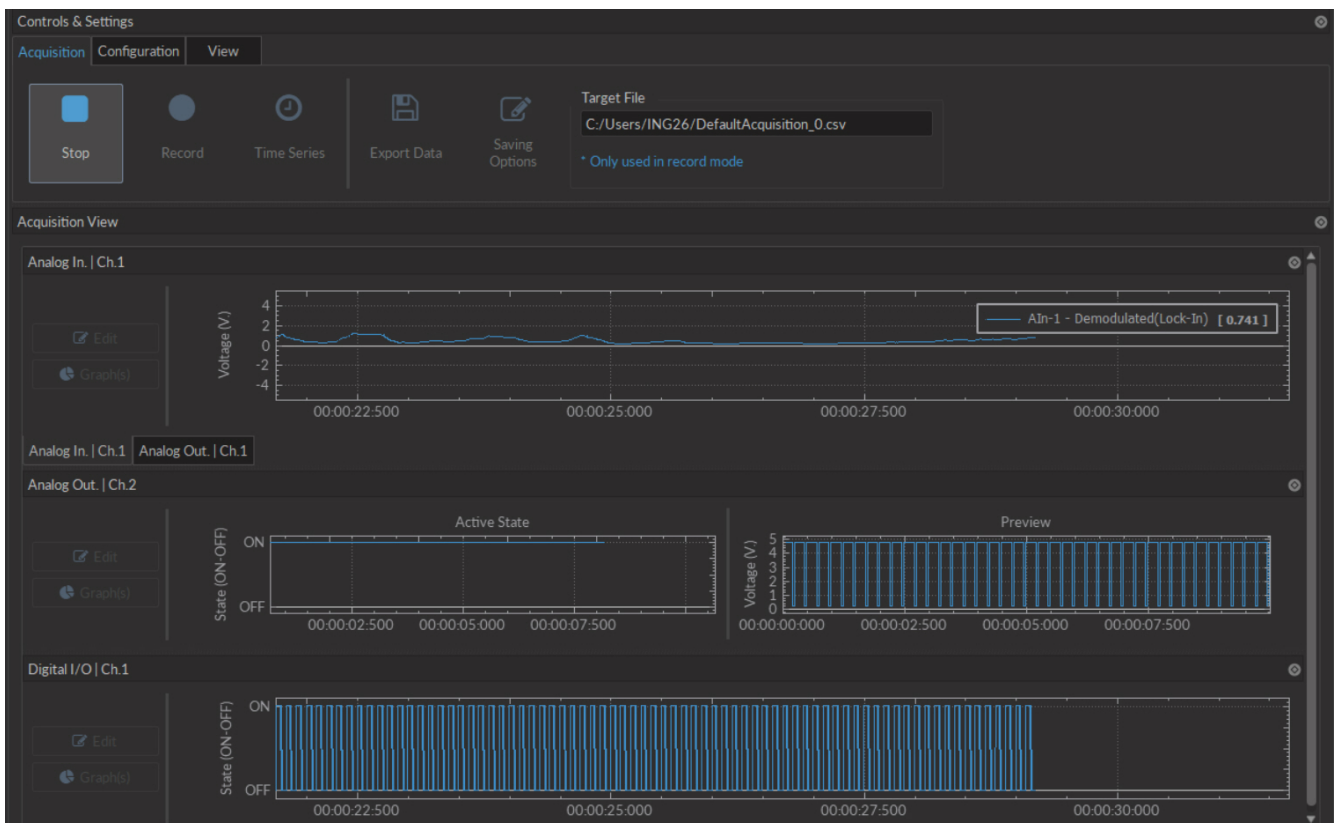


Figure 3.1: Console User Interface

3.1.1 Channels

The console has three different types of channels. The **Digital I/O** (Section 3.1.1) channels allow the input and output of TTL signals. The **Analog Output** (Section 3.1.1) channels allow the output of analog signals. The **Analog Input** (Section 3.1.1) channels allow the input of analog signals. Any number of channels may be added using the **Add channel** button on the **Configuration** tab (Section 3.1.2). The **Channel(s) configuration** window contains the following elements shared by most channel types.

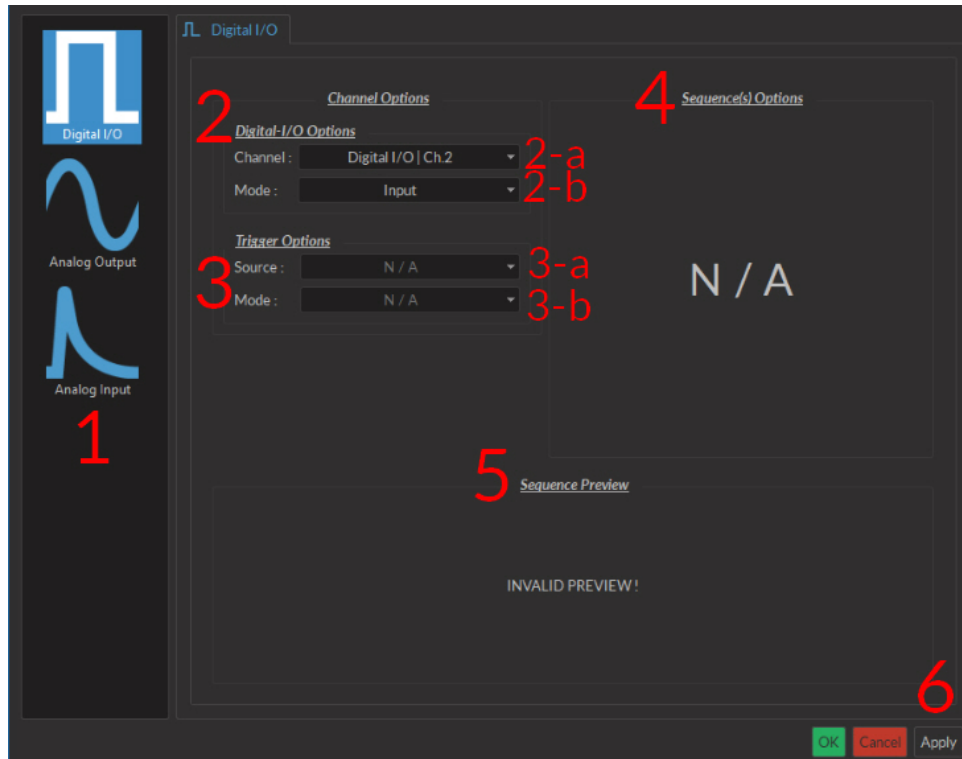


Figure 3.2: Channel(s) configuration window, Digital I/O input

1. The **Channel Types** (Fig. 3.2) are selected in the box on the left side of the window. Selecting the **Digital I/O**, **Analog-Out** or **Analog Input** icons will display the parameters to each respective **Channel type** on the right side of the window.
2. The **Channel Options** (Fig. 3.2) include the **Channel** drop-down list and the channel **Mode** list.
 - a) The **Channel** identifies which of the 4 channels available for each channel type is currently being modified. The channel can be changed by selecting a new one from the drop-down list.
 - b) The **Mode** identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). The specifics of these choices are defined in the section for each channel type.
3. The **Trigger Options** (Fig. 3.2) define the trigger methods. These options include the trigger **Source** and **Mode**.
 - a) The **Source** trigger option allows the choice of a **Manual Trigger** (activated by a user) or an **Input** trigger, coming from an input on a **Digital I/O** channel.
 - b) The **Mode** defines how the trigger activates a sequence. This includes input sequences, which can be triggered/gated by an outside source.
 - In **Triggered** mode, the sequence is started manually or by a trigger source from another digital input channel. Once the trigger source is received, the sequence will continue until the end or until **Stop** is pressed.
 - In **Gated mode**, the sequence will play as long as there is a high TTL signal (4 V or more) on the input modulation BNC. This signal comes from a different light source or device driver. When the TTL signal is

low (0.4 V or less), the sequence stops and waits for another high TTL signal to continue. This mode will cut pulses, with a new pulse restarting once the high signal returns.

- The **Sequence Options** (Fig. 3.2) define the parameters of each pulse sequence for output channels. These parameters are defined with each channel type. Should a parameter chosen be impossible to apply to a sequence (For example, a **Time ON** greater than $1/\text{Frequency}$), the color of the option boxes will turn **RED**.
- The **Sequence Preview** (Fig. 3.2) section allows visualization of the output sequence that will play by selecting the channel in the graph.
- The **Apply** button (Fig. 3.2) will generate the defined channel OR update an already configured channel with any changes.

Digital I/O Channels

With the **Digital I/O** channels, each digital channel can be configured as an output or an input and create TTL (On/Off) pulse sequences. Each numbered channel corresponds to the same number digital channel on the console. Pulse sequences have different parameters depending on the **Channel Mode**, which can be **Continuous** or **Square** for output sequences and **Input** for input signals. The **Channels Configuration** window contains the following elements for a Digital I/O channel.

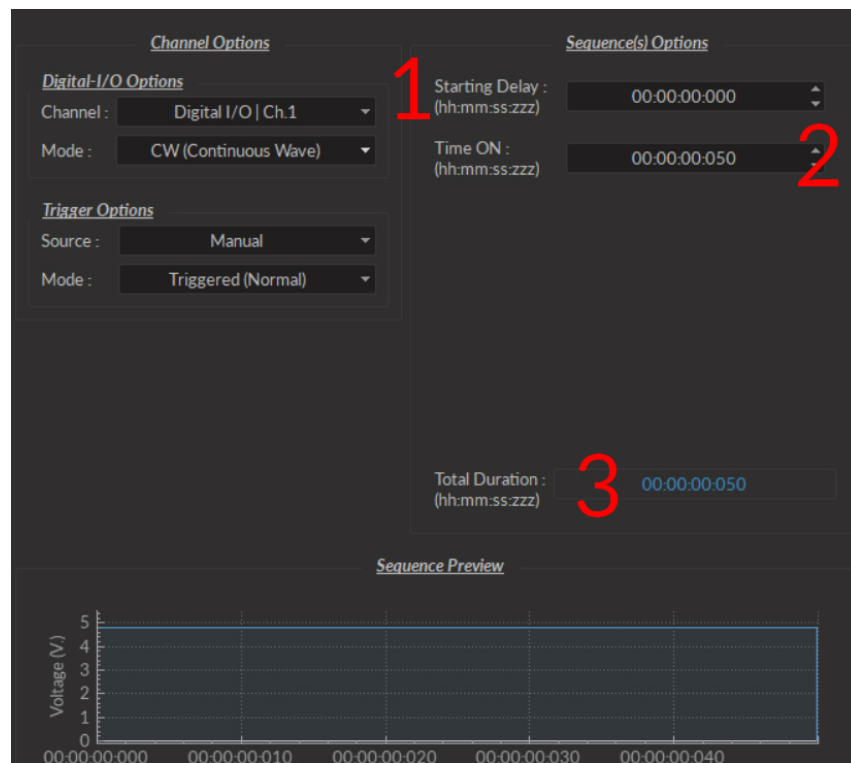


Figure 3.3: Channel(s) configuration window, Digital I/O CW

- The **CW(Continuous Wave)** channel mode (Fig. 3.3) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.
 - The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the signal.
 - The **Time ON** defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse sequence is stopped manually.
 - The **Total Duration** shows the total expected duration of the pulse sequence. Should the duration be infinite, the box will display ∞ . If there is an error in parameter selection, this box will display **N/A**.

- The **Square** channel mode (Fig. 3.4) allows the creation of a square TTL pulse sequence. This includes all sequence options as the **CW** mode, with the following additions.
 1. The **Frequency** sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period**. For example, a signal at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a signal at 0.5 Hz (frequency) will output one pulse every 2 seconds (period).
 2. The **Time ON** defines the length of a single pulse. This time can also be converted to a **Duty Cycle**, which indicates the % of the period the pulse duration corresponds to.
 3. The **Pulse(s) per sequence** set the number of pulses per sequence. If it is set to 0, the number of pulses will be infinite.
 4. The **Number of sequence(s)** sets the number of times that the sequence will be repeated.
 5. The **Delay between sequences** sets the delay between each sequence.
- The **Input** mode (Fig 3.2) receives a signal that is translated to 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the console. No **Sequence Options** or **Sequence Previews** are available for this mode.

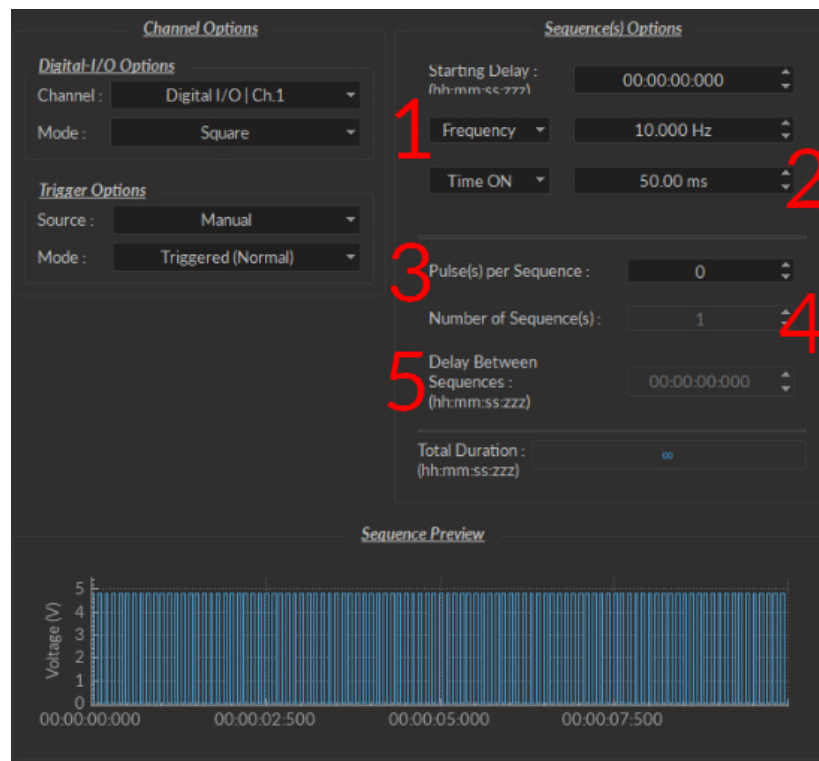


Figure 3.4: Channel(s) configuration window, Digital I/O square

Analog Output

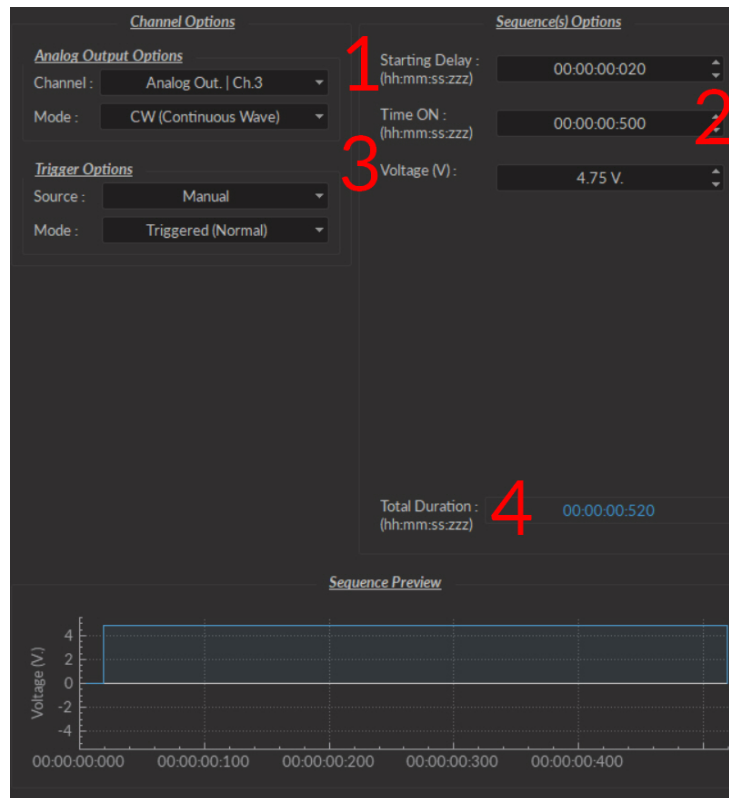


Figure 3.5: Channel(s) configuration window, Analog Output CW

The **Analog Output** channel type creates analog pulse sequences. Each numbered channel corresponds to the same number analog channel on the console. Pulse sequences have different parameters depending on the channel **Mode**, which can be **Continuous**, **Square** or **Sine**. The **Channels Configuration** window contains the following elements for an analog channel.

- The **CW (Continuous wave)** channel mode (Fig. 3.5) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box.
 1. The **Starting Delay** (Fig. 3.5) defines the time between the activation of the pulse sequence and the beginning of the signal.
 2. The **Time ON** (Fig. 3.5) defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse sequence is stopped manually.
 3. The **Voltage** (Fig. 3.5) defines the voltage of the continuous signal, in volts. The signal cannot go beyond ± 4.75 V.
 4. The **Total Duration** (Fig. 3.5) shows the total expected duration of the pulse sequence. Should the duration be infinite, the box will display ∞ . If there is an error in parameter selection, this box will display **N/A**.

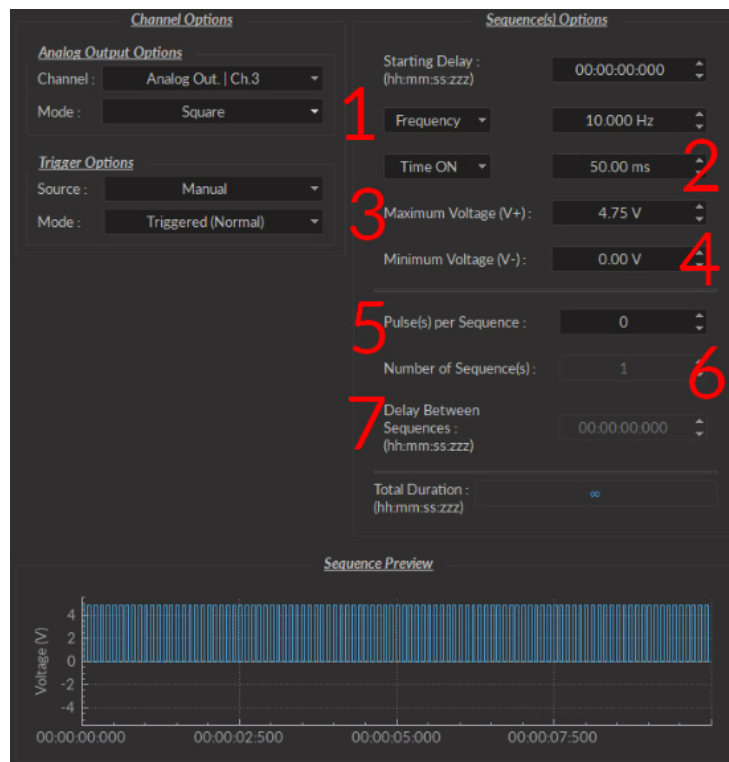


Figure 3.6: Channel(s) configuration window, Analog Output Square

- The **Square** channel mode (Fig. 3.6) creates a sequence of pulses with the minimum of the pulses at **V-** and the maximum of each pulse at **V+**. This includes all sequence options as the **CW** mode, with the following additions.
 1. The **Frequency** (Fig. 3.6) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period**. For example, a signal at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a signal at 0.5 Hz (frequency) will output one pulse every 2 seconds (period).
 2. The **Time ON** (Fig. 3.6) defines the length of a single pulse. This time can also be converted to a **Duty Cycle**, which indicates the % of the period the pulse duration corresponds to.
 3. The **Maximum Voltage (V+)** (Fig. 3.6) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.
 4. The **Minimum Voltage (V-)** (Fig. 3.6) defines the minimum voltage of each pulse, in volts. The signal cannot go beyond -4.75 V.
 5. The **Pulse(s) per sequence** (Fig. 3.6) set the number of pulses per sequence. If it is set to 0, the number of pulses will be infinite.
 6. The **Number of sequence(s)**(Fig. 3.6) sets the number of times that the sequence will be repeated.
 7. The **Delay between sequences** (Fig. 3.6) sets the delay between each sequence.

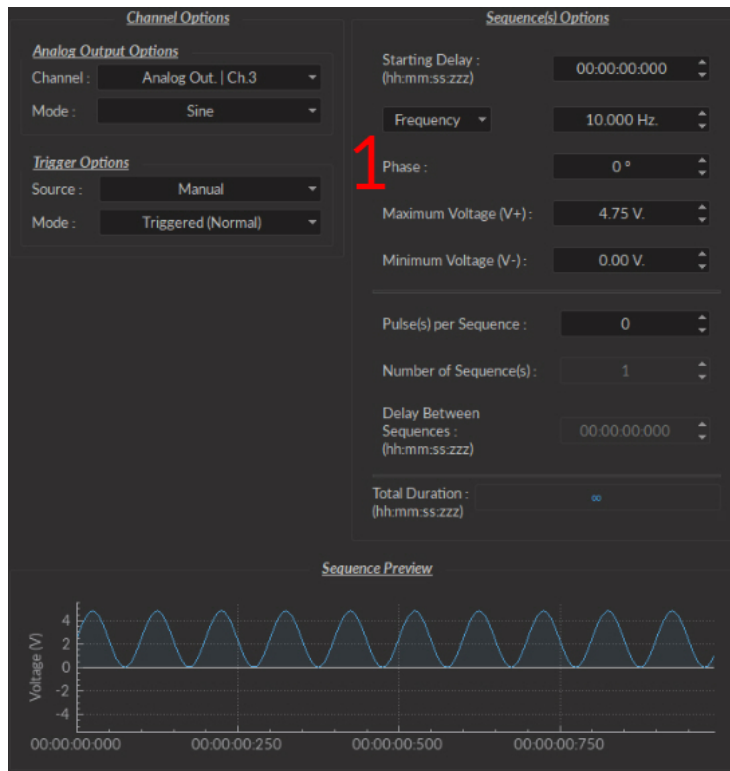


Figure 3.7: Channel(s) configuration window, Analog Output Sine

- The **Sine** mode (Fig. 3.7) creates a sinusoidal pulse sequence with peaks at **V+** and **V-**. This includes all sequence options as the **CW** and **Square** mode, save the following.
 - The **Time ON** option is changed for the **Phase** option (Fig. 3.7). This allows the choice of the sine wave phase, in degrees.

Analog Input

The **Analog Input** channels (Fig. 3.8) acquire from the **Analog Input** BNC connector ports. For these channels, **Sequence Options** are replaced by **Global Options** concerning the acquisition of data. These **Global Options** are applied to all **Analog input** channels. They use different **Channel Modes** unique to the **Analog Input** channels.

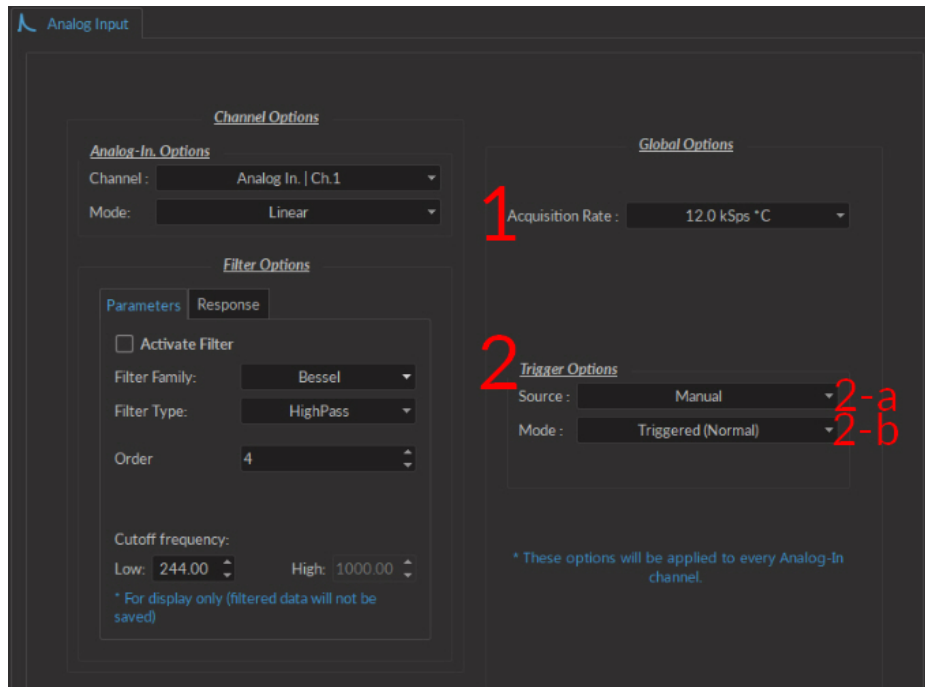


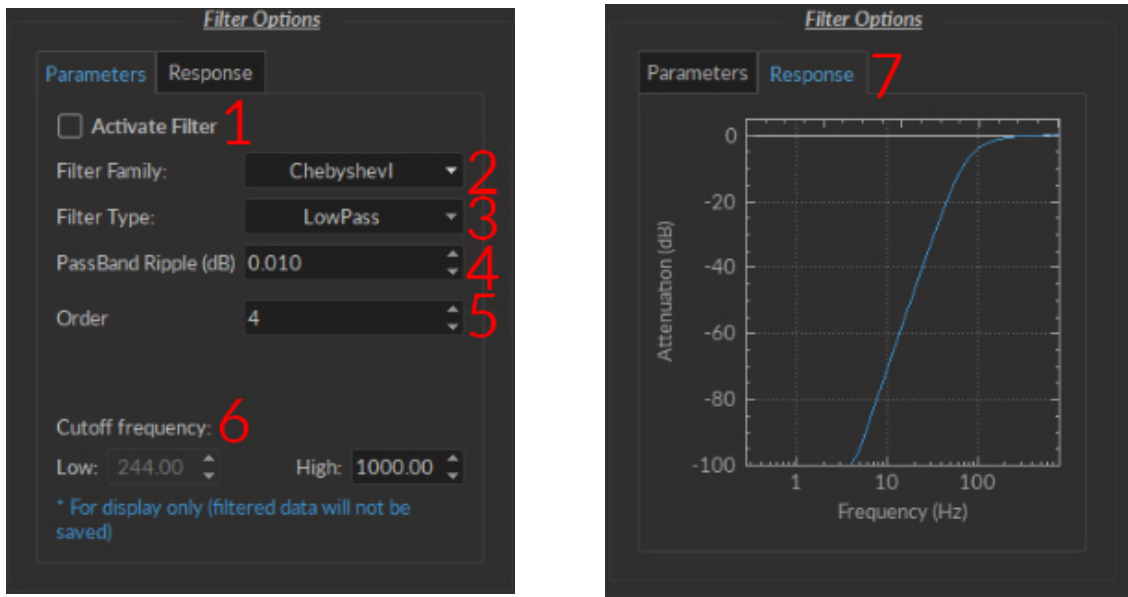
Figure 3.8: Channel(s) configuration window, Analog Input Linear

There are two **Global Options** available to all **Analog-in Modes**.

1. The **Acquisition Rate** (Fig. 3.8) is measured in kilosamples per second. A rate of 1 kSps will acquire 1,000 samples per second per channel. During an acquisition, a maximal amount of $3.6 * \text{rate} [\text{in kSps}] \text{ MB/min}$ of data are created.
2. The **Trigger Options** defines the trigger source and mode.
 - a) The **Trigger Source** is either **Manual** or a **Digital I/O** channels.
 - b) The **Trigger Mode** is either **Triggered** or **Gated**. **Gated** mode is only available when the trigger source isn't **Manual**.

Each **Analog-In Mode** has a specific set of parameters. The function of each **Mode** is described here.

- The **Linear** channel mode (Fig. 3.8) allows the direct measurement of signal received by a channel. The linear mode has the option of a frequency filter, found in the **Filter Options** box.
 1. When the **Activate Filter** checkbox is selected, the defined filter is applied on all input data and displayed on a new trace. The filtered data is for display only, and will not be saved.
 2. The **Filter Family** drop-down list is used to choose the filter type. These include **Bessel**, **Butterworth** and **Chebyshev (I ad II)** filters.
 3. The **Filter Type** drop-down list allows the choice of a filter type from **High-Pass**, **Low-Pass**, **Band-Pass** and **Band-Stop**.
 4. The **Ripples** box is only available for **Chebyshev** filters. The ripple (either in the passband, or in the stopband) defines the acceptable filter response ripples. The higher the ripples, the sharper the filter response.
 5. The **Order** selector is linked to filter sharpness; a higher order will provide a sharper filter.
 6. The **Cutoff Frequency** boxes are used to define the low/high cutoff values for the filter, depending on the type used. The cutoff frequency must be less than half of the sampling rate.



(a) Filter Tab

(b) Response Tab

Figure 3.9: Linear Mode Filter Options

7. The **Response Tab** displays the filter response (attenuation) according to frequency.

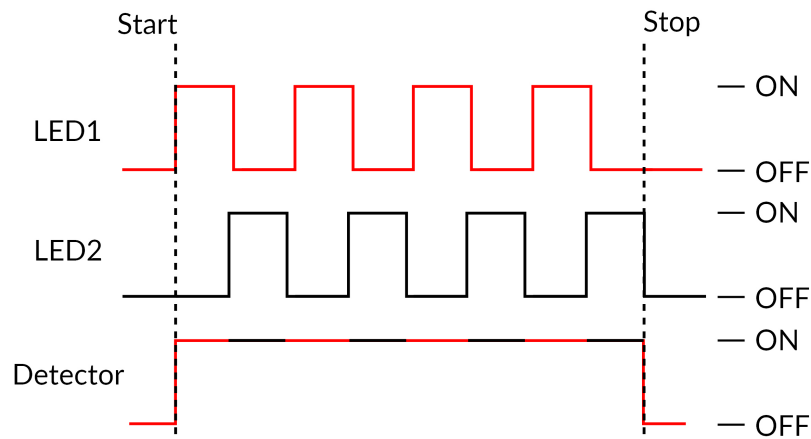


Figure 3.10: Interleaved Acquisition Timing Diagram

- The **Interleaved** channel mode allows 2 channels to send an alternating pulsed signal of opposite phase for two separate light sources. Each source can excite a different fluorophore, which allows the detection of two separate fluorescence signals coming from the same sample using a single channel (Fig. 3.10). A more detailed procedure to use this modality is included in section 3.1.3.

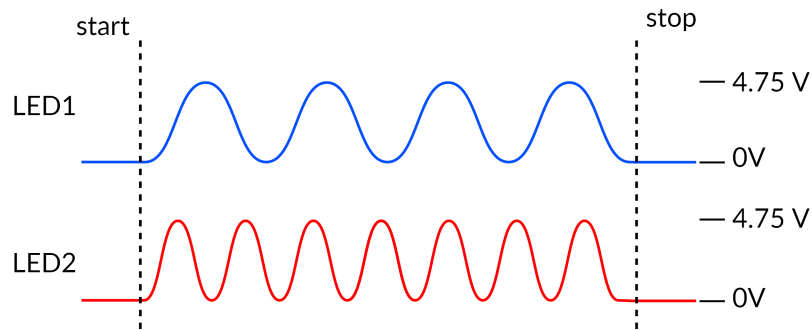


Figure 3.11: Lock-in Acquisition Timing Diagram

- When using the **Lock-In** mode, the input will only record signals at a given reference frequency, filtered by a low-pass filter. This allows the detection of multiple different signals if each is at its own reference frequency (Fig. 3.11). A more detailed procedure to use this modality is included in section 3.1.3.

3.1.2 Control and Settings tabs

The three **Control and settings tabs** are used to manage the different parts of the software. There are three tabs, **Acquisition**, **Configuration** and **View**.

Acquisition Tab

The **Acquisition** tab allows the activation of pulse and recording sequences. Three **Live**, **Record** and **Time Series** buttons are used for activation and will not function if no channels are configured. This includes the saving of data accumulated by these pulse sequences.

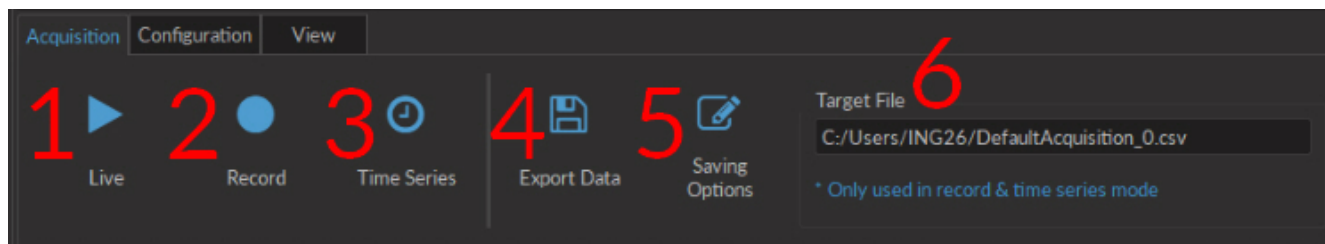


Figure 3.12: Acquisition Tab

1. The **Live** button (Fig. 3.12) activates all prepared channels. This mode does not automatically save data, keeping only the most recent 700 000 data points in memory. This mode is not recommended for long or critical measurement sequences.
2. The **Record** button (Fig. 3.12) activates all prepared channels while periodically saving recorded data to the disk. This mode is recommended for long measurement sequences.

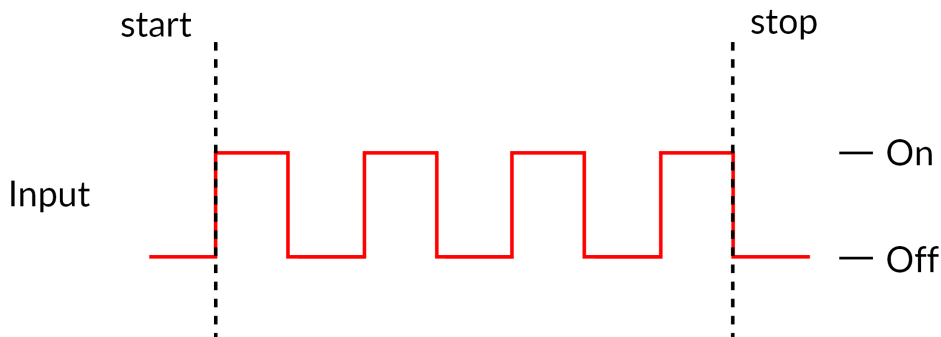


Figure 3.13: Time Series Acquisition Timing Diagram

3. The **Time Series** button (Fig. 3.12) opens the **Time Series Window** (Fig 3.14). This window allows the organisation of timed activation using the following parameters. Each **Time series** sequence is automatically saved to the same .csv file as defined in **Saving Options**.
 - The **Settings** box contains the series settings.
 - The **Number of series** defines the amount of times the series is repeated.
 - The **Time ON** defines the duration of the series.
 - The **Interval Between Series** defines the amount of time between each series, if the **Number of series** is greater than 1.
 - The **Total Duration** displays the total duration of all series.
 - The **Progression** box includes the **Progression bar**, which indicates the progression of the series (in %), while the **Time Elapsed** counter indicates the progression of the series.

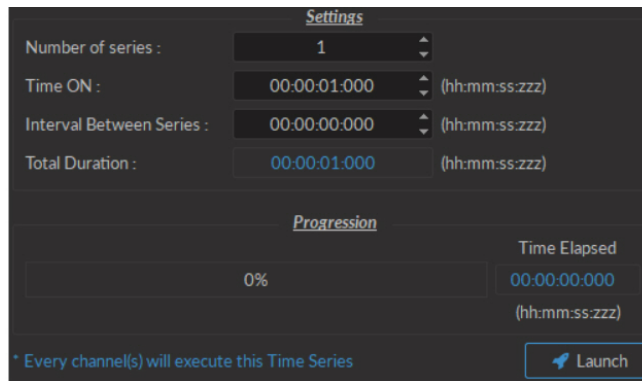


Figure 3.14: Time Series Window

- The **Launch** button start the series. While the series is active, it is impossible to add channels or change the configuration, though **View** settings can be modified.
4. The **Export Data** button (Fig. 3.12) will export all data in the **Acquisition view** into a .csv file. This is the only way to export data obtained will using the **Live** acquisition mode.
 5. The **Saving Options** (Fig. 3.12) button opens the **Saving Menu** window (Fig. 3.15).

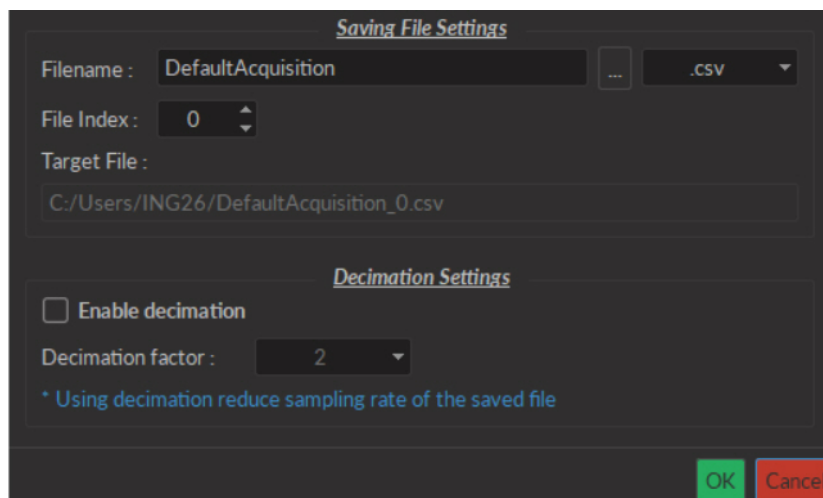


Figure 3.15: Saving Menu Window

- The **Save file settings** box is used to define how and where the file is saved. The name is taken from the **Filename** box, while the save location can be chosen by clicking the **...** button. The **File Index** box is used to define the current indexation number used for multiple files saved during the same measurement session. All this information is summarized in the **Target File** box.
 - The **Decimation Settings** allow the reduction of file sizes. This method conserves points averaged over a number of data points equal to the **Decimation Factor**¹. When the **Enable decimation** checkbox is selected, the **Decimation factor** box is used to define the number of points averaged.
6. The **Target File** box (Fig. 3.12) displays the save location and name used for files obtained using **Record**.

Configuration Tab

The **Configuration** tab is used to configure which channels are used, as well as saving and loading the configuration of the console software.

¹For a data set of 10 points, saved with a **Decimation Factor** of 2, the first two points (1,2) will be averaged, the second two points (3,4)... This produces a file of 5 points of data

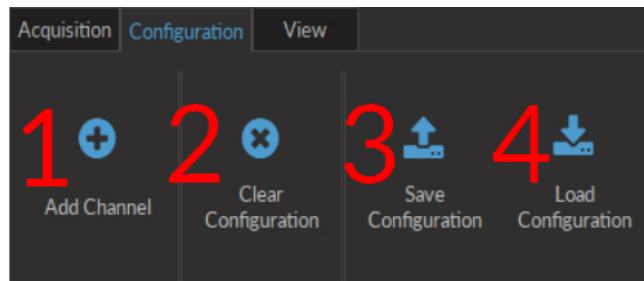


Figure 3.16: Configuration Tab

1. The **Add Channel** button (Fig. 3.16) opens the **Channels configuration** window. This window is detailed in section 3.1.1.
2. The **Clear configuration** button (Fig. 3.16) resets the acquisition view and all other parameters set. Any configurations already set will be lost.
3. The **Save configuration** button (Fig. 3.16) allows a console configuration to be saved in the **.doric** format. This file will only preserve the configuration of each current channel.
4. The **Load configuration** button (Fig. 3.16) allows a console configuration in **.doric** format to be loaded.

View Tab

The **View Tab** (Fig. 3.17) is used to modify the presentation of graphs in the **Acquisition view**.

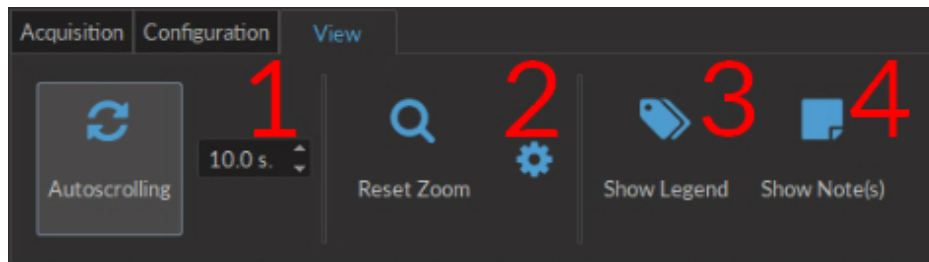


Figure 3.17: View Tab

1. The **Autoscrolling** button (Fig. 3.17), when clicked, makes the graphs scroll as new data appears. The duration (in seconds) kept on display is defined in the box beside the button.
2. The **Reset Zoom** button (Fig. 3.17) sets the graph zoom to a pre-defined value. This value is defined using the **Zooming menu** (Fig. 3.18), accessed using the **Gear** button.

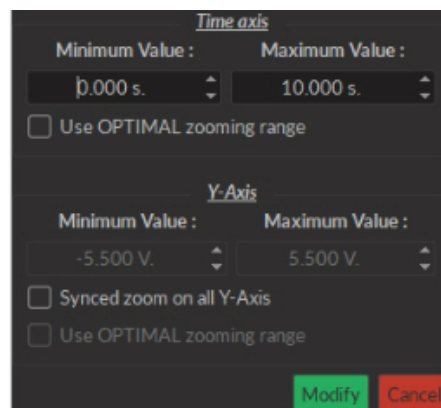


Figure 3.18: Zooming Menu Window

- The **Time axis** box is used to define the minimum and maximum values of the horizontal axis. If the **Use OPTIMAL zooming range** checkbox is selected, these values will be set to display all data currently in memory.
 - The **Y-Axis** box is used to define the minimum and maximum value of the vertical axis. If the **Synced zoom on all Y-Axis** checkbox is selected, these values can be changed. This only allows the **Voltage** axes to be changed, to the same value for all. The **Use OPTIMAL zooming range** will set the axes to a value displaying all data points in memory.
 - The **Modify** button will apply any changes to the axis values. The **Cancel** button cancels all changes.
3. The **Show Legend** button (Fig. 3.17) will open the **Legend** window (Fig. 3.19). This window display each configured **Channel**, as well as its **Name**, its **Channel Mode**, **Trigger Mode** and **Trigger Source** if applicable. Selecting the button while a window is open will close the window.

Channel	Name	Type	Trigger Mode	Trigger Source
▼ Digital I/O Ch.1	Digital I/O Ch.1	-	N / A	N / A
▼ Graph(s)				
DI/O-1		Input	-	-
▶ Digital I/O Ch.2	Digital I/O Ch.2	-	Triggered (Normal)	Manual
▼ Analog Out. Ch.4	Analog Out. Ch.4	-	Triggered (Normal)	Manual
▶ Graph(s)				
▼ Analog In. Ch.1	Analog In. Ch.1	-	Triggered (Normal)	Manual
▼ Graph(s)				
Aln-1		Physical	-	-

Figure 3.19: Legend Window

4. The **Show Notes** button (Fig. 3.17) will open the **Note Viewer** window (Fig. 3.20). Selecting the button while a window is open will close the window.

Note(s) List			Note Information
Title	Time	Graph	Title
Note_0005	00:00:03:042	Aln-1 - Raw	Note_0005
Note_0006	00:00:05:363	Aln-1 - Raw	
			Time (hhmm:ss:zzz)
			00:00:03:042
			Note Text
			Spike
Delete			Apply

Figure 3.20: Note Viewer Window

- The **Note(s) List** displays the **Title**, **Time** and **Graph** on which a note is marked. Selecting the **Delete** button will delete any note selected on the list.
- The **Note Information** box displays the information of a note selected in the **Note(s) List**. The **Title**, **Time** and **Note Text** can be edited. The **Apply** button applies these changes to the graph.

3.1.3 Acquisition View

The **Acquisition View** box displays all information concerning active channels. Each channel chosen using **Add Channel** is displayed in this window, occupying a rectangular box.

Each **Channel box** shows the following basic elements, with additional elements available for specific channel types.

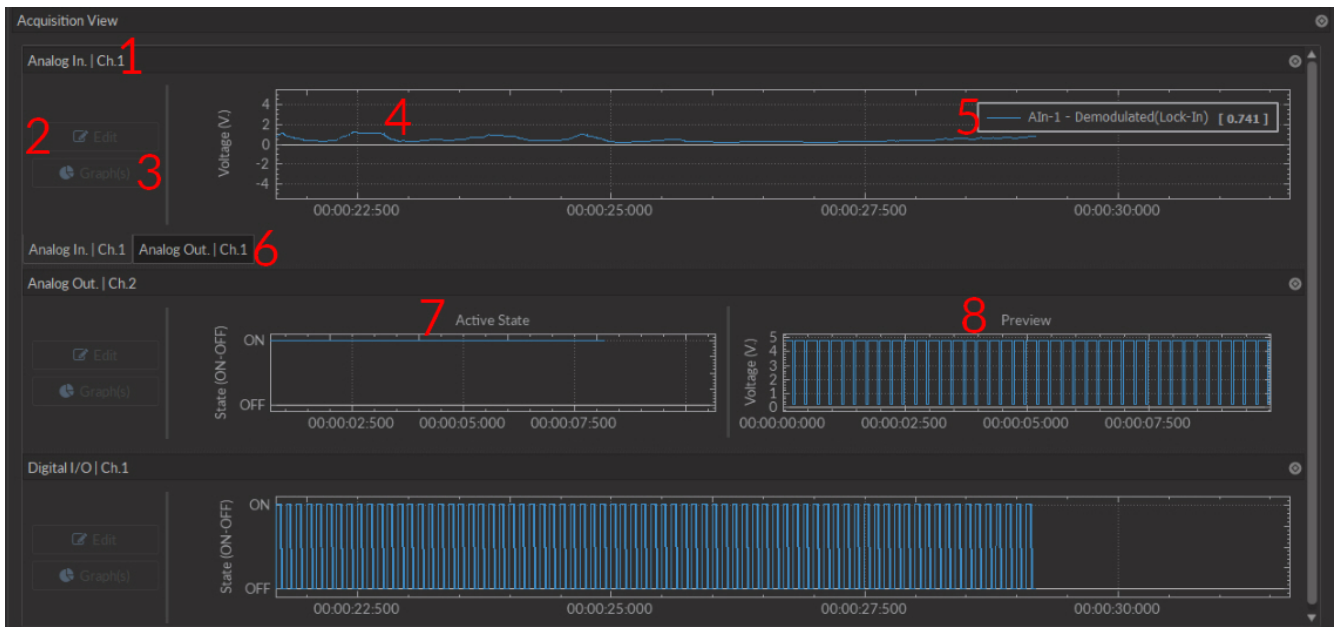


Figure 3.21: Acquisition View Box

1. The **Channel name** is located on the upper left of the **Channel box** (Fig. 3.21), identifying the type of channel and its number, corresponding to that on the console. This name can be modified in the **Graph options** window.
2. The **Edit** button (Fig. 3.21) allows the editing of channel parameters, opening the channel configuration windows. For further information, see section 3.1.1.

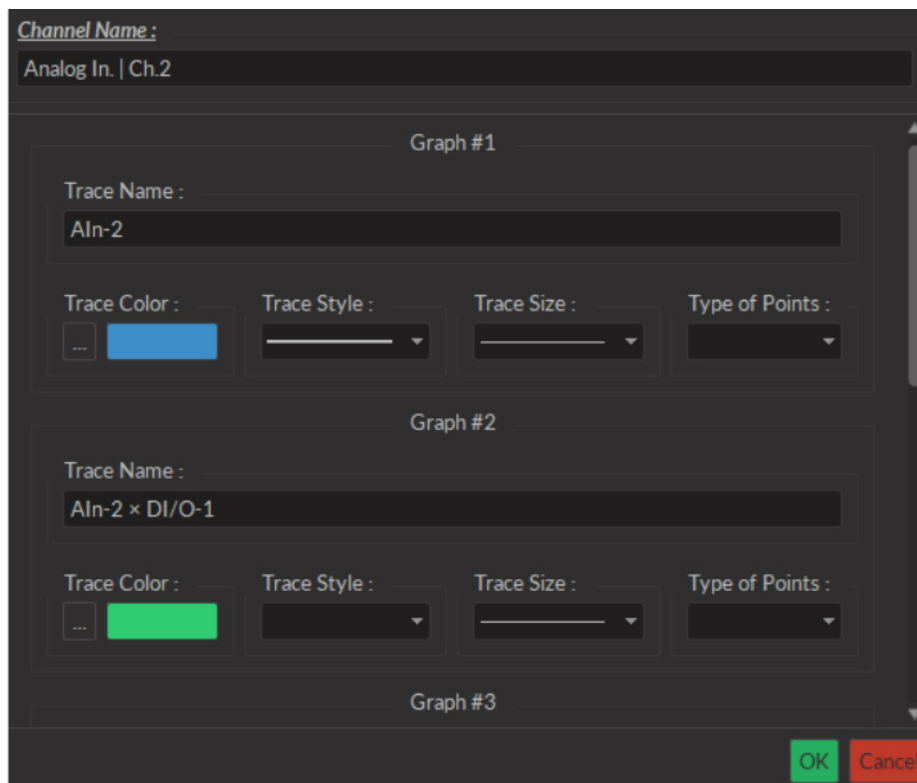


Figure 3.22: Graphs Window

3. The **Graph(s)** (Fig. 3.21) button opens the **Graph Options** window.

- The **Channel Name** (Fig. 3.22) is indicated in the upper left.
- The **Trace # X** box (Fig. 3.22) (where **X** is the number of the trace) show the visual options available for a single trace on the graph. A trace box appears for each trace on the graph.
 - The **Trace Name** box contains a standard name generated by the software. The name can be changed here.
 - The **Trace Color** button (...) opens the **Color Select** window (Fig. 3.23), which allows the selection of a trace color from a wide palette. The **Pick screen color** in this window allows the selection of any color displayed on the computer screen.

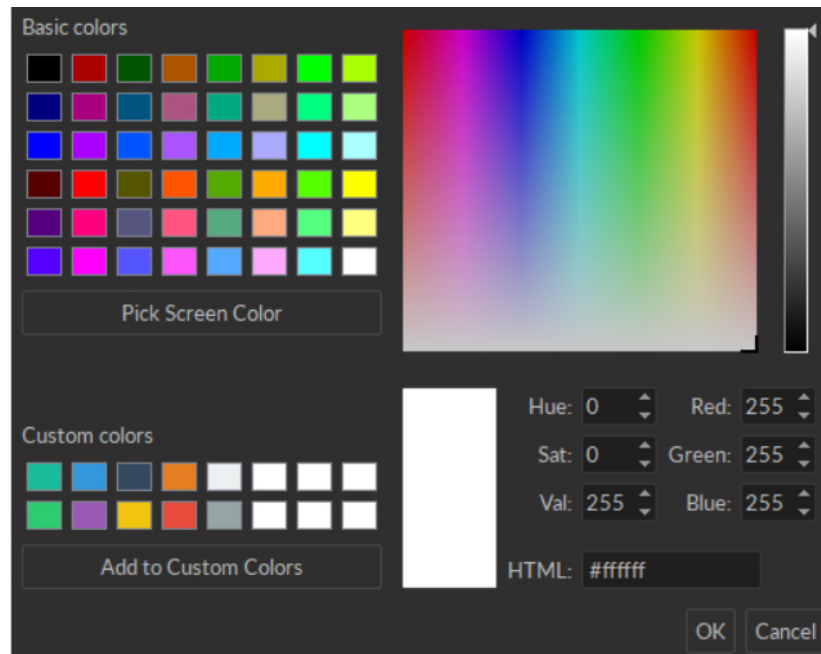


Figure 3.23: Select Color Window

- The **Trace style** drop-down list allows the selection of the type of trace, from full to dashed lines. If the style chosen is empty, the trace will not be displayed.
 - The **Trace size** drop-down list allows the selection of the trace size. Using a bigger **Trace size** than the default may result in slower display and performance degradation.
 - The **Type of points** drop-down list allows the selection of what type of point used to indicate data points on the trace. Using different point types than the default (none) may result in slower display and performance degradation.
4. The **Graph** (Fig. 3.21) box displays the graph of a channel, with **Voltage** or **State** as the vertical axis and **Time** as the horizontal axis. Double-clicking either axis will open a **Change Axis Range** window that allows the axis limits to be changed, as in the **Zooming Menu**. Any changes done on a horizontal axis will change the axis limits for each channel.
 5. The **Instant values** box (Fig. 3.21) can be activated by right-clicking the **Input graph** box and selecting **Show instant values**. This box shows the current value detected by the console for each trace on the selected channel. This box cannot be activated on **Preview graphs**.
 6. The **Channel tabs** (Fig. 3.21) appear in certain input modes (such as **Interleaved** and **Lock-in**) where the input automatically sets the output values on separate channels. It is possible to create a **Channel tab** by undocking one channel and moving it above another until it turns blue, then releasing it.
 7. Analog output channels display an **Active state** graph (Fig. 3.21). This graph displays whether the channel is outputting a signal (On, $V \neq 0$) or not (Off, $V = 0$).
 8. Output channels display a **Preview** graph (Fig. 3.21), showing a preview of the pulse sequence.

The following section describes the usage of the **Interleaved** measurement mode for **Analog Input** channels. Once the **Interleaved** channel mode is chosen, the the **Interleaved/Sequential options** box (Fig. 3.24) appears, containing the following parameters.

1. The **Channel #** drop-down lists allows the choice of interleaved outputs. Once **Channel #1** has been selected, **Channel #2** only allows the same type of output (analog or digital) to be selected.
2. The **Preconfiguration** drop-down list allows the choice of a pre-configured frequency for the interleaved channels. The previously selected channels are configured to function at the chosen frequency.

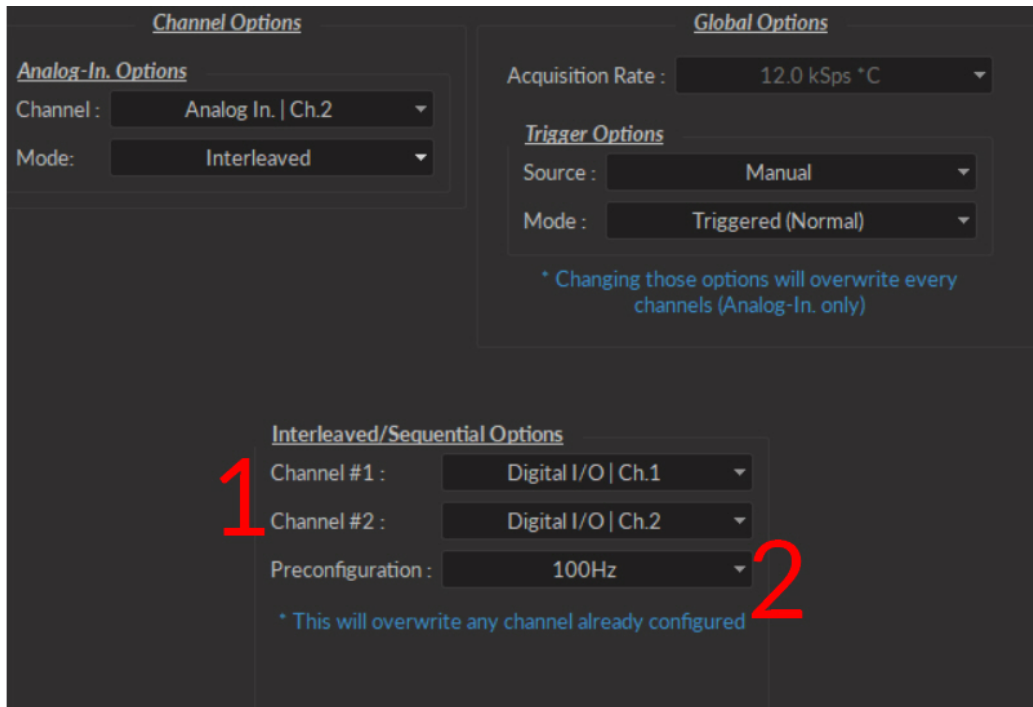


Figure 3.24: Channels configuration, Analog Input Interleaved Mode

The following steps describe the basic manner in which to use **Interleaved Mode**.

1. Select two channels from the **Channels #** lists.
2. Select a frequency from the **Preconfiguration** list.
3. Connect the *Fiber Photometry Console* to at least two different light sources using the outputs preconfigured for interleaved mode in the previous step. Ensure the driver(s) are in **TTL** mode (for digital channels) or **MOD** mode (for analog channels), with the driver set at the desired maximum current level (in mA).
4. Connect the detector to the appropriate analog input channel(s) corresponding to the input.
5. Start measurement using **Play** or **Record** on the **Acquisition** tab.
6. After a measurement is made, 3 traces are available in the **Graphics/Traces List**.

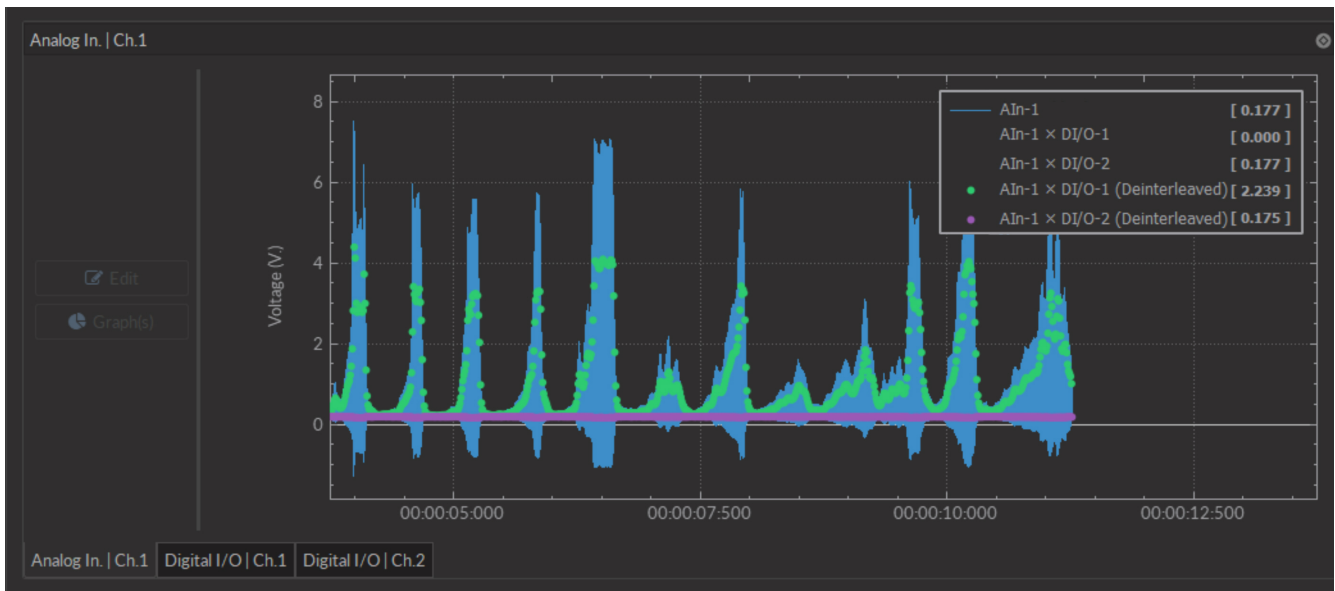


Figure 3.25: Acquisition View, Interleaved Mode Traces

- The **Raw Data** (AIn-1) (Fig. 3.24) shows the raw signal received on the input channel.
- The **Intermediate Data** (AIn-1 x DI/O-1) (Fig. 3.24) traces show the fluorescence signal caused by each light source, obtained by multiplying the output signals by the input signal.
- The **Deinterleaved Data** (AIn-1 x DI/O-1(mean)) (Fig. 3.24) shows the averaged signal of each demodulated pulse. This leaves a single averaged point per pulse sent by the output channels.

The following section describes the usage of the **Lock-in** mode for **Analog Input** channels. Once the **Lock-in** channel mode is chosen, the **Lock-in options** box (Fig. 3.26) appears, containing the following parameters.

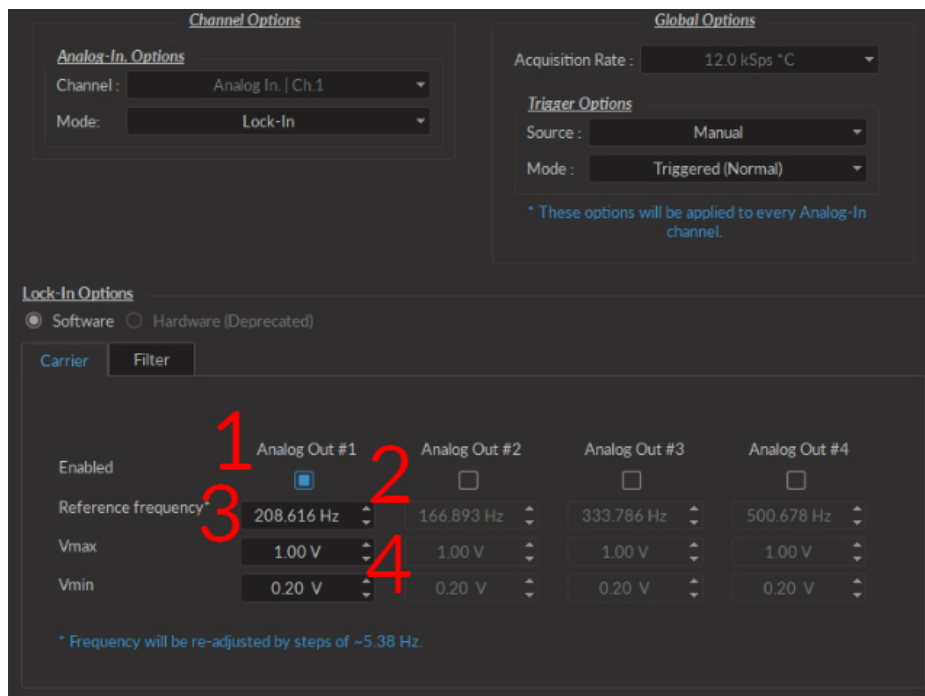


Figure 3.26: Channels configuration, Analog Input Lock-in Mode, Carrier Tab

1. The **Channel List** (Fig. 3.26) indicates which channels are used to output a reference frequency. Multiple output channels can be selected for a single input channel. Any output channels already configured by a different input channel will be usable, but not configurable. The output configuration must be changed in the initial input channel.
2. The **Enabled** checkboxes are used to select which output channels are in use.
3. The **Reference frequency** (Fig. 3.26) allows the choice of a reference frequency. The reference frequency should not be a multiple of a known noise frequency (e.g. 60 Hz), or a multiple of another reference frequency.
4. The **Vmax/Vmin** (Fig. 3.26) defines the voltage sent from the console to the device. The minimum possible difference is of 0.1 V for the **LEDD**. The **Vmin** value is 0.1 V for the **LEDD** and 0.2 V for the **LEDRV**.
5. The **Cutoff Frequency** (Fig. 3.27) (the frequency at which a -3 dB attenuation will occur), found on the **Filter** tab is kept fixed at 12 Hz for optimal filtering results.
6. The **Filter response** is displayed on the **Filter** tab.

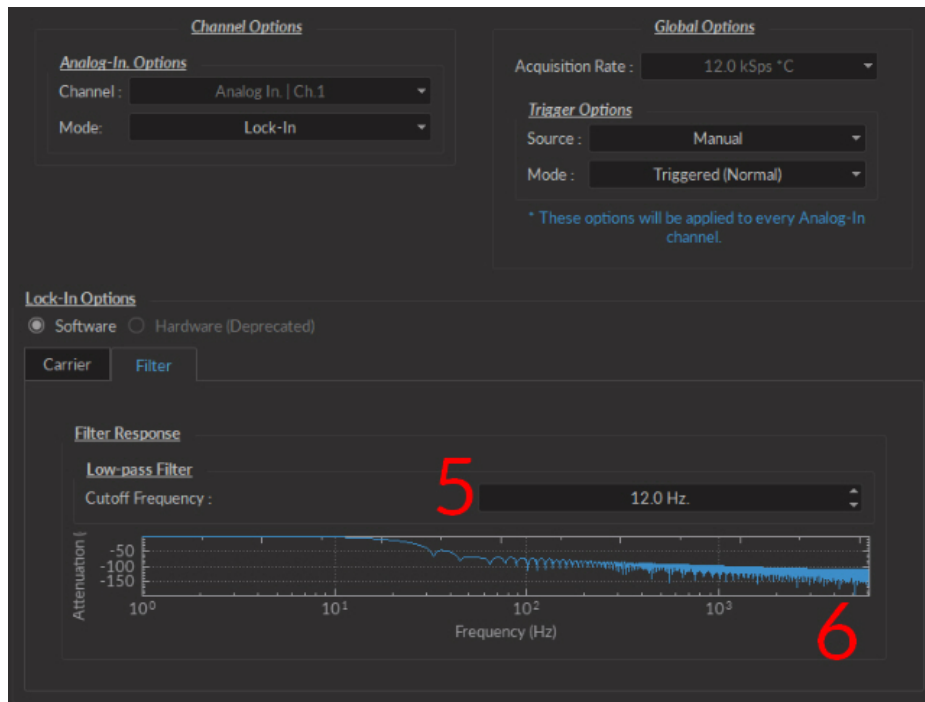


Figure 3.27: Channels configuration, Analog Input Lock-in Mode, Filter Tab

3.2 Photometry Analyser

This module provides an easy means to extract relevant data from the data acquired by the Doric Fiber Photometry Console. The software loads data in .CSV or .doric format, implements signal processing functions and saves the traces in .CSV or .doric format. This software does not replace standard analysis tools such as Matlab, ImageJ or Excel, but aims to offer useful processing algorithms developed for the photometry data.

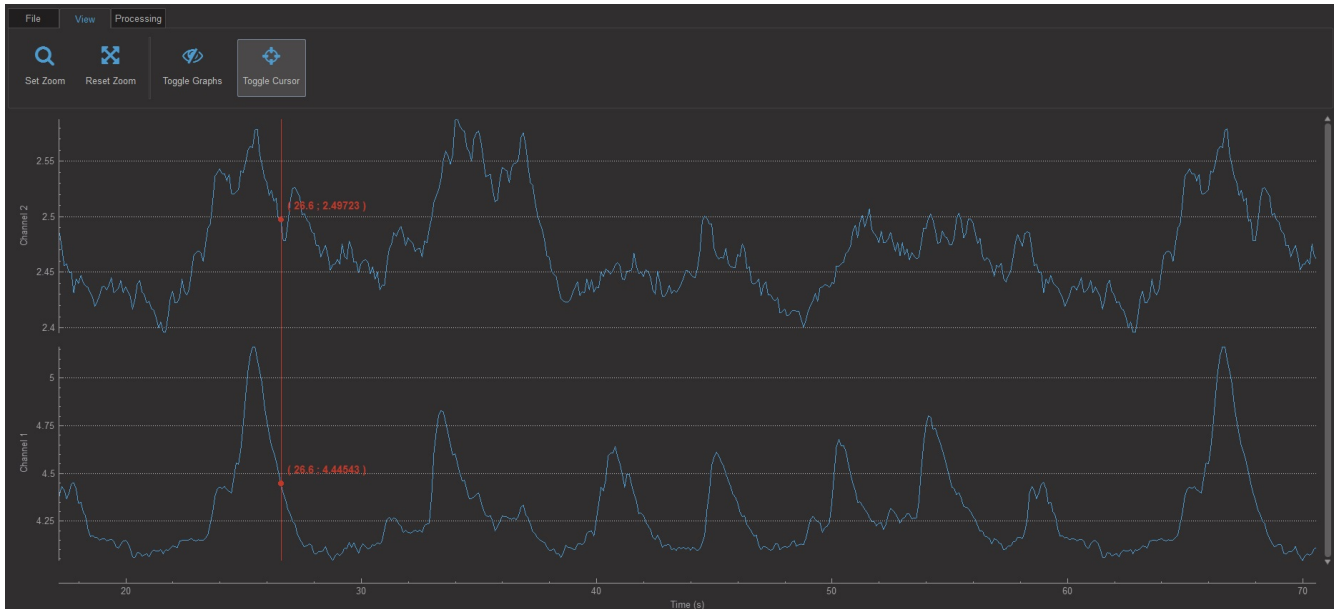


Figure 3.28: Photometry Analysis Module Interface

1. The **Graph Box** displays the loaded data, allows navigation through the points by zooming and dragging.
2. The **Function Toolbar** contains all the buttons and the functions accessible.

3.2.1 Function Toolbar

1. The **File Tab** (Fig. 3.29) is used to load/save data,

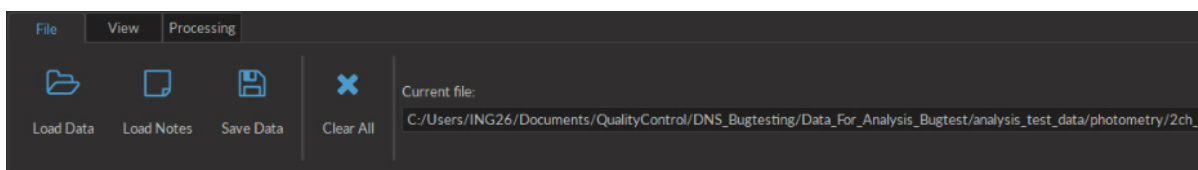


Figure 3.29: File tab

- The **Load Data** button loads a .CSV or .doric file with the first row being the headers, and the first column being the x-axis. The imported column(s) can be chosen.
 - The **Save Data** button saves the user-defined data to a .CSV or .doric file.
 - The **Clear all** button clears all loaded data from the module. This clearing cannot be undone.
2. The **View tab** (Fig. 3.30) is used to manipulate the appearance of the displayed data.

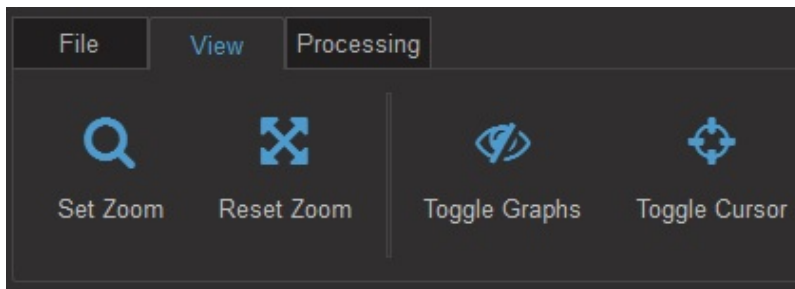


Figure 3.30: View Tab

- The **Set Zoom** button opens the **Set custom display range** window (Fig. 3.31). This window allows the selection of the axis values associated with the **Reset Zoom** button. The **Automatic** option will choose values to display all loaded data.

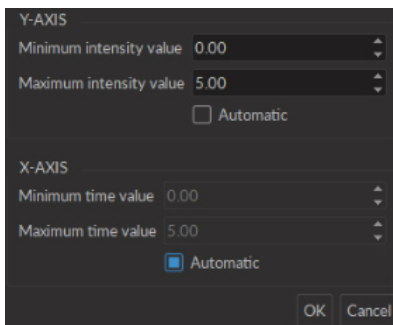


Figure 3.31: Set Custom Display Range Window

- The **Reset Zoom** button rescales the axes to values set in the *Set Zoom* function.
- The **Toggle graphs** button opens the **Show/Hide Graph(s)** window (Fig. 3.32). The list in the window is used to toggle current graphs on or off.

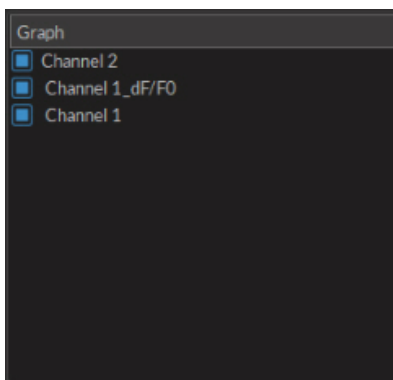


Figure 3.32: Show/Hide Graph(s) Window

- The **Toggle cursor** button adds a cursor to the graphs that shows local coordinates of time and intensity, as displayed in red on figure 3.28.
3. The **Processing tab** is used to control the various processing functions.

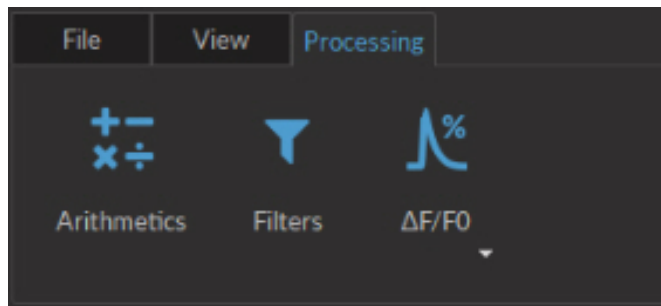


Figure 3.33: *Processing Tab*

- The **Arithmetics** function applies basic arithmetic operations (+, −, ×, ÷) between 2 channels. The result is shown in a new graph.

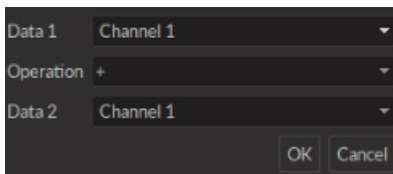


Figure 3.34: *Arithmetics Window*

- The **Filter** function opens the **Filter** (Fig. 3.35) window uses a butterworth filter to remove unwanted frequencies from the data. These filters can be **Bandpass**, **Bandstop**, **Low-pass** or **High-pass**.

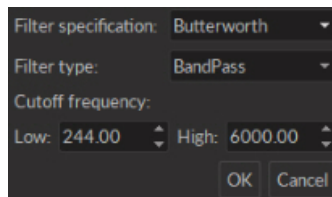


Figure 3.35: *Filters Window*

- The $\Delta F/F_0$ function opens the **Select Which Traces to Process** window (Fig. 3.36). It is used to calculate the normalized fluorescence variation of the data and displays the results in a new graph. See section 3.2.2 for more details.
 - **Reference channel** is chosen using the channel list. The $\Delta F/F_0$ can either be calculate for a channel with itself, or with another channel as the reference (e.g. if one channel represents the signal, and the other channel represents the reference). In the latter, the $\Delta F/F_0$ is calculated independently for each channel, and the signal and the reference are subtracted.
 - **F₀ calculation method** shows the method used to evaluate F₀, either using a running average or the least mean squares method.
 - The **Time Range (s)** allows a segment of time to be chosen for processing from the loaded dataset.

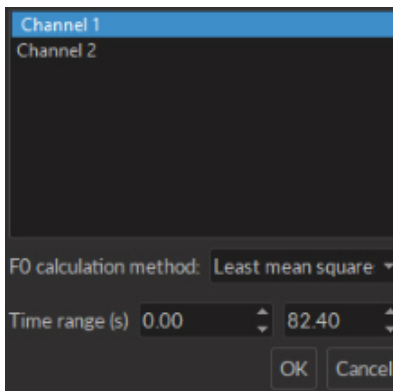


Figure 3.36: $\Delta F/F_0$ Options

3.2.2 Algorithms

$\Delta F/F_0$

For each point, the processed fluorescence intensity I_t is defined as $I_t = (F_t - F_0)/F_0$, where F_t represent the fluorescence intensity at time t . For the *running average* F_0 calculation option, the algorithm is inspired from G. Cui, S. B. Jun, G. Luo, M. D. Pham, S. S. Vogel, R. M. Costa, *Deep brain optical measurements of cell type-specific neural activity in behaving mice*, Nature Protocols 9, 1213-1228 (2014). Briefly, F_0 is calculated as the running average fluorescence intensity variation over a window of 1 minute. If less than 1 minute is available, the algorithm will use the average of all the data. If the calculation method is *least mean square*, the algorithm is inspired from T. N. Lerner, C. Shilyansky, T. J. Davidson, L. Luo, R. Tomer, K. Deisseroth *Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits*, Cell 162, 635-647 (2015). The algorithm will calculate the least mean square fit of the whole data series, and use that fit as the F_0 .

Specifications

Table 4.1: General Specifications

SPECIFICATION	VALUE	NOTE
Power supply	110 - 240 VAC ; 50 - 60 Hz	Power supply adapter included
DC power supply	12 VDC	
Mass	797 g	
Dimensions	36 cm x 3.63 cm x 9.82 cm	Depth includes connectors
Digital input voltage	0 to +5 V	Min Hi Level: 4 V; Max Low Level: 0.55 V
Digital output voltage	0 to +5 V	
Digital input impedance	3 k Ω	
Digital output impedance	30 Ω	
Digital time propagation delay	4.6 ns	
Digital time input resolution	82 μ s	
Digital time output resolution	10 μ s	
Analog output voltage	\pm 4.75 V	
Analog output sample frequency	25 MS/s	
Analog output max. frequency	10 khz	
Analog output impedance	6 Ω	
Analog time propagation delay	20 ns	
Analog input voltage	\pm 10 V	
Analog input sample frequency	15 kS/s	
Channel-to-channel isolation	110 dB	
Analog input impedance	100 to 124 k Ω	

¹Chopping Enabled: This mode provides very low noise with lower output rates. Chopping does not eliminate the offset error and drifts caused by input resistors. Noise performance depends on the selected analog input range and the chopping mode. The analog input is always operated with chopping mode in order to optimise the offset drift and to allow a better noise performance. When starting an acquisition, input data rate (Samples per second) can be selected according to the needs of each test. Higher data rate provides higher RMS noise. Analog inputs are reversed and then calculated as an average of two conversions in order to reduce the offset error.

Table 4.2: Analog input noise and resolution specifications with chopping mode enabled

Data rate	Real Rate	Time resolution	- 3dB Frequency	RMS noise	Effective resolution ¹
0.3 kSps *C	372 Hz	2686 μ s	200 Hz	9.6 μ V	20.0 bits (9.5 μ V/bit)
1.0 kSps *C	1 001 Hz	999 μ s	520 Hz	15.5 μ V	19.3 bits (15.5 μ V/bit)
2.5 kSps *C	2 534 Hz	395 μ s	1 300 Hz	26.1 μ V	18.5 bits (27.0 μ V/bit)
6.0 kSps *C	6 041 Hz	166 μ s	3 100 Hz	46.0 μ V	17.7 bits (47.0 μ V/bit)
12. kSps *C	12 166 Hz	82 μ s	6 300 Hz	120 μ V	16.3 bits (123.9 μ V/bit)

¹ Effective resolution for linear mode at ± 10 V input range. Input resolution is 16 bits (0.152 mV). Lower data rates provides higher effective resolution. The lock-in mode allows higher effective resolution. In this case, effective resolution is higher than RMS noise for every listed frequency.

Table 4.3: Recommended Environmental Specifications

DESCRIPTION	OPERATION	STORAGE
Use	Indoor	Indoor
Temperature	0-40 ° C	0-40 ° C
Humidity	40-60% RH, non condensing	40-60% RH, non condensing

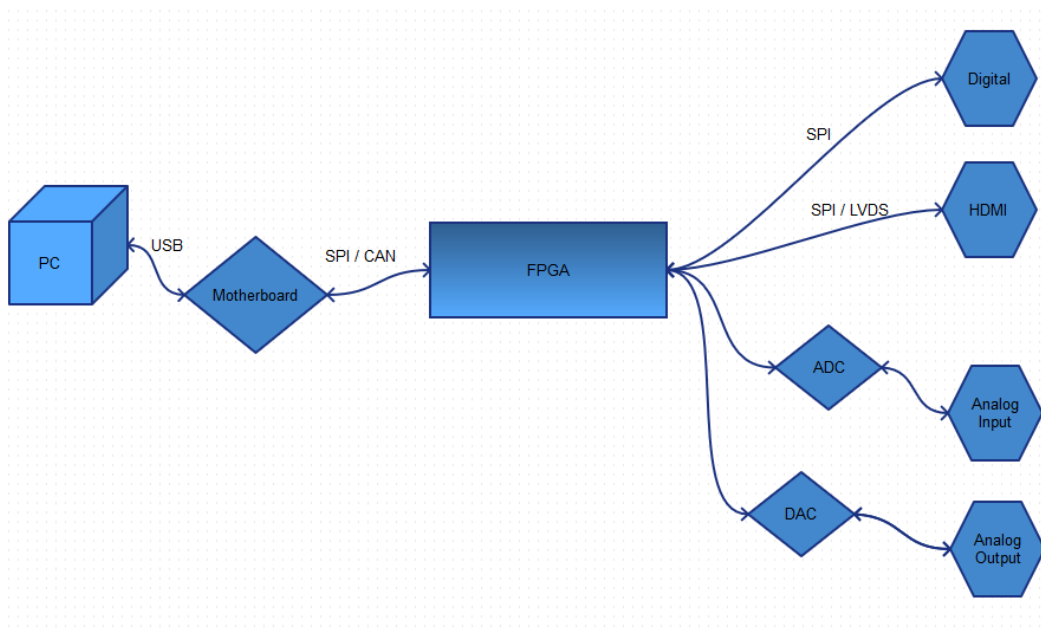


Figure 4.1: Electronic Bloc diagram

5.1 Important handling information

Warning: Do not open the box to avoid electrical injury.

5.2 Maintenance

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

5.3 Warranty

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

5.4 Contact us

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

Phone 1-418-877-5600

Email sales@doriclenses.com



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357 rue Franquet - Quebec, (Quebec)
G1P 4N7, Canada

Phone: 1-418-877-5600 - Fax: 1-418-877-1008

www.doriclenses.com