Doric Neuroscience Studio

User Manual

Version 5.3.4

Contents

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Software Overview

1.1 Doric Neuroscience Studio

Our vast product line allows users to build for different applications such as optogenetics, fluorescence microscopy, electrophysiology and fiber photometry. In order to implement the best applications, our engineers have created an intuitive software which allows control of the hardware and the acquisition of data.

The free Doric Neuroscience Studio Software incorporates different modules for each device connected. The existing modules allow:

- Control of our Programmable LED Drivers
- Control of our Laser Diode Module Drivers
- Control of our Ce:YAG Fiber Light Sources Drivers
- Acquisition of the voltage of a chosen light source input BNC
- Acquisition of data from our Fiber Photometry Console
- Acquisition of a live feed from our Behavioral Tracking Camera
- Control of our Fluorescence Microscope Driver
- Control of our Optogenetics TTL Pulse Generator (OTPG) to create complex pulse protocols
- Control our Optogenetically Synchronized Electrophysiology System
- Analyse image data from the microscope.
- Analyse photometry data.
- Analyse electrophysiology data.
- Analyse behavioral tracking data.
- Simulate optrodes in brain tissue.

1.2 System requirements

Windows

- Operating system: Microsoft Windows 7, 8, 10; 64-bit
- Processor speed: 2 GHz and 4 cores minimum, 3 GHz and 8 cores recommended
- Memory: 4 GB RAM minimum, 8 GB RAM recommended
- Hard drive: 500 MB of free hard disk space

2

Getting Started

2.1 Installing the software

1. **Run** the Doric Neuroscience Studio Installer from the supplied USB key or download the latest version of the software from our website. See Table 5.2 for computer requirements.
2. **Select** the language to use during the installation.
3. In the license agreement window (Fig. 2.1), accept the agreement and click **Next** to continue the process.

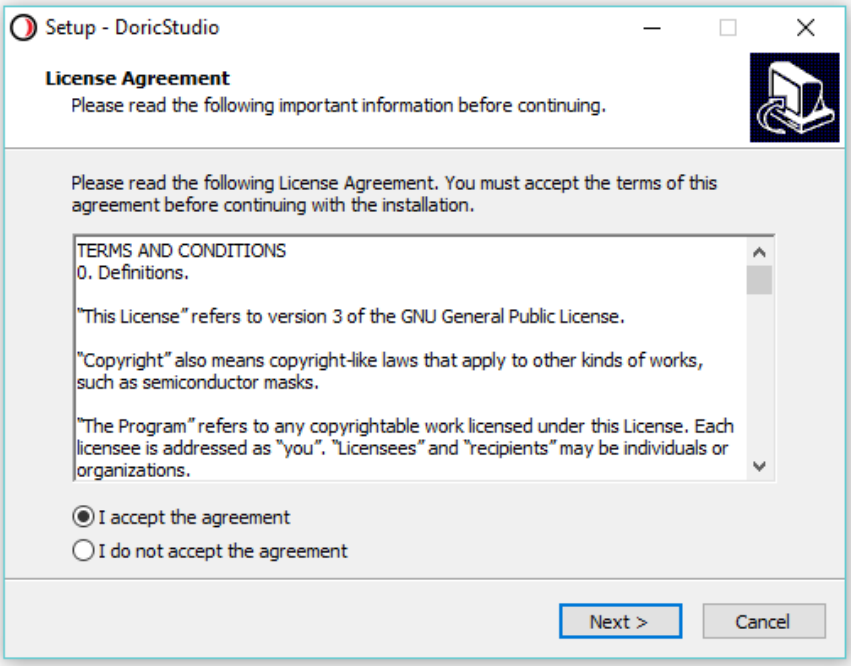


Figure 2.1: *Doric Neuroscience Studio License Agreement*

4. Click **Next** in the Information window.
5. **Choose** where to install the software (Fig. 2.2) and click **Next**.

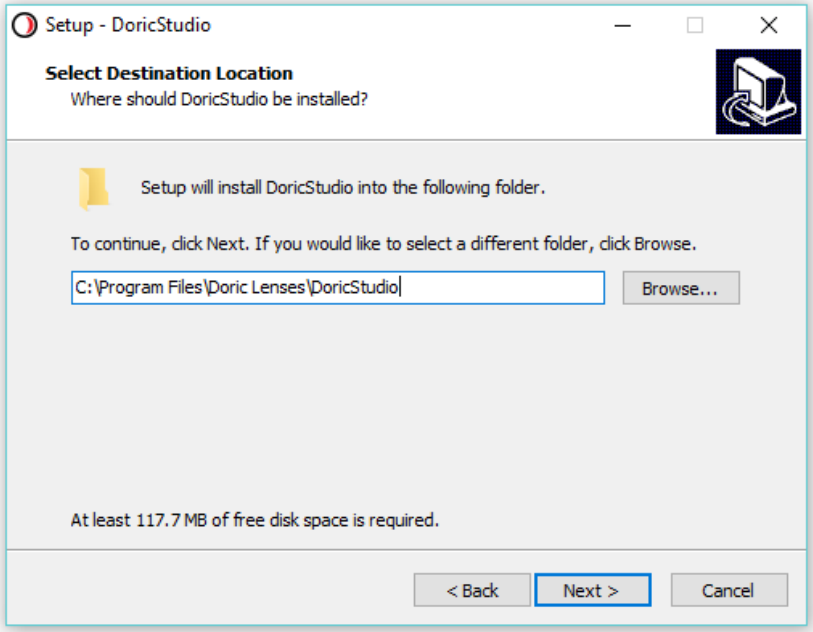


Figure 2.2: *Select Destination Location*

6. **Choose** if desired to create a shortcut in the Start Menu folder and click **Next**.
7. **Choose** if desired to create a desktop icon and click **Next**.
8. When ready, click **Install** to begin the process. This should take a few moments. When the installation is done, the message in figure 2.3 will show up.

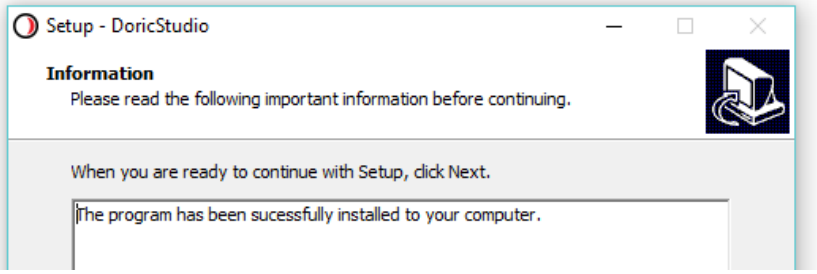


Figure 2.3: *Successful Installation of the Doric Neuroscience Studio*

9. Click **Next** and **Finish** to exit the setup.
10. Now the software is ready for use.

2.2 Launching the Doric Neuroscience Studio

Connecting cables to the computer before powering the device(s) may lead to an incorrect driver installation.

Now that the software is properly installed, the new device(s) can be controlled from the computer. When the software is launched and the Doric device(s) connected, a new configuration can be created or a saved one can be loaded. Once a device is connected, a **Device Tab** will appear. These tabs are used to configure the behaviour of each device. The

connection is indicated at the lower left of the window in the format "*Device* is ready", for any given device. The analysis modules can also be displayed as additional tabs, allowing for on-the-spot data analysis. The details of each type of tab are found in section 2.3.

2.3 Software Organization

On Figure 2.4, the different menu options available at all times in the studio are shown.

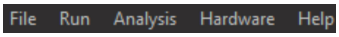
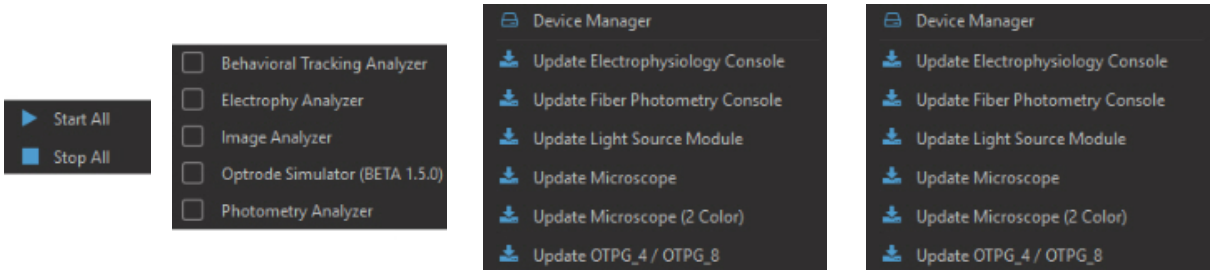


Figure 2.4: *Menu Options*

- **File** is used to quit the software
- **Run** (Fig. 2.5a) allows the activation/deactivation of all **Device Tabs** with the **Start All** and **Stop All** selections.



(a) *Run* (b) *Analysis* (c) *Hardware* (d) *Help*

Figure 2.5: *Menu Drop-down Lists*

- **Analysis** (Fig. 2.5b) is used to open analysis modules. These modules are detailed in Chapter 4.
- **Hardware** (Fig. 2.5c) is used to manage any Doric devices linked to the computer.

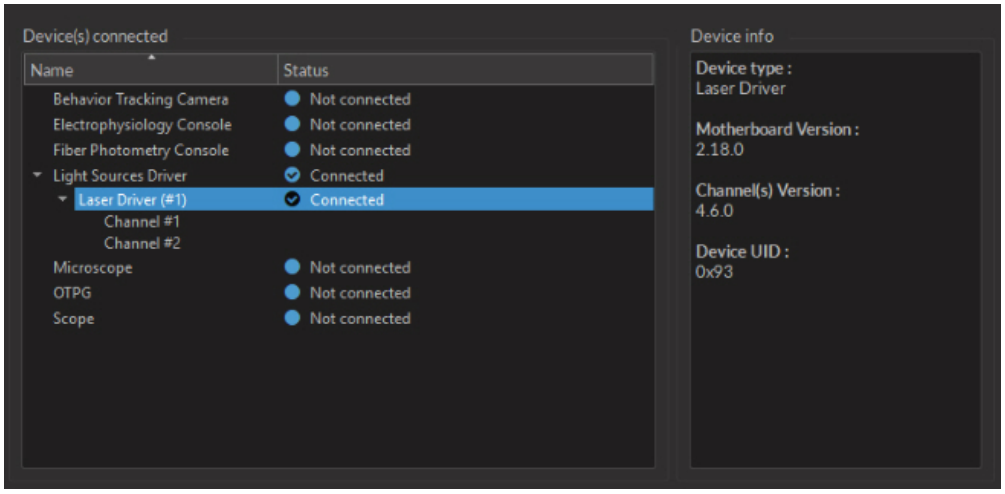
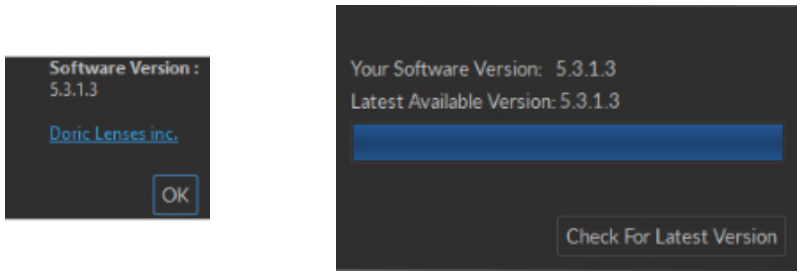


Figure 2.6: *Device Manager Window*

  - The **Device Manager** selection opens the **Device Manager** window (Fig. 2.6). This window shows the connected devices as well as their respective firmware versions.
  - The **Update** selections are used to update device firmware. Under normal circumstances these should not be used.

- **Help** provides information on the different features included in the software.



(a) *About Window* (b) *Updates Window*

Figure 2.7: *Help Windows*

  - When the **Help** buoy is selected, extra information will be displayed when the *Help Cursor* appears. A small box describing the feature will appear. If the item cannot be interacted with, the invalid cursor will appear. These cursors will change depending on the cursor package used by the computer. The standard windows AERO cursor guidelines are used for the snapshots in this manual.
  - The **User Manual** selection will open the manual associated with this version of the software.
  - The **About** selection will open the **Software Info** window (Fig. 2.7a), which shows the current version of the software.
  - The **Check for updates** selection open the window (Fig. 2.7b) of the same name. It can be used to check if the software can be updated. This function requires an Internet connection.

2.4 System Tabs

Once a device is connected to the computer, the following tabs may appear depending on the device.

1. The **Device Tabs** will change depending on which device is connected. The tab is named *DX-Y: Title*, with *X* being the device number, *Y* being the module number for a device using multiple tabs, and *Title* being the module's name. This is where the application is configured and controlled. Each type of device is a different module. Each one is explained in the next chapter of this document.
2. The **Analysis Tabs** can be activated from the **Analysis** menu. These tabs offer various analysis modules to perform additional data processing on results obtained through Doric Lenses devices.

2.5 Software Features

There are several features that are found in the software to improve ease of use.

2.5.1 Widgets

A number of widgets within the Studio can be undocked by clicking the symbol. Double clicking the title bar will return the window to its original position.

2.5.2 Graphs, Plots and Traces

Most plot displays feature similar functions. The **Reset Zoom** button resets the plot to its default viewing format. Active plots often have the **Autoscroll** function, which will move the plotted data to keep a fixed duration plotted. The **Clear Data** button clears the data in the plot. After the data is cleared, it cannot be recovered.

To change the scale of the axes in any graph, **Shift+Mouse Wheel** changes the Y-axis and **Ctrl+Mouse Wheel** changes the X-axis. The scale changes around the position of the mouse cursor, and the scale cannot be changed unless the mouse cursor is on the graph.

Clicking and dragging graphs will change the position in 2-D space. If multiple graphs are present, the X-axis (representing time) will change equally on all graphs, while the Y-axis is changed for each graph individually.

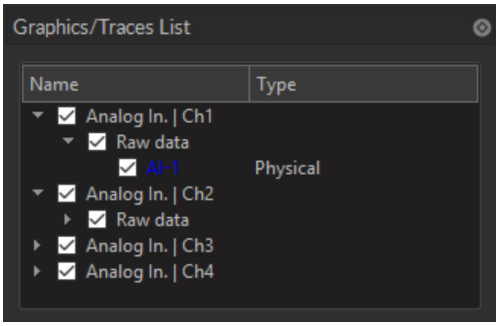


Figure 2.8: *Graphs/Traces List*

The **Graphs/Traces List** (Fig. 2.8) can be found on most input tabs. This list will show the various traces associated with each graph. These choices can be expanded by clicking the arrow to the left of the graph name. By unchecking individual traces, they are removed from the graph.

2.5.3 .doric Format

The **.doric** format, based on the HDF5 is used both to save data recorded by the software, as well as record pulse sequences. This format is preferable for large data sequences.

2.6 Updating Software & Firmware

Doric electronic devices, such as drivers and consoles, require periodic updates to their firmware for optimal performance. The *Doric Neuroscience Studio* also requires periodic updates to integrate new features. The following section shows how to keep your Doric devices and software up to date.

2.6.1 Updating Doric Neuroscience Studio

1. Make sure to keep the software regularly updated. By selecting **Help — Check for updates** the **Check for updates** window will appear.
2. Should the installed version be older than the version online, a hyperlink will appear allowing you to download the update from our website.

3. Disconnect all Doric devices from the computer before starting the update.
4. Once downloaded, run the file. It will immediately detect the previous version and present the option of uninstalling it (Fig. 2.9). **Click Yes**.

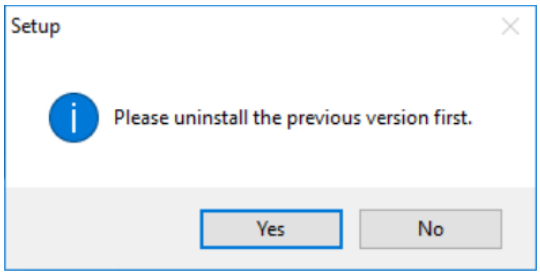


Figure 2.9: *Uninstall Window*

5. When the program asks if you are certain (Fig. 2.10), **Click Yes**.

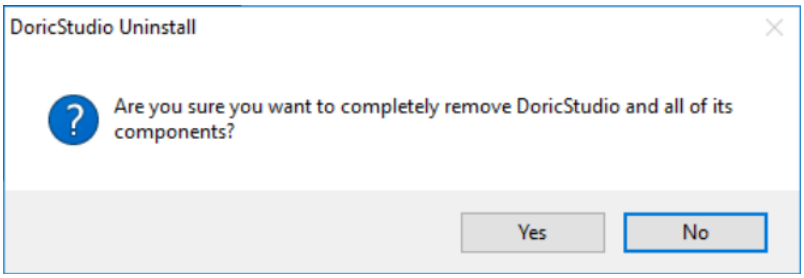


Figure 2.10: *Uninstall Confirmation*

6. When the previous version is uninstalled, the following (Fig. 2.11) will appear. **Click Ok**, and the installation of the new version of the software should start, as shown in section 2.2.

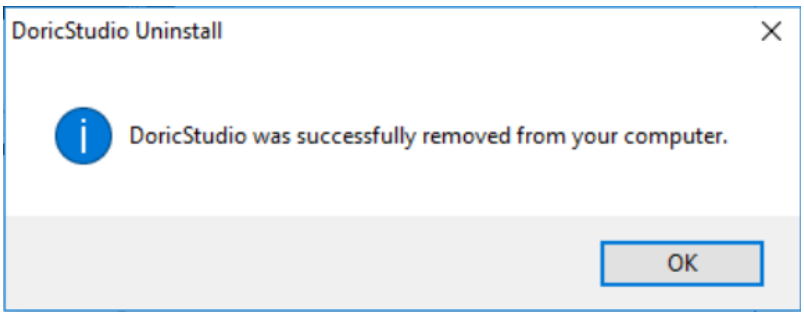


Figure 2.11: *Uninstall Completion*

7. Once the installation is finished, the update is complete.

2.6.2 Updating Firmware

To update device firmware, contact us at sales@doriclenses.com. Instructions and firmware files will be provided.

3

Device Modules

3.1 Light Sources

Doric Light Sources can be controlled by the Doric Neuroscience Studio. These include *LED Modules*, *Laser Diode Modules* and *Ce:YAG Fiber Light Source*. The interface is separated into two main sections, **Control & settings** and the **Acquisition View**. Each light source driver has a number of **Channels**, each one controlling a light source of its given type. These channels, accessible using the **Add Channel** will be the first detailed.

Figure 3.1: *Light Source Driver Tab*

3.1.1 Channels

Each light source driver is separated into a number of **Channels**. Each channel controls a single light source. While each channel can be controlled in **Stand-alone** mode by the driver, additional functions can be accessed for these channels when the driver is connected to the Doric Neuroscience Studio. These function are used through the **Channel Configuration** window (Fig. 3.2).

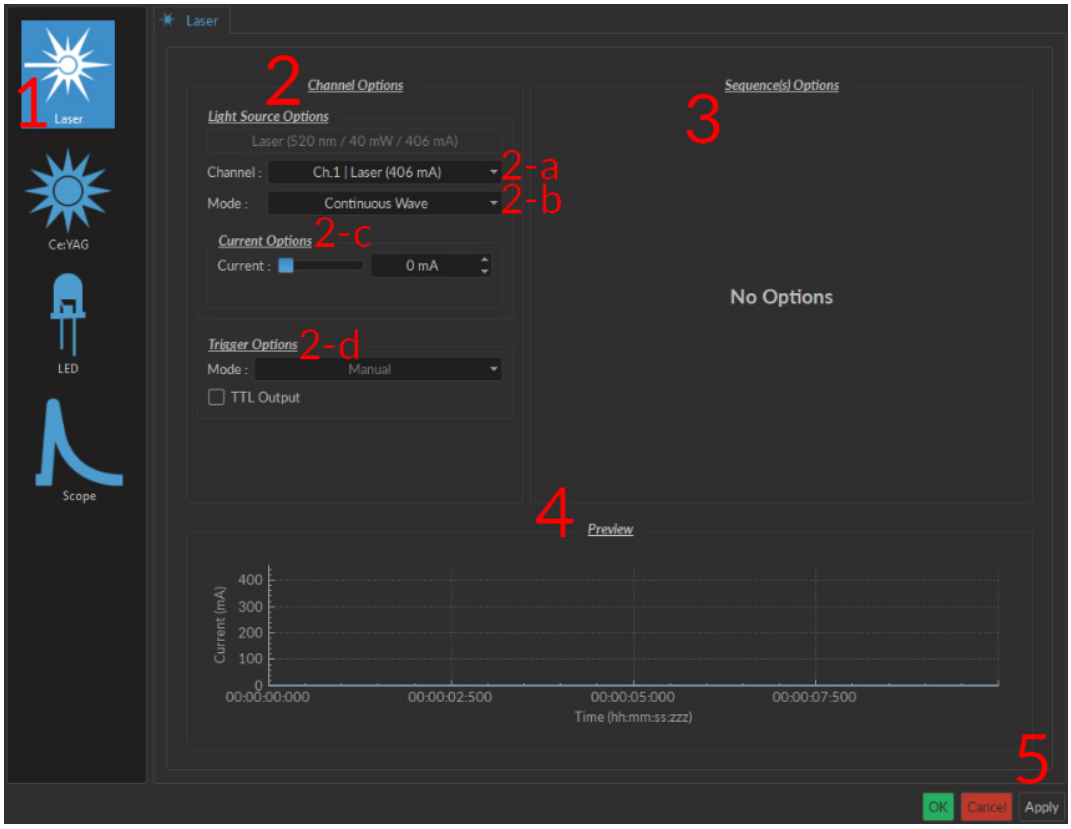


Figure 3.2: *Light Source Channel Configuration Window*

1. The **Channel Types** (Fig. 3.2) are displayed on the left side of the window. These include **Laser** light sources, **Ce:YAG** light sources and **LED** light sources, as well as the **Scope** to measure signal using the driver.

2. The **Channel Options** box (Fig. 3.2) includes **Light Source Options** and **Trigger Options** for the given channel.

    a) The **Channel** (Fig. 3.2) drop-down list identifies which driver channel is currently being edited, assuming a driver with multiple channels.

    b) The **Mode** (Fig. 3.2) drop-down list includes each possible driver mode. These are used to control the pulse sequences emitted by the light source. The options related to this mode are detailed with the **Sequence Options**.

    c) The **Current Options** (Fig. 3.2) includes the slider used to control the current sent to the light source.

    - When using a *LED Driver* module, the **Overdrive** checkbox will appear. When selected, this allows the system to exceed the normal safe current limit of the light source. **THIS SHOULD ONLY BE USED WITH PULSED SIGNALS, AS IT CAN OTHERWISE DAMAGE THE LIGHT SOURCE.**
    - When using a *LEDD*, the **Low-Power** checkbox will appear. When selected, this allows reduced-power signaling for the same voltage. This mode is only available for *CLED* modules. This allows low-power signals to be more stable in time. The maximal current is reduced to one tenth of light source's normal maximal current. If the **BNC Output** is used, the voltage of the signal is proportional to the current passing through the light source, and not the voltage sent to it. For example, a driver with a normal maximum current of 2000 mA for a 5 V signal (400 mA/V) will have a maximum current of 200 mA for a 5 V signal (40 mA/V). The **BNC output** of the driver will still relate LED current with a 400 mA/V conversion factor.

    d) The **Trigger Options** (Fig. 3.2) allow the selection of a number of trigger modes to activate a pulse sequence.

    - The **Manual** trigger mode is standard, and allows direction activation by the user.
    - The **Triggered** trigger mode is active when an input greater than 4 V is detected on the BNC input. Following input pulses will be ignored while the sequence is running. The sequence will restart with the arrival of the first input pulse after the sequence has finished.

- The **Gated** trigger mode is active 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.
- If the **TTL Output** option is checked, the output BNC channel can be used as a TTL generator. The monitoring signal will provide a TTL signal instead of an analog voltage output proportional to the LED current. The output will send out a 5 V signal whenever the input current is >0 mA. This can be used even if a light source is not connected.

3. The **Sequence options** box (Fig. 3.2) is where sequence options are defined depending on the mode. The **Continuous wave, External TTL** and **External Analog** modes have no additional sequence options.

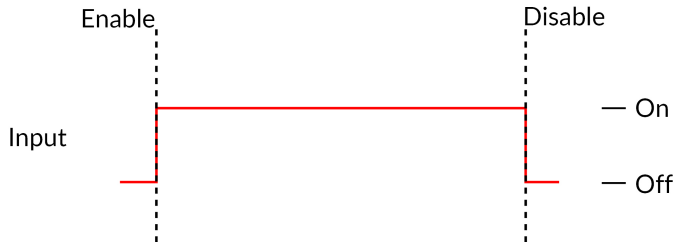


Figure 3.3: *Constant Current Mode Driver Signal*

a) The **Continuous Wave** mode (Fig. 3.3) produces a continuous signal at the chosen current. This mode can only be triggered manually. When this mode is active, the driver channel will show **CW** under **MODE**. This mode has no additional sequence options.

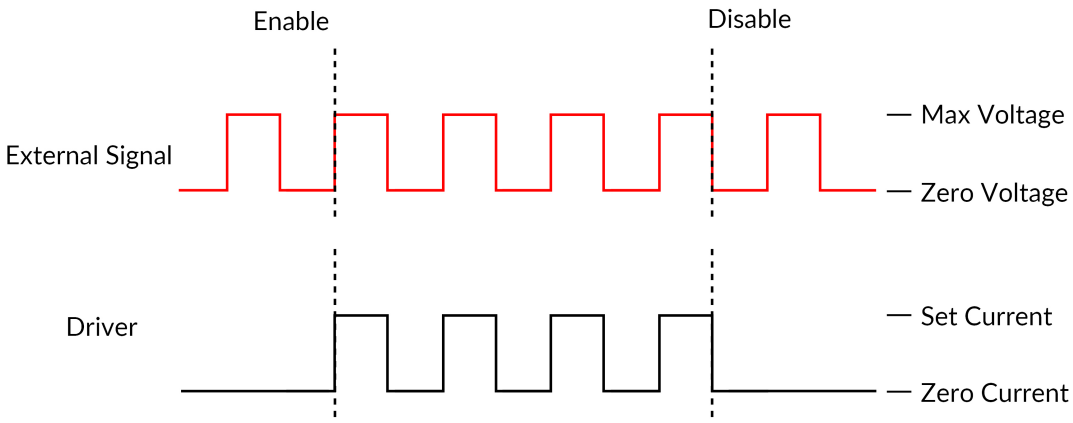


Figure 3.4: *Driver Signal Response to External Source in External TTL Mode*

b) The **External TTL** mode (Fig. 3.4) has the light source follow a TTL signal provided by an external source connected to the **BNC Input**. When this mode is active, the driver channel will show **TTL** under **MODE**. This mode has no additional sequence options.

c) The **External Analog** mode (Fig. 3.5) is similar to the External TTL, except that the current will be set by the voltage on the BNC input (Fig. 3.5, top). On the input BNC, a maximum voltage signal corresponds to a maximum driver current. Should the current set on the light source be less than the maximum current, any voltage corresponding to a higher current will clip the output waveform (Fig. 3.5, bottom). To avoid any clipping of the output waveform, the maximum current setting must be equal to or greater than the corresponding maximum analog input voltage. See the corresponding light source manual to find the voltage/current relationship. This mode has no additional sequence options.

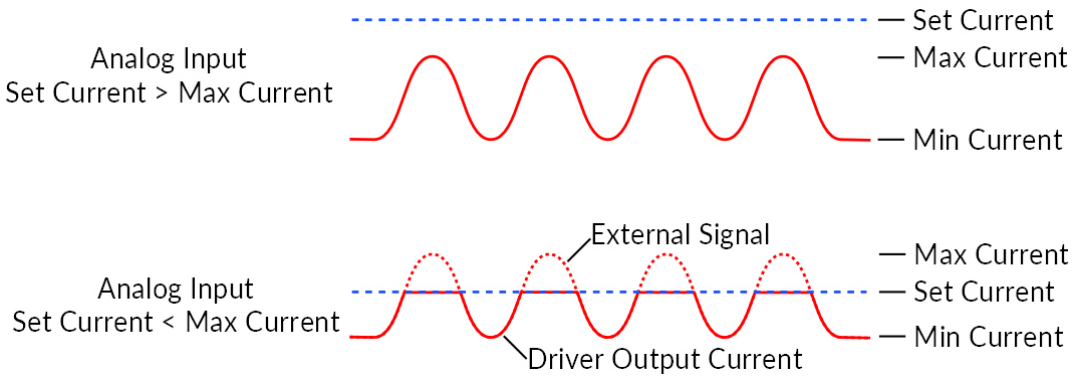


Figure 3.5: *Driver and Light Source in External Analog Mode*

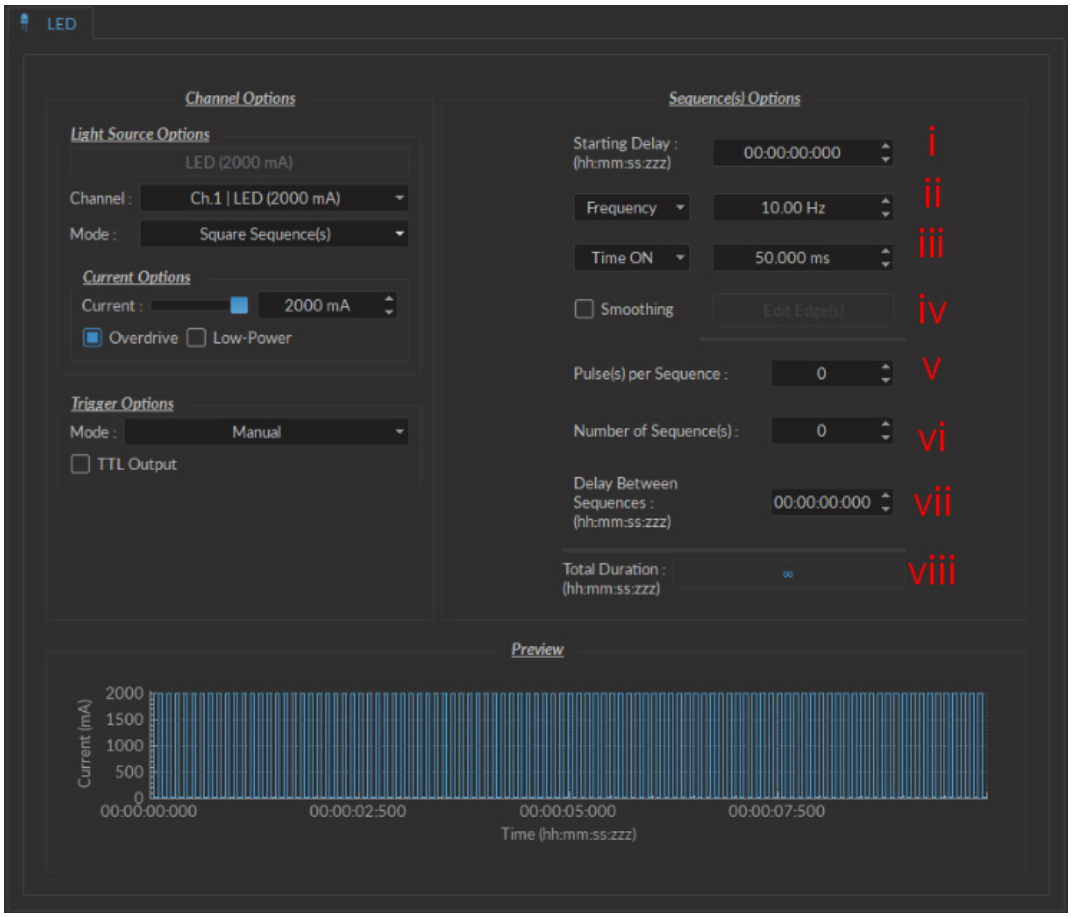


Figure 3.6: *Light Source Channel Configuration Window, square sequence options*

d) The **Square sequences** mode has the light source follow a square pulse sequence.
   i. The **Starting Delay** (Fig. 3.6) sets the delay (in hh:mm:ss:zzz format) before the first pulse.
   ii. The **Frequency/Period** (Fig. 3.6) sets the frequency (in Hz) or period (in ms) for the pulse sequence. For example, a signal at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a pulse sequence at 0.5 Hz (frequency) will output one pulse every 2000 ms (period).
   iii. The **Time ON/Duty Cycle** (Fig. 3.6) sets the time (in ms) or the duty cycle (in %) for each pulse. The **Time ON** must be lower than (1/frequency)+0.005 ms, while the **Duty cycle** must be below 100 %. These squares will appear red should an impossible **Frequency**/**time ON** be selected. Should the **Smoothing** option be selected, this feature becomes inaccessible.

iv. The **Smoothing** option is used to change the pulse slope in square pulse sequences. The **Edit Edges** button opens the **Smoothing Edge(s)** window (Fig. 3.60).

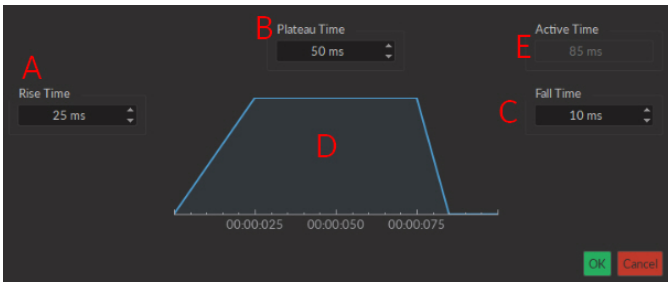


Figure 3.7: *Light Source Smoothing Edge(s) Window*

A. The **Rise Time** box is used to define the duration to rise from 0 to the pulse maximum.
B. The **Plateau Time** box is used to defined the duration the pulse is at its maximum value.
C. The **Fall Time** box is used to define the duration to descend from the pulse maximum to 0.
D. The **Pulse Graph** displays the pulse shape.
E. The **Active Time** box displays the total duration of the pulse. While the **Smoothing** option is active, the **Time ON** is fixed at this value.

v. The **Pulses per sequence** (Fig. 3.6) sets the number of pulses per sequence. If it is set to 0, the pulse will be repeated indefinitely.

vi. The **Number of sequences** (Fig. 3.6) sets the number of times that the sequence will be repeated. If it is set to 0, the sequence will be repeated indefinitely.

vii. The **Delay between sequences** (Fig. 3.6) sets the delay (in hh:mm:ss:zzz format) between each sequence if the **Number of Sequences** is greater than 1.

viii. The **Total Duration** (Fig. 3.6) displays the total time of the experiment. The different values can be *Inf* for infinite, a valid time value or *Err* if the **Time ON** value is greater than 1/frequency.

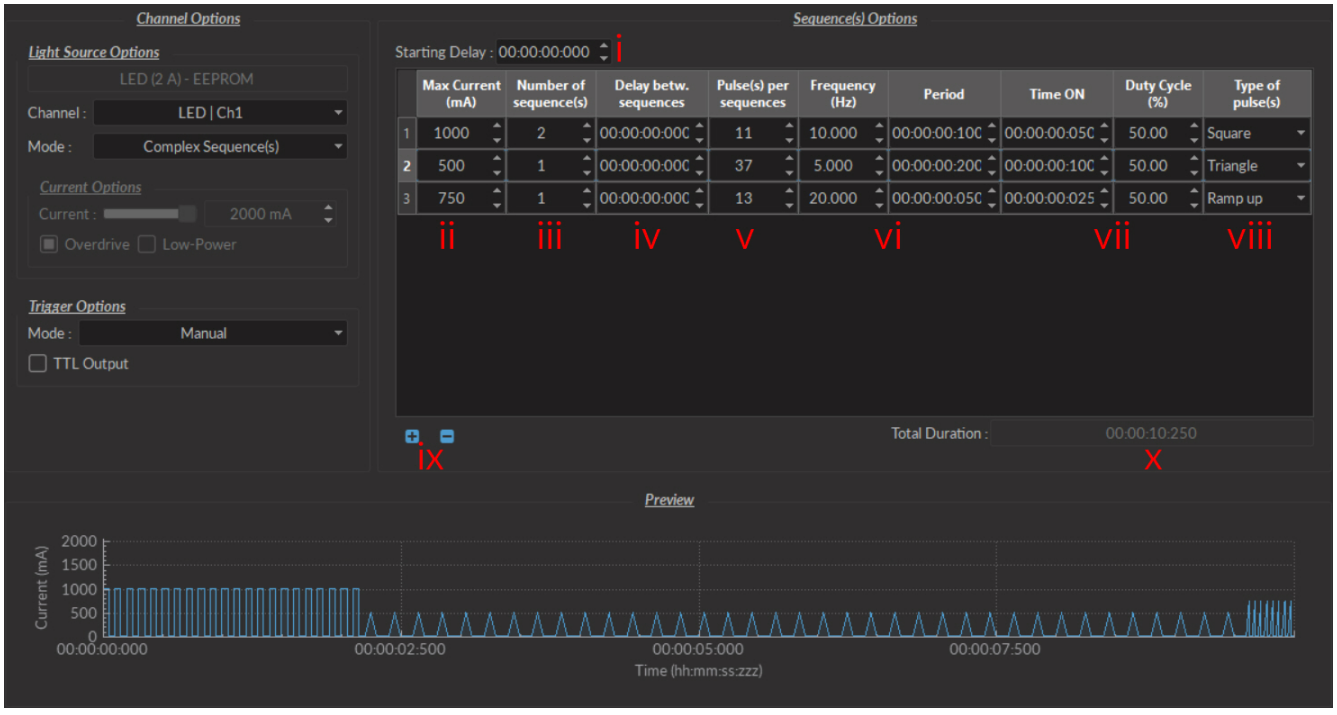


Figure 3.8: *Complex Sequences Window*

e) The **Complex Sequences** mode mode allows the design of complex pulse sequences. Multiple sequences can be combined to create a more elaborate pulse sequence. These are displayed in a spreadsheet format.

i. The **Starting Delay** (Fig. 3.8) sets the delay (in hh:mm:ss:zzz format) before the first pulse sequence.
ii. The **Max Current** (Fig. 3.8) sets the maximum current (in mA) for the given sequence.
iii. The **Number of sequences** (Fig. 3.8) sets the number of times that the sequence will be repeated, with a minimum of 1.
iv. The **Pulses per sequence** (Fig. 3.8) sets the number of pulses per sequence, with a minimum of 1.
v. The **Delay between sequences** (Fig. 3.8) sets the delay (in hh:mm:ss:zzz format) between each sequence if the **Number of Sequences** is greater than 1.
vi. The **Frequency/Period** (Fig. 3.8) sets the frequency (in Hz) or period (in ms) for the pulse sequence. These two values are linked, and when one is changed the other will adjust automatically. For example, a signal at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a pulse sequence at 0.5 Hz (frequency) will output one pulse every 2000 ms (period).
vii. The **Time ON/Duty Cycle** (Fig. 3.8) sets the time (in ms) or the duty cycle (in %) for each pulse. These two values are linked, and when one is changed the other will adjust automatically. The **Time ON** must be lower than (1/frequency)+0.005 ms, while the **Duty cycle** must be below 100 %.

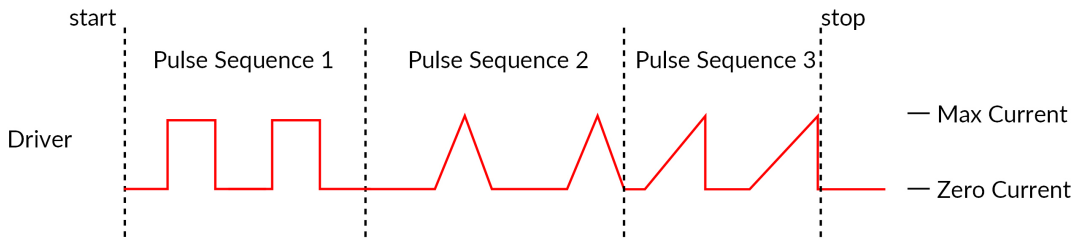


Figure 3.9: *Internal Complex Mode Pulse Sequences*

viii. The **Types of pulses** (Fig. 3.8) sets the pulse type. Pulses can be **Square**, triangular (**Triangle**), **Ramp up Ramp down** or **Delay** (Fig. 3.9). The **Delay** pulse type is used to create a delay between different sequence
ix. The **Sequence controls** (Fig. 3.8) allow the addition (**+**) or removal (**-**) of sequences to the spreadsheet.
x. The **Total Duration** (Fig. 3.8) displays the total time of the experiment. The different values can be *Inf* for infinite, a valid time value or *Err* if the **Time ON** value is greater than 1/frequency.

f) The **Scope** mode allows the measurement of electrical signal using the driver (Fig. 3.10). The signal is received by the Input BNC of the chosen channel on the light source driver.

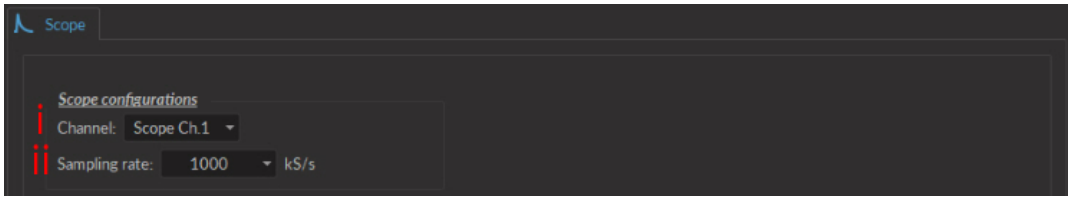


Figure 3.10: *Scope*

i. The **Channel** drop-down list indicated which driver channel will be used to receive signal. The chosen can be used to drive a light source while serving as a scope.
ii. The **Sampling Rate** drop-down list allows the selection of the rate (in kilosamples per second) at which measurements are taken.

4. The **Preview** box (Fig. 3.2) displays a preview of the chosen sequence while in the **Continuous Wave**, **Square Sequences** and **Complex Sequences** mode.

5. The **Apply** button (Fig. 3.2) will generate the defined channel OR update an already configured channel with any changes.

3.1.2 Control & Settings

The **Control & settings** sections is used to control the light source. It includes the following elements.

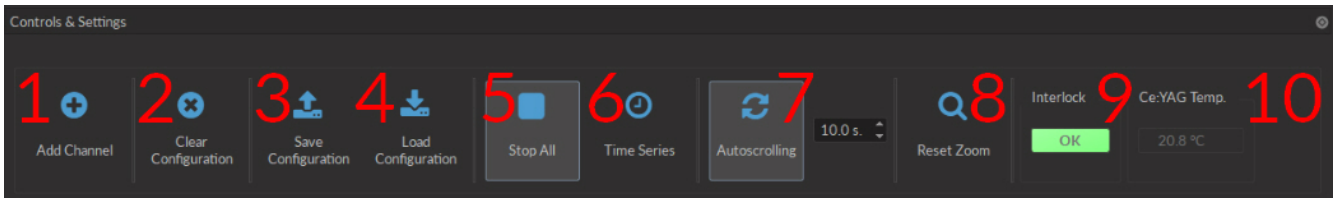


Figure 3.11: *Control & Settings*

1. The **Add channel** button (Fig. 3.11) opens the **Channel Configuration** window 3.2. See section 3.1.1 for more details.
2. The **Clear Configuration** button (Fig. 3.11) clears all configuration channels. Cleared channels cannot be recovered unless previously saved.
3. The **Save configuration** button saves all currently configured channels in **.doric** format.
4. The **Load configuration** button loads a file in **.doric** format that contains a previously saved set of configured channels.
5. The **Start All** button (Fig. 3.11) starts all currently configured channels.
6. The **Time Series** button opens the **Time Series** window. This tool allows all channels to share the same timing.

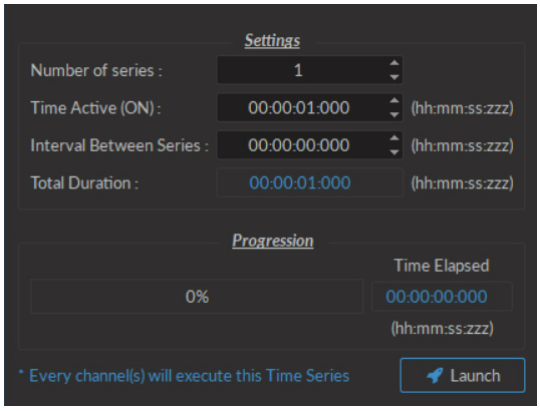


Figure 3.12: *Control & Settings, Time Series Window*

- The **Number of series** (Fig 3.12) sets the number of times that the sequence will be repeated, with a minimum of 1.
- The **Time Active** sets the duration of each series in hh:mm:ss:zzz format. If the **Time series** is used in combination with a sequence, the **Time Active** should be greater than the sequence **Total Time** If the **Time Active** is shorter, the sequence will be stopped after the **Time Active**.
- The **Interval between series** sets the duration between each series in hh:mm:ss:zzz format.
- The **Total Duration** displays the total duration of the sequence in hh:mm:ss:zzz format.
- The **Progression** bar displays the progression of the sequence in %, while the **Time Elapsed** counter displays the progression in hh:mm:ss:zzz format.
- The **Launch** button starts the sequence.

7. The **Autoscrolling** button activates the autoscroll function. When active, the **Graph** in the **Acquisition View** will follow a section as wide as the time defined beside the button.

8. The **Reset Zoom** button resets the axes in the **Graph** to their standard values.

9. The **Interlock** indicator displays OK when the interlock is correctly connected, and OPENED when disconnected.

10. The **Ce:YAG Temp** indicator displays the temperature of the *Ce:YAG source* in real time. This indicator will only appear when a *Ce:YAG driver* is connected to the computer. Should the temperature be too high the temperature will appear in red. Should the temperature be too low, the temperature will appear in blue.

3.1.3 Experiment View

The **Experiment View** box is used to display information related to the usage of each channel. This section allows limited control of the light source while it is active.

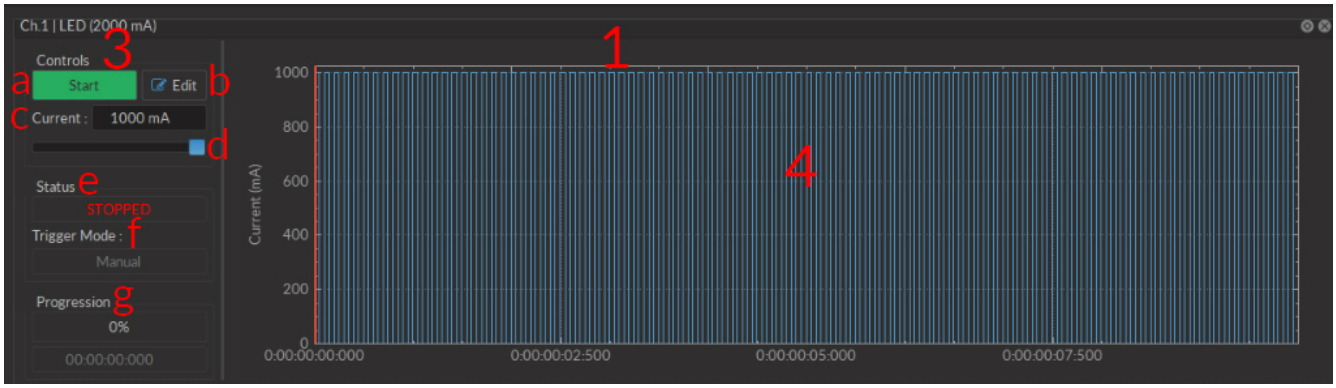


Figure 3.13: *Experiment View, Light Source Channel*

1. The **Light Source Channel** box (Fig. 3.13) contains all elements related to a single light source channel.

2. The **Scope Channel** box (Fig. 3.14) is used to control and configure an active **Scope**.

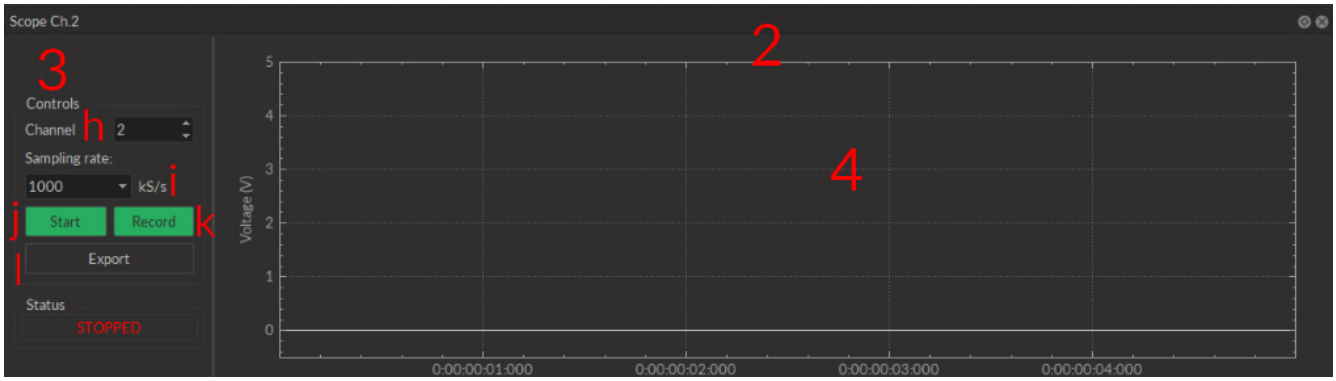


Figure 3.14: *Experiment View, Scope Channel*

3. The **Controls View** displays all elements to control/configure the channel.

   a) The **Start/Stop** button activates/deactivates the light source connected to the **Light Source Channel**.

   b) The **Edit** button opens the **Channel configuration** window to edit the pulse sequence. This button is only accessible when the channel is deactivated.

   c) The **Current Box** box allows the current to be changed exactly (in mA).

   d) The **Current Slider** allows the light source current to be adjusted.

   e) The **Status** box displays the status of the channel (**Light source** or **Scope**). The **Status** will display RUNNING... when active and STOPPED when deactivated.

f) The **Trigger Mode** of the light source is displayed in this box.

g) The **Progression** box displays the progression of the pulse sequence.The advancement of the sequence is displayed % on the **Progression bar**, and in hh:mm:ss:zzz format on the **Time Elapsed** box.

h) The **Channel** drop-down list is used to chose the channel used as a scope.

i) The **Sampling Rate** drop-down list allows the selection of the rate (in kilosamples per second) at which measurements are taken.

j) The **Start** scope channel button activates a live measurement sequence. Important measurements should not be made as a live measurement, as these only conserve a small amount (60 s) of data.

k) The **Record** scope channel button starts a recorded measurement sequence.

l) The **Export** scope channel button allows the recording of a live measurement sequence on the scope.

4. The **Graph View** displays either a preview of the pulse sequence for **Light Source Channels** or the received signal for the **Scope Channel**.

3.2 Fiber 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.

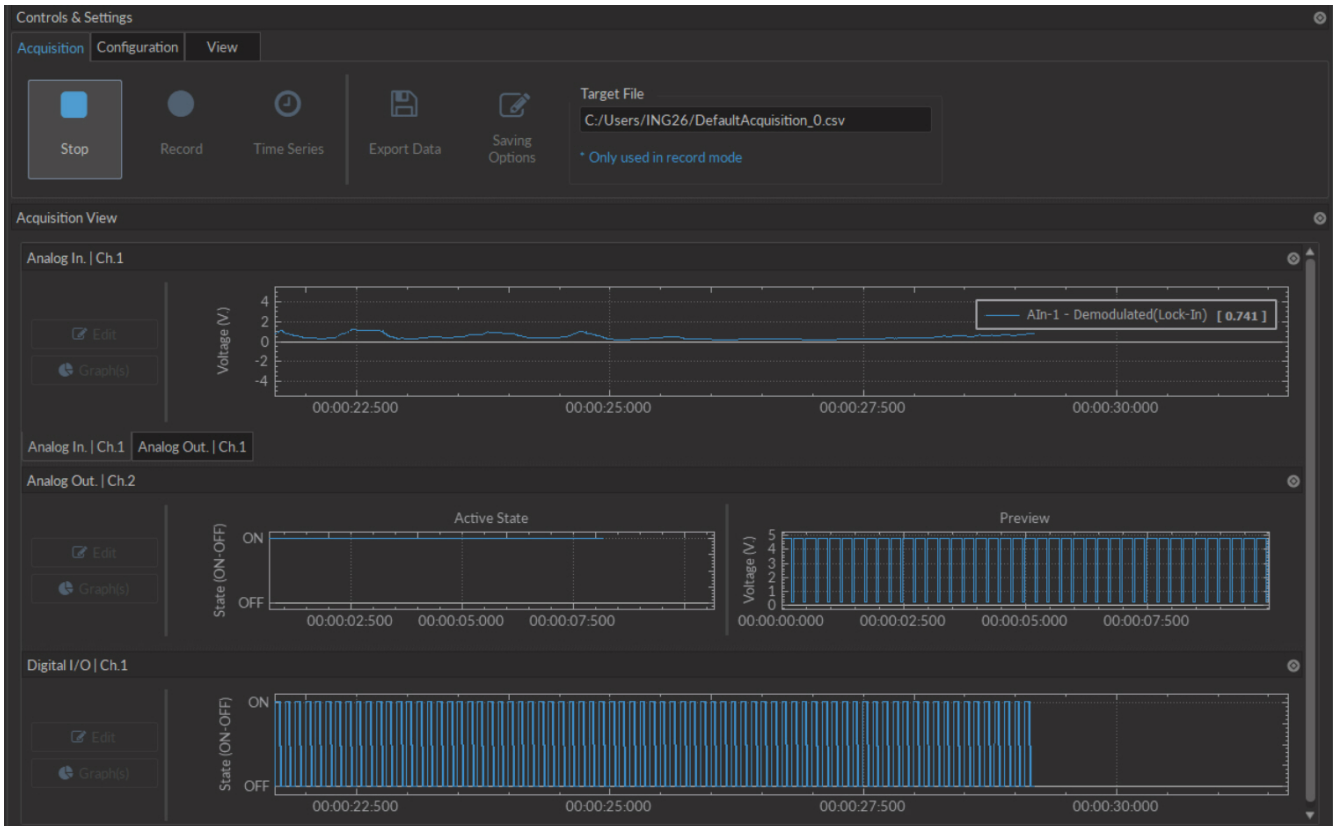


Figure 3.15: *Console User Interface*

3.2.1 Channels

The console has three different types of channels. The **Digital I/O** (Section 3.2.1) channels allow the input and output of TTL signals. The **Analog Output** (Section 3.2.1) channels allow the output of analog signals. The **Analog Input** (Section 3.2.1) channels allow the input of analog signals. Any number of channels may be added using the **Add channel** button on the **Configuration** tab (Section 3.2.2). The **Channel(s) configuration** window contains the following elements shared by most channel types.

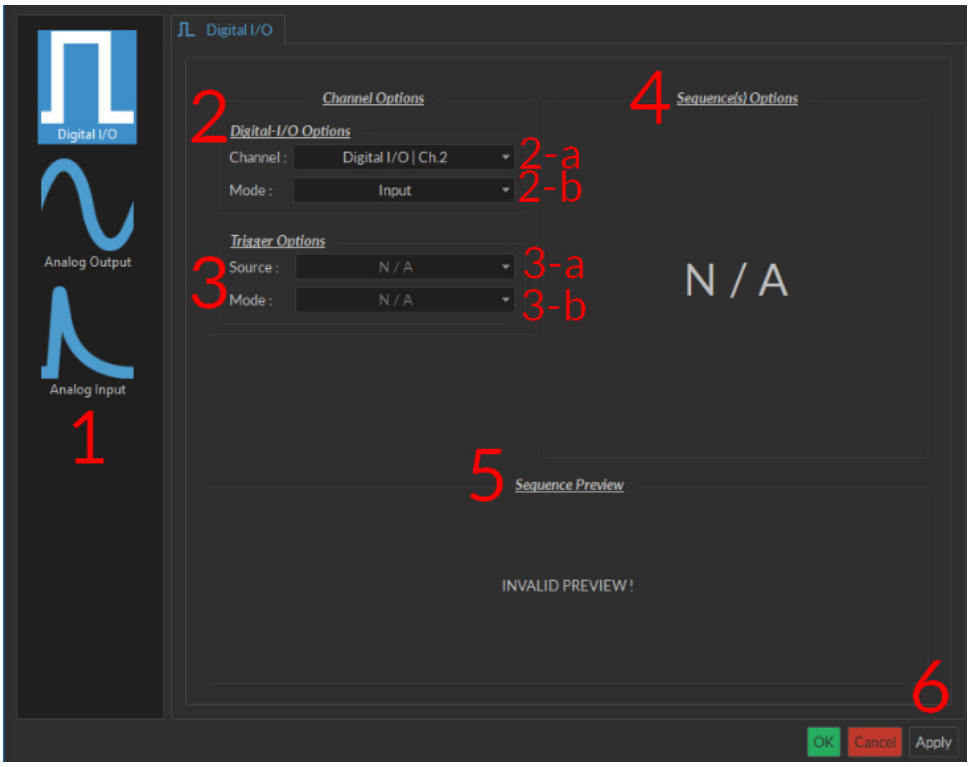


Figure 3.16: *Channel(s) configuration window, Digital I/O input*

1. The **Channel Types** (Fig. 3.16) 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. 3.16) 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. 3.16) 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.

4. The **Sequence Options** (Fig. 3.16) 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/**Frequency**), the color of the option boxes will turn **RED**.
5. The **Sequence Preview** (Fig. 3.16) section allows visualization of the output sequence that will play by selecting the channel in the graph.
6. The **Apply** button (Fig. 3.16) 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.

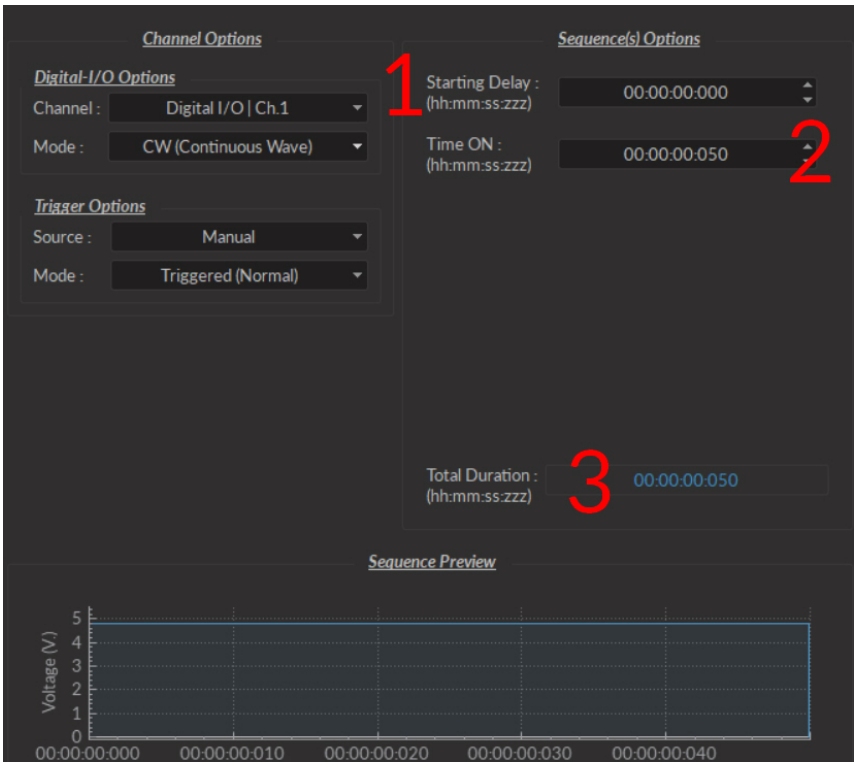


Figure 3.17: *Channel(s) configuration window, Digital I/O CW*

- The **CW(Continuous Wave)** channel mode (Fig. 3.17) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.
    1. The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. 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.
    3. 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. 3.18) 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 3.16) 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.

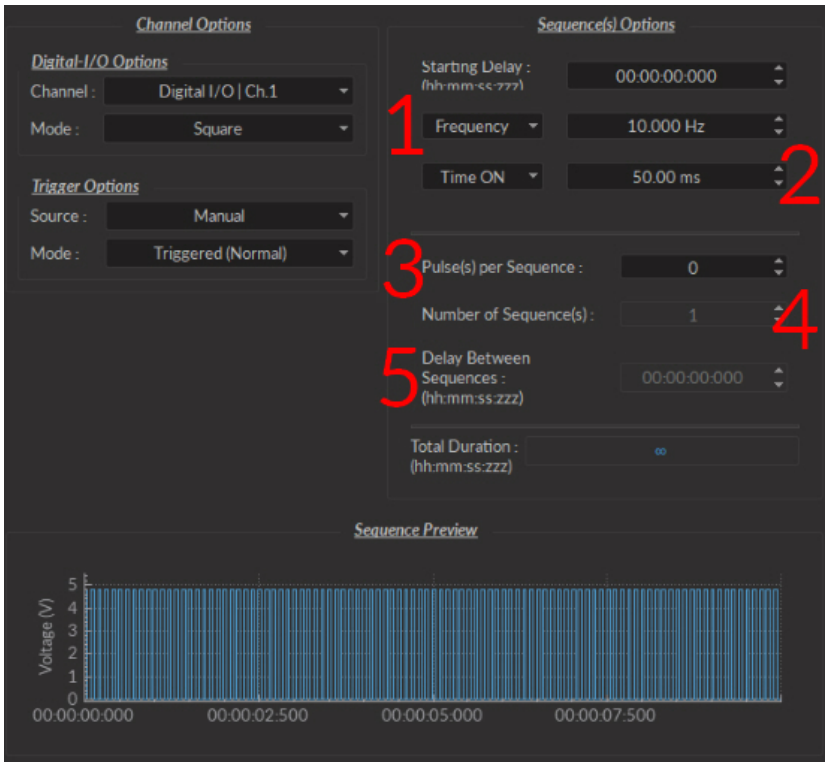


Figure 3.18: *Channel(s) configuration window, Digital I/O square*

Analog Output

Figure 3.19: *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. 3.19) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box.
    1. The **Starting Delay** (Fig. 3.19) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Time ON** (Fig. 3.19) 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. 3.19) defines the voltage of the continuous signal, in volts. The signal cannot go beyond ±4.75 V.
    4. The **Total Duration** (Fig. 3.19) 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**.

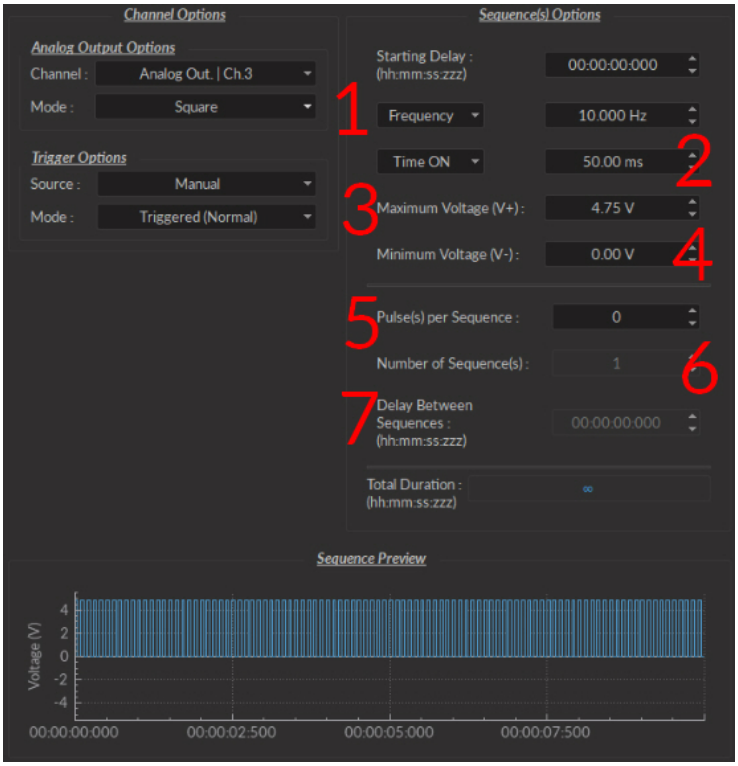


Figure 3.20: *Channel(s) configuration window, Analog Output Square*

- The **Square** channel mode (Fig. 3.20) 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. 3.20) 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. 3.20) 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. 3.20) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.
    4. The **Minimum Voltage (V-)** (Fig. 3.20) defines the minimum voltage of each pulse, in volts. The signal cannot go beyond -4.75 V.
    5. The **Pulse(s) per sequence** (Fig. 3.20) 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. 3.20) sets the number of times that the sequence will be repeated.
    7. The **Delay between sequences** (Fig. 3.20) sets the delay between each sequence.

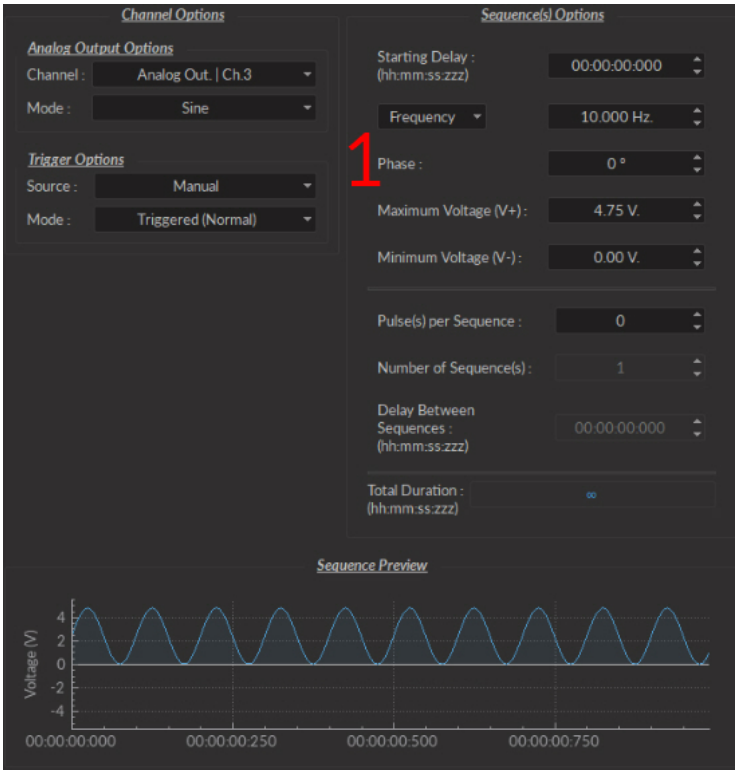


Figure 3.21: *Channel(s) configuration window, Analog Output Sine*

- The **Sine** mode (Fig. 3.21) 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. 3.21). This allows the choice of the sine wave phase, in degrees.

Analog Input

The **Analog Input** channels (Fig. 3.22) 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.

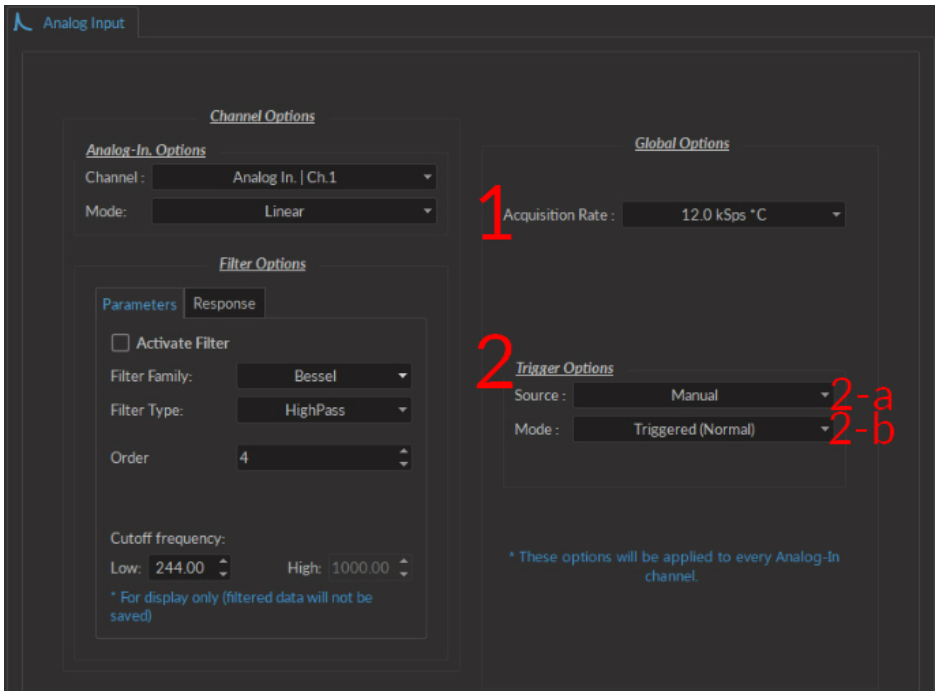


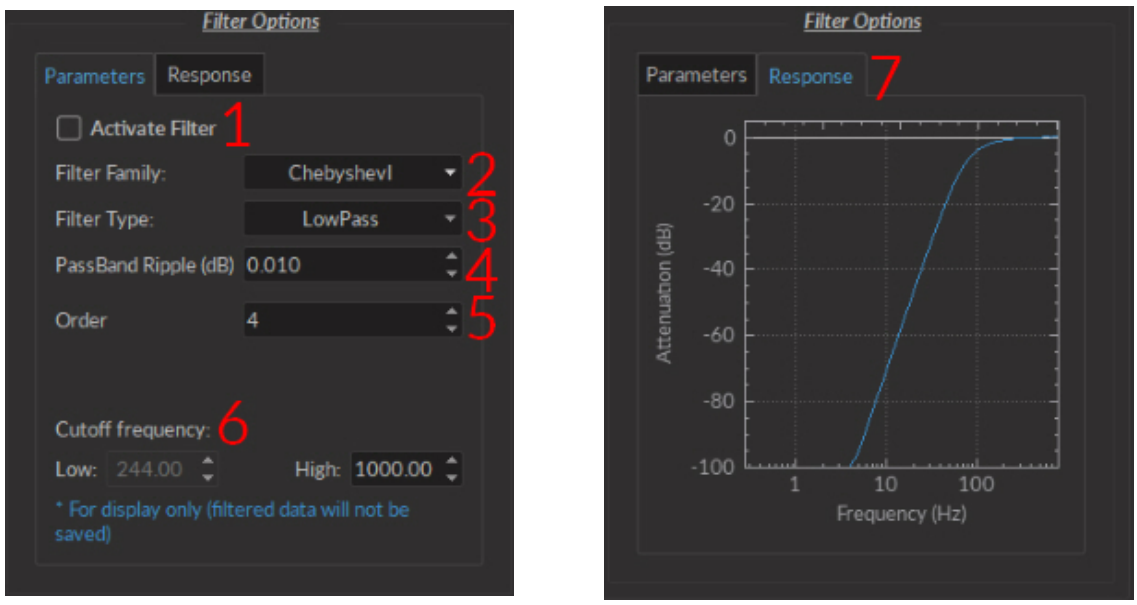
Figure 3.22: *Channel(s) configuration window, Analog Input Linear*

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

1. The **Acquisition Rate** (Fig. 3.22) 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 * rate [in kSps] 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 paramters. The function of each **Mode** is described here.

- The **Linear** channel mode (Fig. 3.22) 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 I)** 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 3.23: *Linear Mode Filter Options*

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

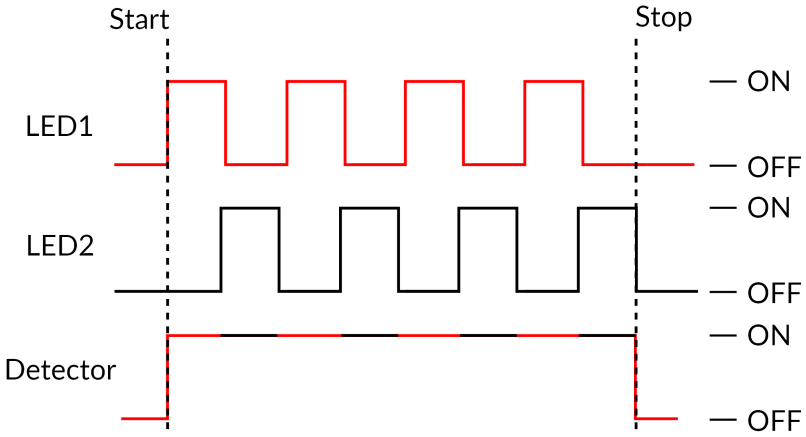


Figure 3.24: *Interleaved Acquisition Timing Diagram*

- 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. 3.24). A more detailed procedure to use this modality is included in section 3.2.4.

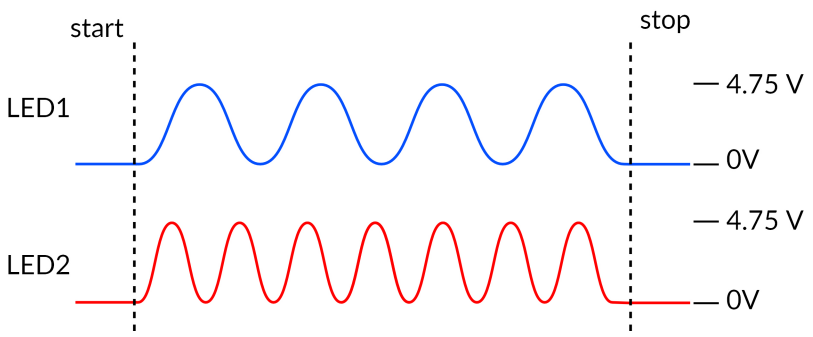


Figure 3.25: *Lock-in Acquisition Timing Diagram*

- 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. 3.25). A more detailed procedure to use this modality is included in section 3.2.5.

3.2.2 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.

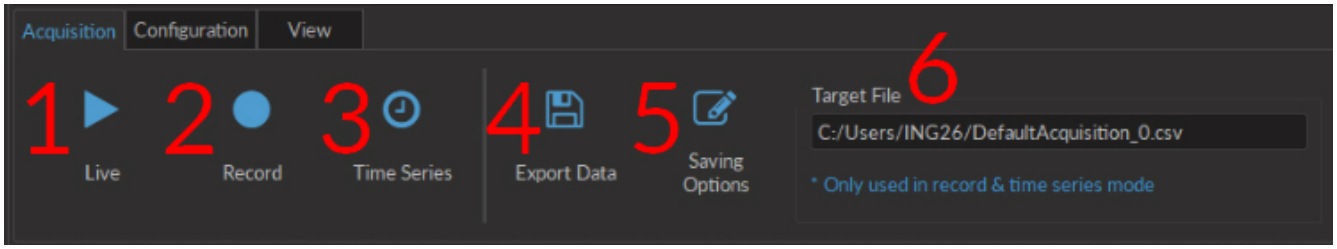


Figure 3.26: *Acquisition Tab*

1. The **Live** button (Fig. 3.26) 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. 3.26) activates all prepared channels while periodically saving recorded data to the disk. This mode is recommended for long measurement sequences.

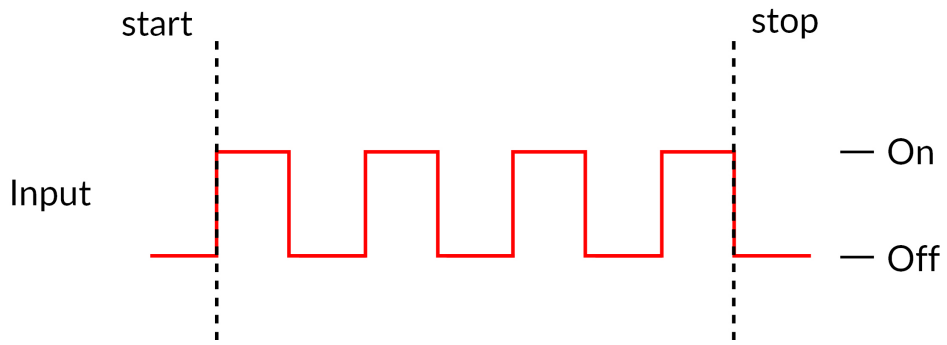


Figure 3.27: *Time Series Acquisition Timing Diagram*

3. The **Time Series** button (Fig. 3.26) opens the **Time Series Window** (Fig 3.28). 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.

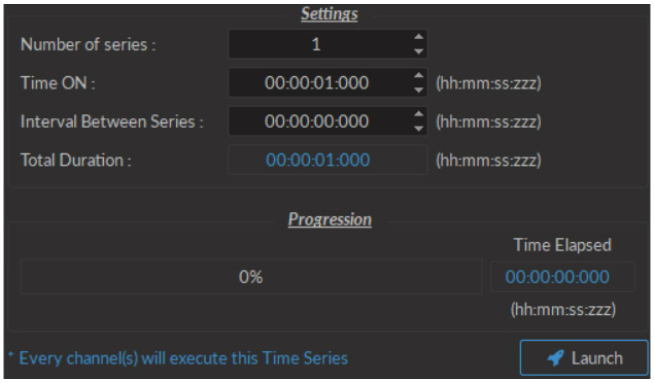


Figure 3.28: *Time Series Window*

- 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. 3.26) 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. 3.26) button opens the **Saving Menu** window (Fig. 3.29).

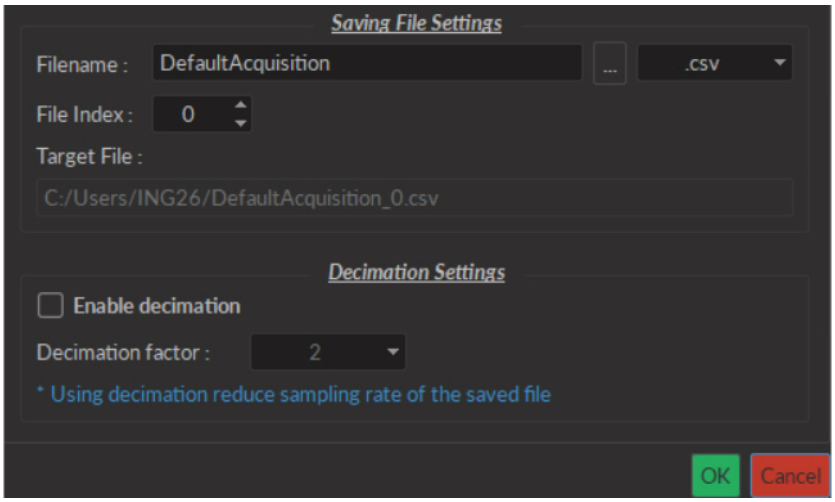


Figure 3.29: *Saving Menu Window*

- 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. 3.26) 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.

[1]For 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

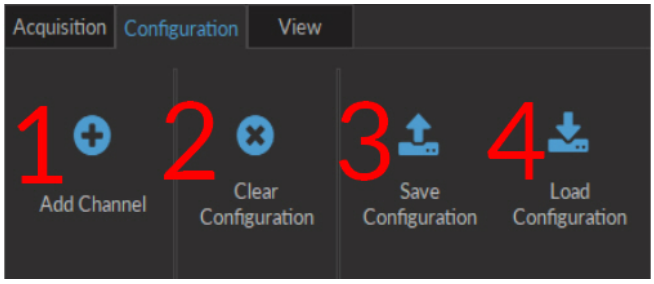


Figure 3.30: *Configuration Tab*

1. The **Add Channel** button (Fig. 3.30) opens the **Channels configuration** window. This window is detailed in section 3.2.1.
2. The **Clear configuration** button (Fig. 3.30) resets the acquisition view and all other parameters set. Any configurations already set will be lost.
3. The **Save configuration** button (Fig. 3.30) 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. 3.30) allows a console configuration in **.doric** format to be loaded.

View Tab

The **View Tab** (Fig. 3.31) is used to modify the presentation of graphs in the **Acquisition view**.

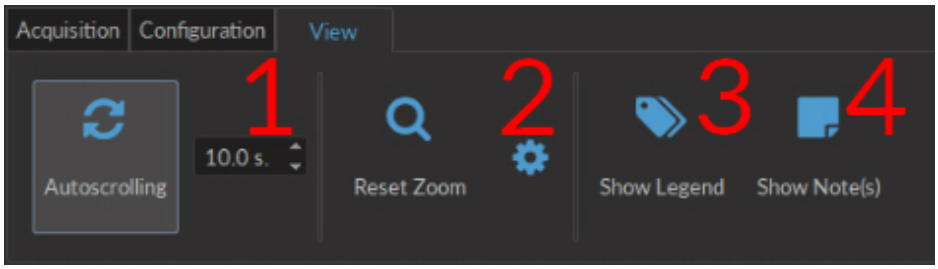


Figure 3.31: *View Tab*

1. The **Autoscrolling** button (Fig. 3.31), 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. 3.31) sets the graph zoom to a pre-defined value. This value is defined using the **Zooming menu** (Fig. 3.32), accessed using the **Gear** button.

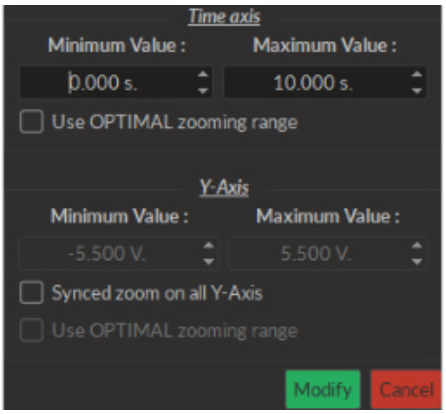


Figure 3.32: *Zooming Menu Window*

- 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. 3.31) will open the **Legend** window (Fig. 3.33). 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)				
AIn-1		Physical	-	-

Figure 3.33: *Legend Window*

4. The **Show Notes** button (Fig. 3.31) will open the **Note Viewer** window (Fig. 3.34). Selecting the button while a window is open will close the window.

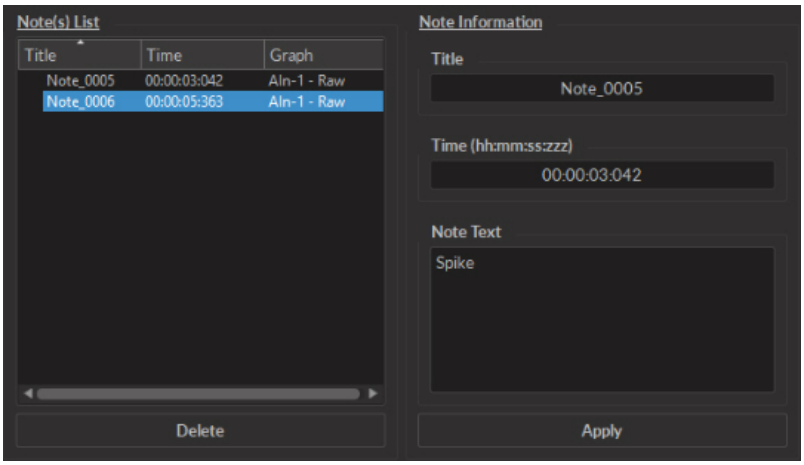


Figure 3.34: *Note Viewer Window*

- 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.

3.2.3 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.

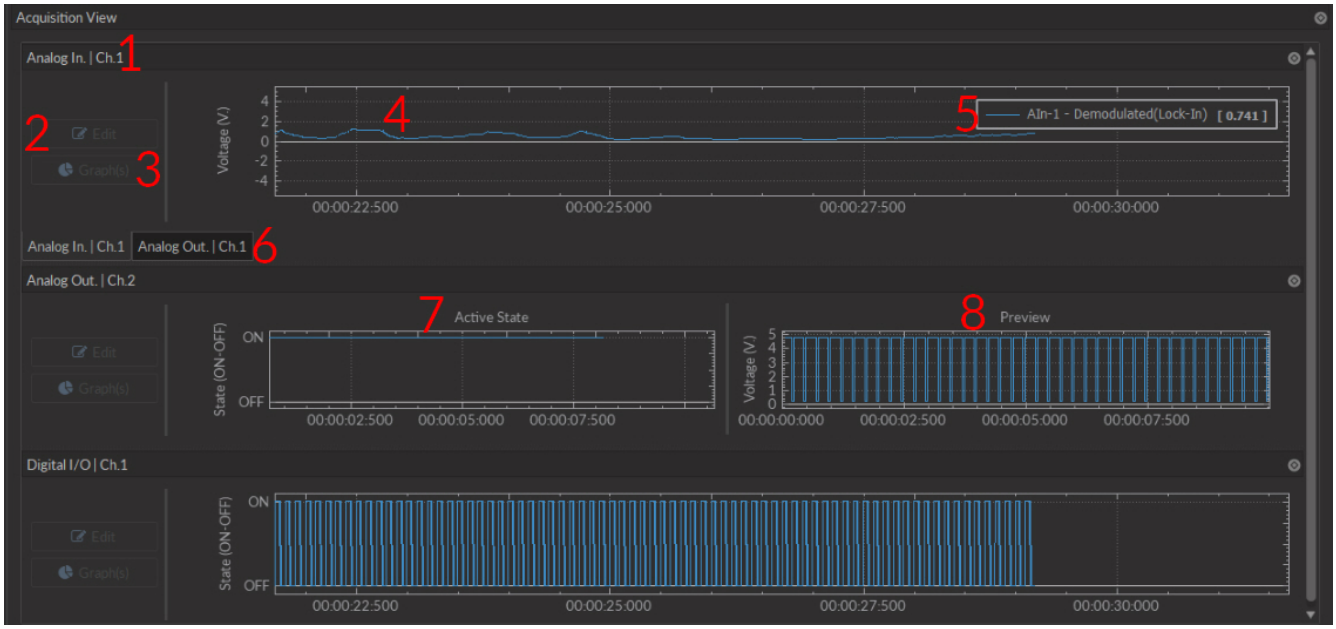


Figure 3.35: *Acquisition View Box*

1. The **Channel name** is located on the upper left of the **Channel box** (Fig. 3.35), identifying the type of channel and it's number, corresponding to that on the console. This name can be modified in the **Graph options** window.
2. The **Edit** button (Fig. 3.35) allows the editing of channel parameters, opening the channel configuration windows. For further information, see section 3.2.1.

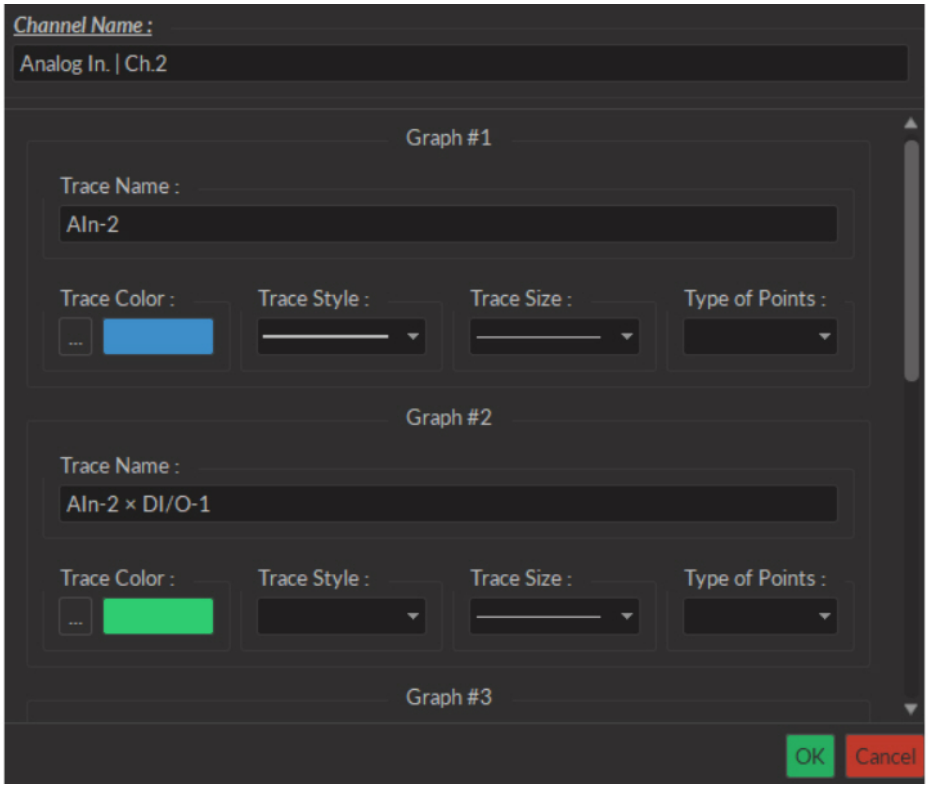


Figure 3.36: *Graphs Window*

3. The **Graph(s)** (Fig. 3.35) button opens the **Graph Options** window.

- The **Channel Name** (Fig. 3.36) is indicated in the upper left.
- The **Trace # X** box (Fig. 3.36) (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. 3.37), 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.

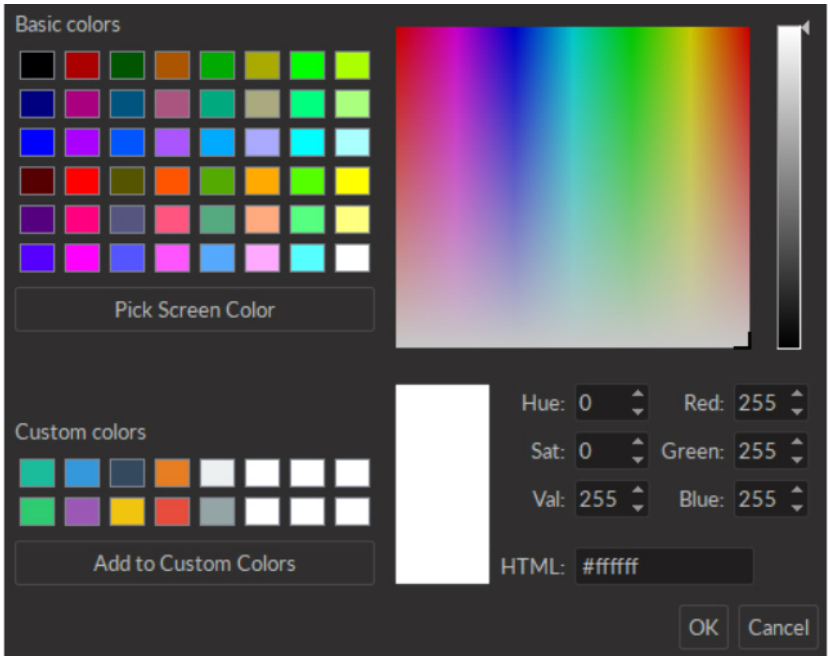


Figure 3.37: *Select Color Window*

  - 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 nd 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. 3.35) 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. 3.35) 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. 3.35) 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. 3.35). 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. 3.35), showing a preview of the pulse sequence.

3.2.4 Interleaved measurement

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. 3.38) 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.

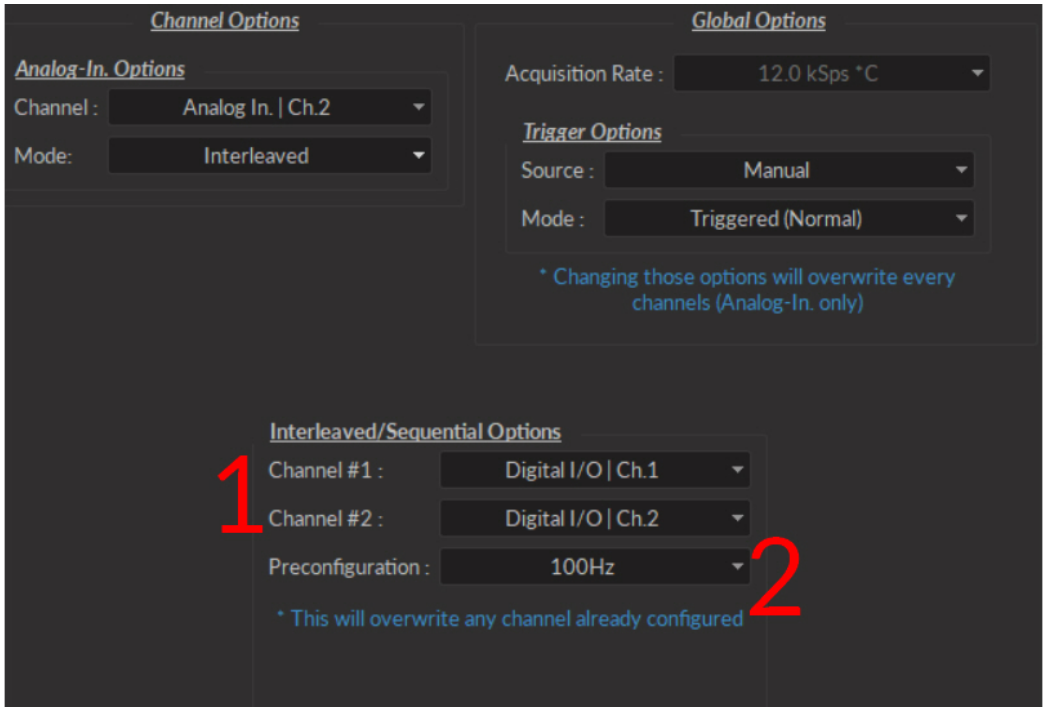


Figure 3.38: *Channels configuration, Analog Input Interleaved Mode*

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**.

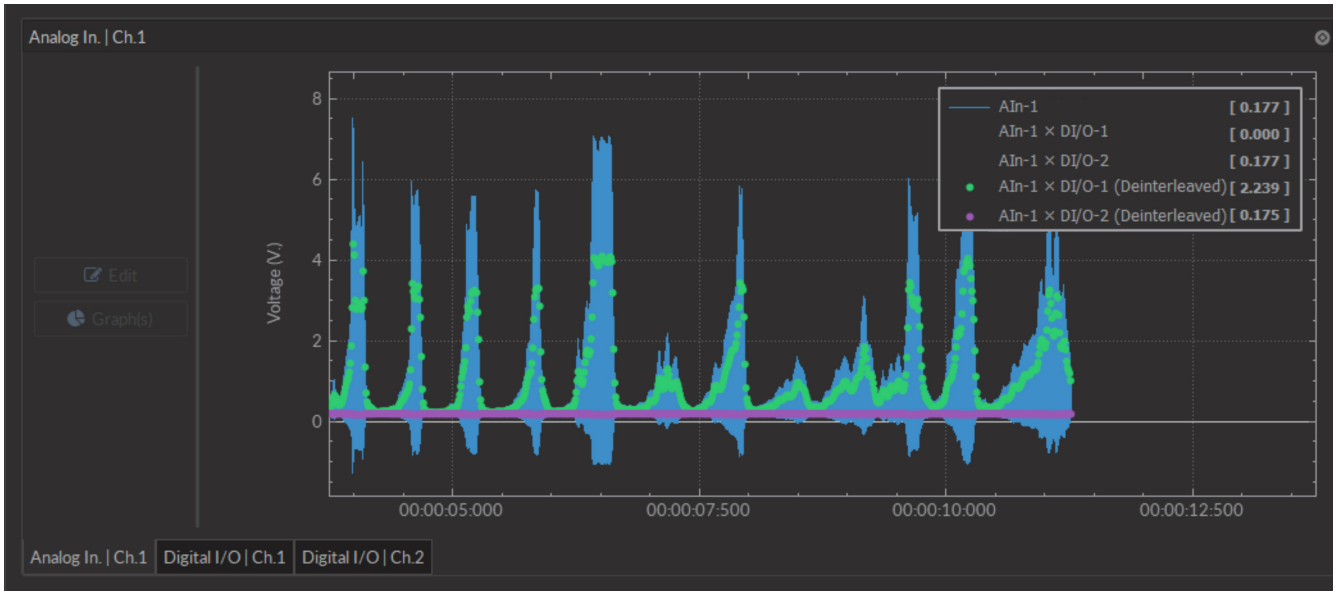


Figure 3.39: *Acquisition View, Interleaved Mode Traces*

- The **Raw Data** (AIn-1) (Fig. 3.38) shows the raw signal received on the input channel.
- The **Intermediate Data** (AIn-1 x DI/O-1) (Fig. 3.38) 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. 3.38) shows the averaged signal of each demodulated pulse. This leaves a single averaged point per pulse sent by the output channels.

3.2.5 Lock-in Measurement

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. 3.40) appears, containing the following parameters.

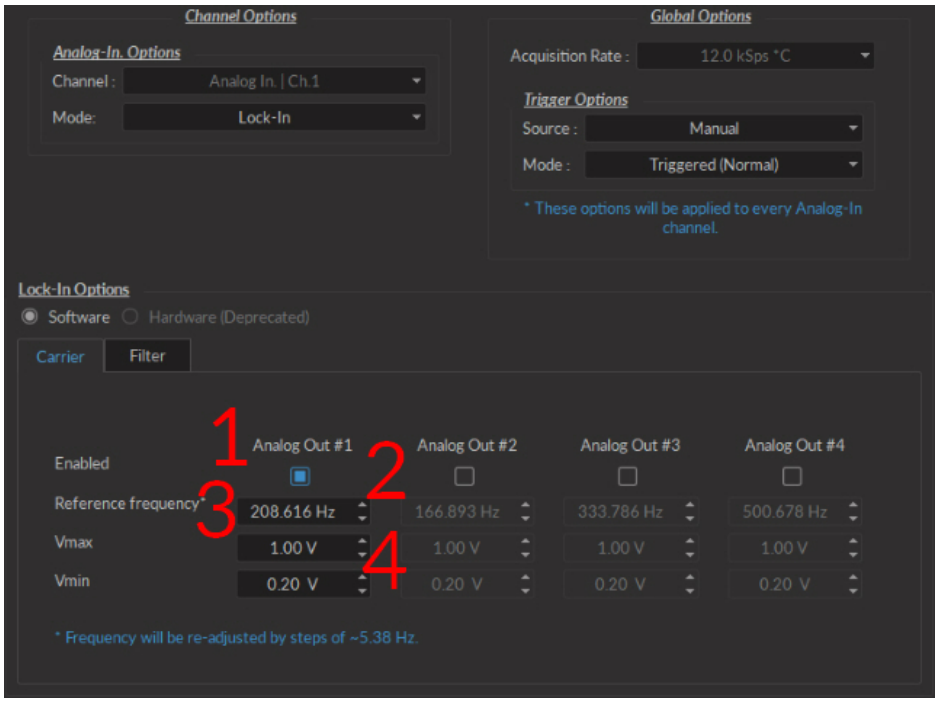


Figure 3.40: *Channels configuration, Analog Input Lock-in Mode, Carrier Tab*

1. The **Channel List** (Fig. 3.40) 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. 3.40) allows the choice of a reference frequency. The reference frequency should not be a multiple a of known noise frequency (e.g. 60 Hz), or a multiple of another reference frequency.
4. The **Vmax/Vmin** (Fig. 3.40) 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. 3.41) (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.

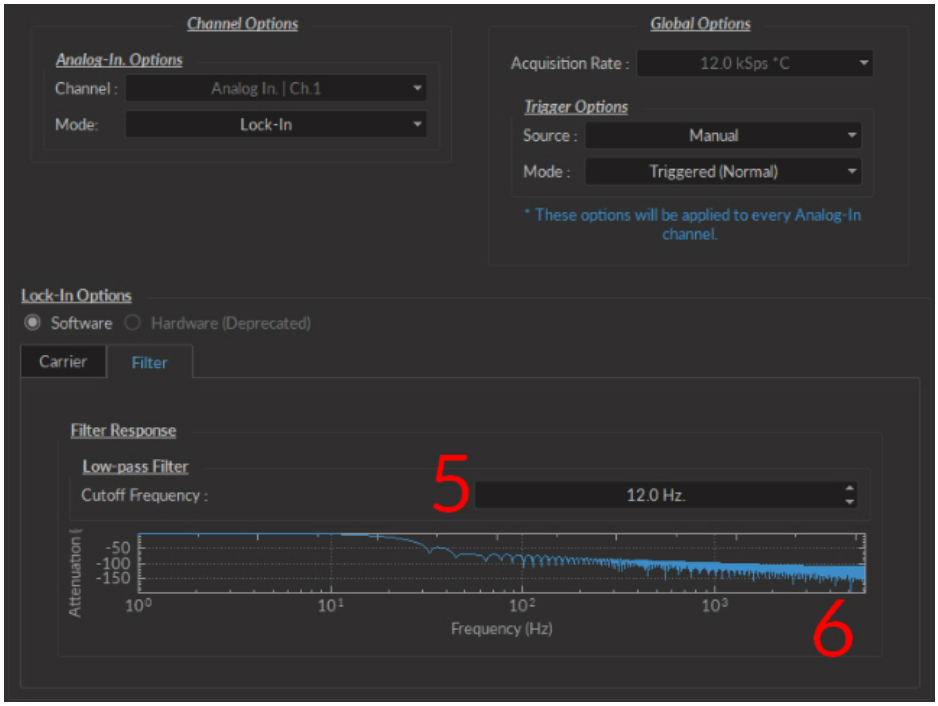


Figure 3.41: *Channels configuration, Analog Input Lock-in Mode, Filter Tab*

3.3 Behavior Tracking Camera

Our Behavior Tracking Camera is a great addition to any experiment. The filming of the animal is complementary information needed to establish a correlation between the neuronal activity of a specific brain region and animal behavior. The interface from the software (Fig. 3.42) provides a framework for streaming high-speed video and related control data over computer networks.

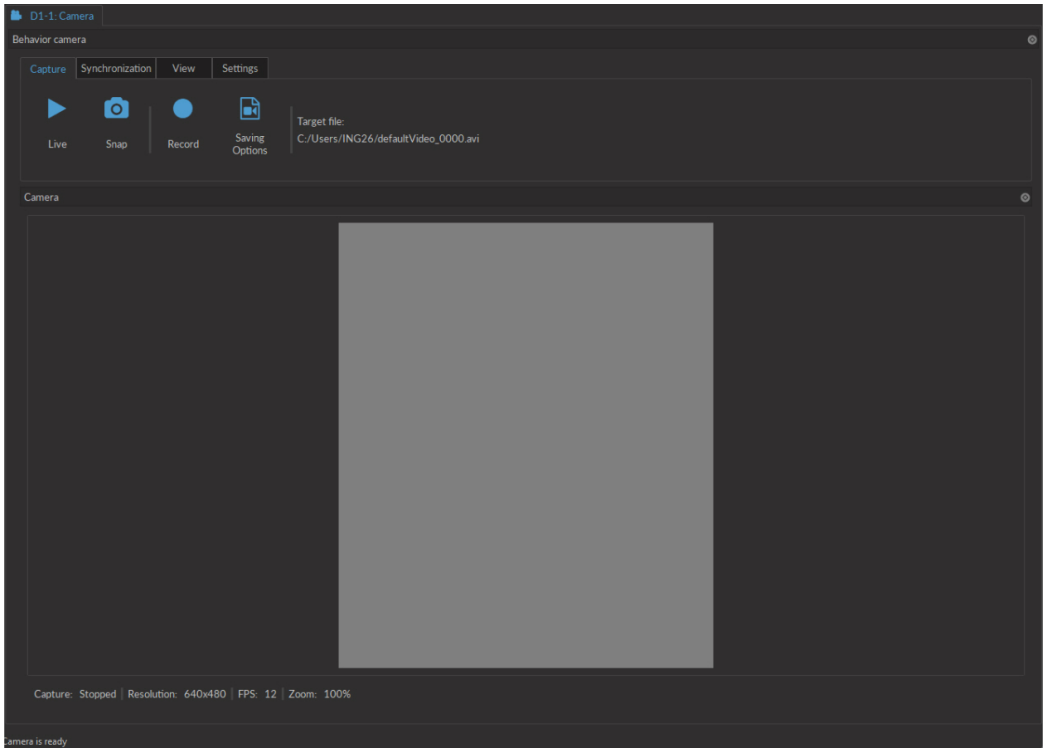


Figure 3.42: *Camera Module*

The constant live feed allows the status of the camera to be followed. On the bottom left are shown **Resolution** (in X by Y pixels), **FPS** (in Frames Per Second) and **Zoom** (in %). In addition to the constant live feed, the module contains four tabs allowing the the configuration and control of the camera.

1. The **Capture** tab (Fig. 3.43) contains the controls related to image and movie capture, and saving.

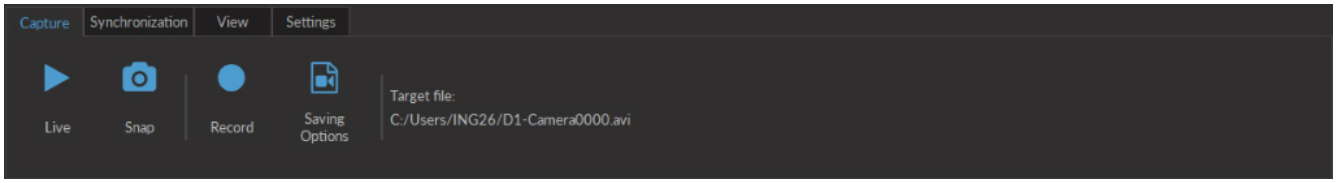
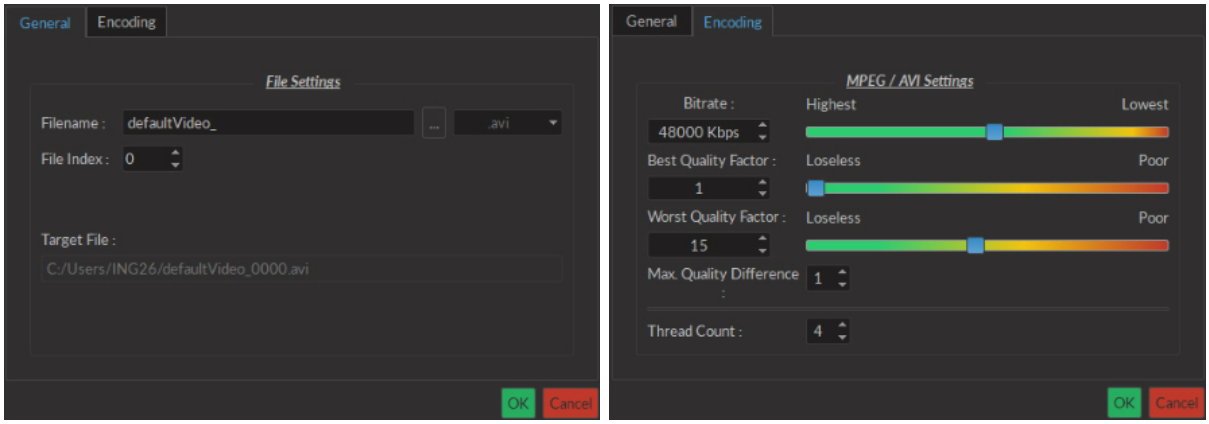


Figure 3.43: *Camera Module - Capture Tab*

- The **Live** button acquires images and displays them. These images are for display only and cannot be saved.
- The **Snap** button saves one image to a user-defined file. Live mode must be active to acquire images.
- The **Record** button acquires a continuous image stream, and saves it to a user-defined file as one .AVI file.
- The target file for recording is defined by the **Saving Options** window and shown in the **Target File** label.
  a) The **General** tab is used to define basic file setting.
     – The **Filename** box is used to define the name of the recorded video file. Currently, all videos are saved in *.avi* format.



(a) *Saving Options Window-General* (b) *Saving Options Window-Encoding*

Figure 3.44: *Saving Options Window*

- The **...** button is used to define the target directory where the video will be saved.
- The **File Index** box is used to choose the index that follows the **Filename**.

b) The *Encoding* tab is used to choose video encoding quality. Most elements can be changed either using the appropriate **Text Box** or **Slider**.
   - The **Bitrate** sets the number of bits recorded per second. Larger, and larger-resolution images require a higher **Bitrate**.
   - The **Best Quality Factor** and **Worst Quality Factor** are used to define the compression of saved video, with a factor of 1 implying no compression, and a factor of 31 for maximal compression. The **Best Quality Factor** indicates the lowest-compression frames accepted, while the **Worst Quality Factor** indicated the highest-compression frames accepted.
   - The **Max Quality Difference** box indicated the maximal compression difference between two subsequent video frames.
   - The **Thread Count** defines the number of processing threads (real and virtual) used on the CPU. There is a maximum of 16 threads. Using more threads can provide better resolution and FPS though is more demanding on the CPU.

2. The **Synchronization** tab (Fig. 3.45) contains the controls related to synchronization with other Doric devices. The software will allow for the synchronized triggering of the experiment. To synchronize the frame acquisition between the camera and the microscope, it is important to set the same frame rate in both devices (*e.g.* Camera FPS: 20 and Microscope exposure: 50 ms).

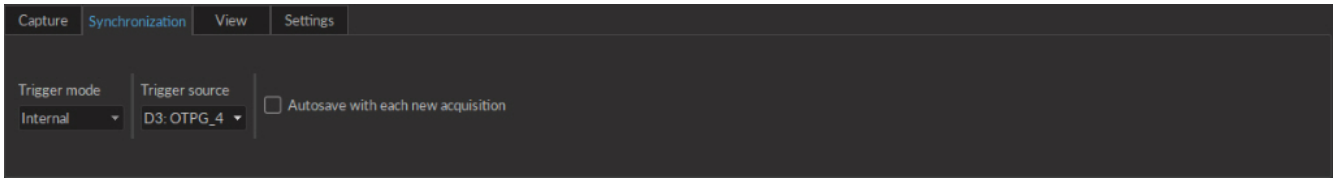


Figure 3.45: *Camera Module - Synchronization Tab*

- The **Trigger Mode** drop-down list allows the **Manual**, **Internal** or **External** modes to be chosen. In **Manual** mode, the camera controls are used to acquire the images. In **Internal** mode, the camera follows a signal coming from the program. In **External** mode, the camera follows an outside signal, with each pulse corresponding to a frame taken.
- **Trigger Source** is used to select the master device when the **Internal** trigger mode is selected.
- While using **Autosave**, every video started by the acquisition of the master device will be automatically saved to the target file determined in the **Capture** tab.

3. The **View** tab (Fig. 3.46) allows the zoom of the video to be modified.

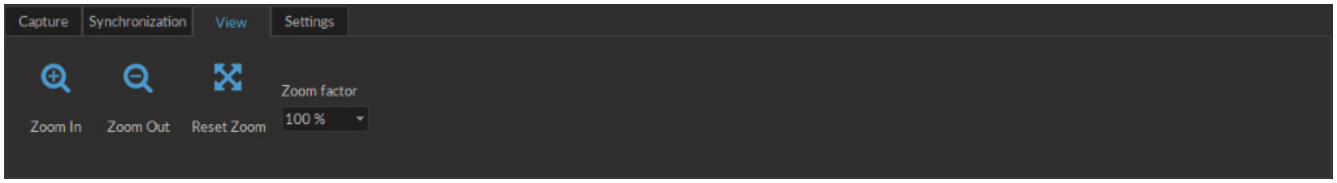


Figure 3.46: *Camera Module - View Tab*

- The **Zoom In** button increases the zoom factor for the image display.
- The **Zoom Out** button decreases the zoom factor for the image display.
- The **Reset Zoom** button resets the zoom factor to 100%.
- The **Zoom factor** button selects the zoom factor directly.

4. The **Settings** tab (Fig. 3.47) contains the controls related to the camera functions.

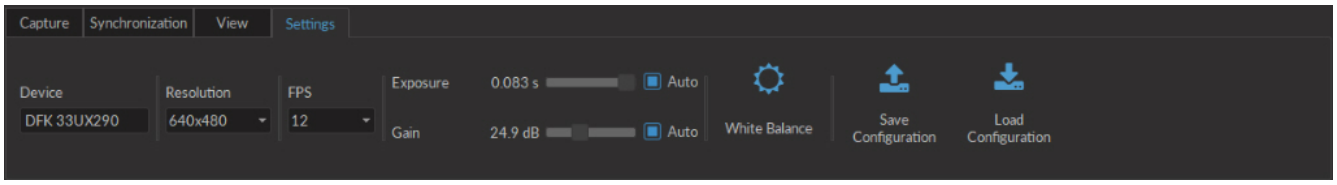


Figure 3.47: *Camera Module - Settings Tab*

- The **Device** box displays the camera serial number.
- The **Resolution** drop-down list selects the resolution of the camera.
- The **FPS** drop-down list selects the frame per second value of the camera. The FPS is dependent on the resolution.
- The **Exposure** (in ms) slider adjusts the exposure time of the pixels. If **Auto** is checked, the exposure time is calculated automatically.
- The **Gain** (in dB) slider adjusts the gain of the pixels. If **Auto** is checked, the gain is calculated automatically.
- The **White balance** button automatically adjusts the white balance for 5 seconds.
- The **Save Configuration** button is used to save the setting configuration in .doric format for future use.
- The **Load Configuration** button is used to load setting configurations in .doric format.

3.4 Microscope

The Microscope module of the Doric Neuroscience Studio provides an interface to control our Fluorescence Microscope Driver. The module enables image acquisition and its export in 16 bit .tif or in .doric (hdf5-based) files. The TIF format can easily be read with any standard imaging software. Doric files can be read by the Doric Neuroscience Studio **Image Analysis Module** or using an HDF5 library . Despite the fact that the images are saved with a 16 bit pixel depth, the true image pixel depth is 10 bit, so pixel gray values are contained between 0 and 1020 counts.

Below is the user interface (Fig. 3.48) and a complete description of all the functions.

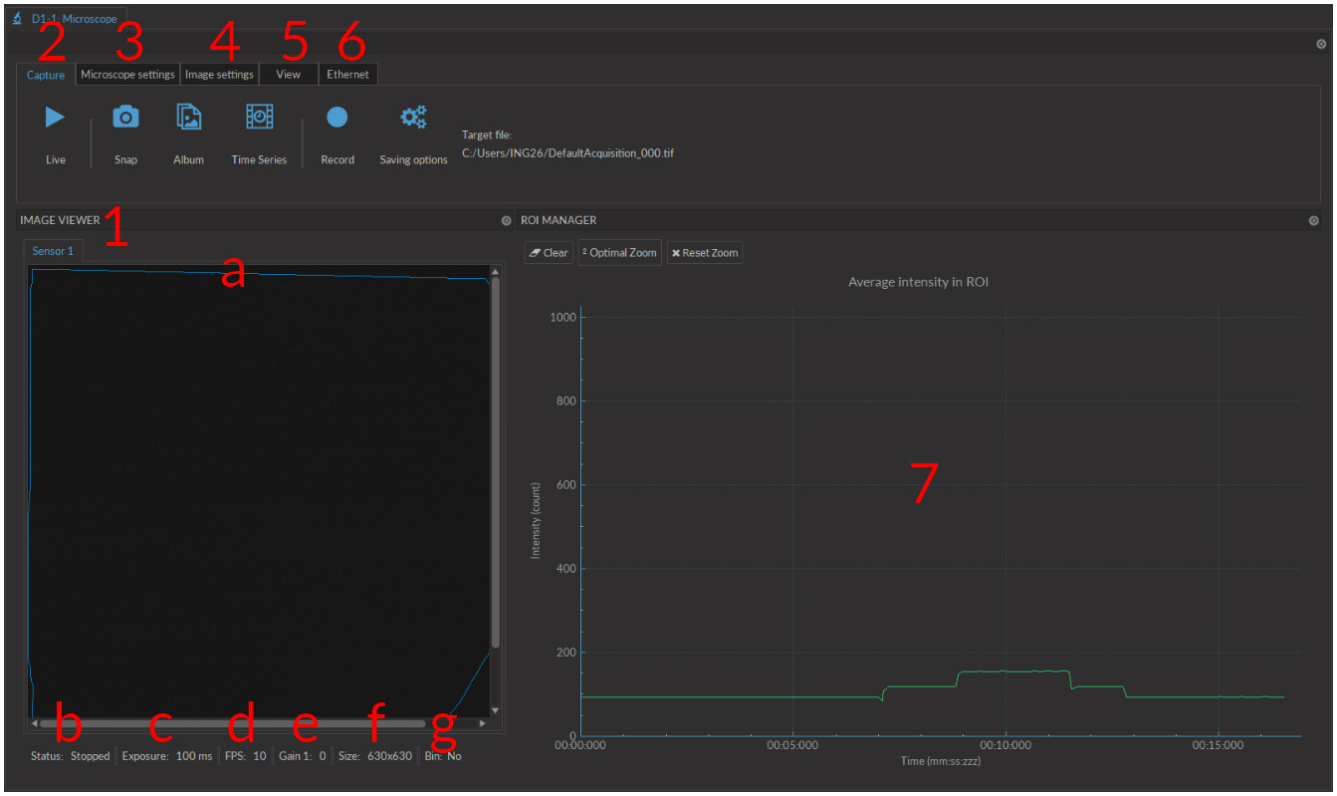


Figure 3.48: *Microscope Module Interface*

1. The **Image Box** (Fig. 3.48) displays images from the microscope and allows region of interest (ROI) drawing by clicking and dragging the mouse over the image.
    a) The **Sensor Tabs** (Fig. 3.48) display the sensors available to view. For multi-sensor microscopes, changing tabs allows you to see the image available to each.
    b) The microscope **Status** (Fig. 3.48) will indicate the current microscope state (Live/Stopped).
    c) The **Exposure** (in ms) (Fig. 3.48) indicates the exposure time of the microscope sensor.
    d) The **FPS** (Frames Per Second) (Fig. 3.48) indicates the number of frames per second taken by the sensor.
    e) The **Gain** (Fig. 3.48) indicates the electrical gain of the sensor.
    f) The **Size** (Fig. 3.48) indicates the resolution of the sensor images (in Pixels x Pixels).
    g) The **Bin** (Fig. 3.48) status indicates whether or not the sensor image is being binned (yes/no).
2. The **Capture** tab (Fig. 3.48) contains different image-capturing functions of the microscope.

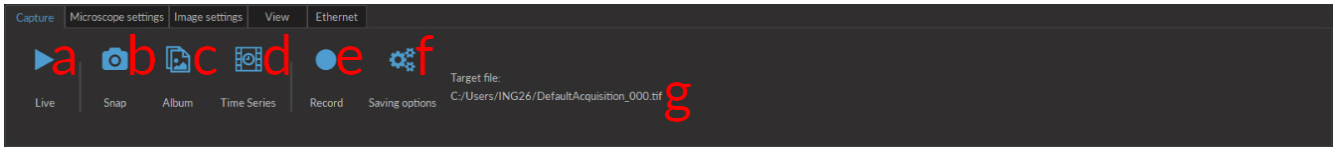


Figure 3.49: *Capture Tab*

a) The **Live** button (Fig. 3.49), when pressed, displays images from the microscope. These images are not saved.

b) The **Snap** button (Fig. 3.49), when pressed, takes a snapshot of the current image and saves it in the requested directory with the desired name (**Saving Options**) as a single image.

c) The **Album** button (Fig. 3.49), when pressed, acquires a snapshot and adds it to an album stack. The whole stack can be saved as one image stack.

d) The **Record** button (Fig. 3.49), when pressed, acquires a continuous image stream, until **Stop** is pressed, and saves it in the requested directory with the desired name (**Saving Options**) as one image stack.

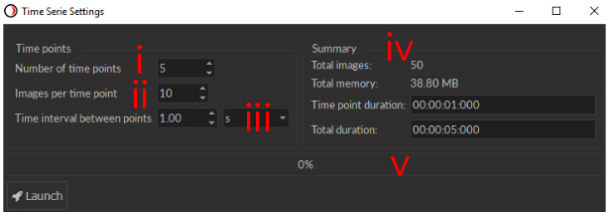


Figure 3.50: *Time Series Window*

e) The **Time Series** button (Fig. 3.49), when pressed, opens the time series interface (Fig. 3.50).

   i. The **Number of time points** (Fig. 3.50) defines the number of moments when a set of images will be recorded.

   ii. The **Images per time point** (Fig. 3.50) defines the number of images taken in each set.

   iii. The **Time interval between points** (Fig. 3.50), defined in ms, s and min, defines the duration between each image set. This duration always has a minimum value of **Exposure time**x**Images per time point**.

   iv. The **Summary** box (Fig. 3.50) shows many values related to the time series, including the **Total images** recorded, the **Total memory** occupied by the full series, the **Time point duration** and the **Total duration** of the full series.

   v. The **Progression bar** (Fig. 3.50) displays the progress (in %) of the time series.

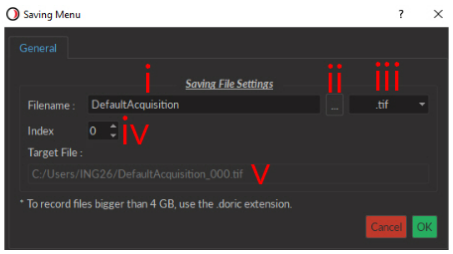


Figure 3.51: *Saving Options Window*

f) The **Saving options** button opens the **Saving options window**.

   i. The **Filename** box (Fig. 3.51) is used to define the recorded file name.

   ii. The **...** button (Fig. 3.51) opens a window used to choose the save file location.

   iii. The **File type** drop-down menu (Fig. 3.51) is used to decide which file type is used to save images. For files larger than 4 GB, the .doric extension is recommended.

   iv. The **Index** box (Fig. 3.51) displays the current index that will be added to the filename.

v. The **Target File** box (Fig. 3.51) shows the full location and name of the file being saved when an image sequence is recorded.

3. **Microscope settings** tab (Fig. 3.52) is used to set parameters related to the microscope recording images.

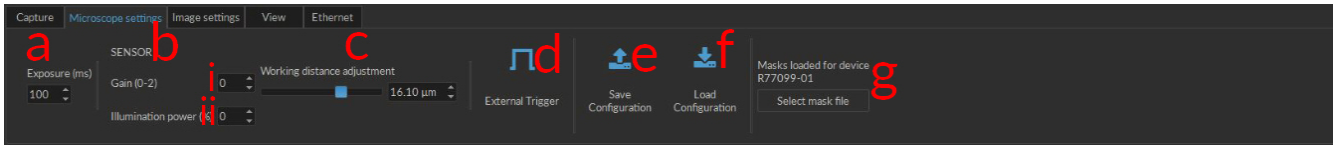


Figure 3.52: *Microscope Settings Tab*

a) The **Exposure** box (Fig. 3.52) sets the exposure time of the sensor. The time can be set between 22 and 1000 ms.

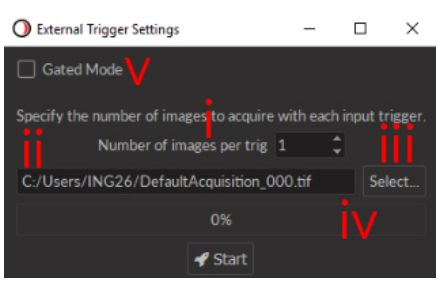
b) The **SENSOR** section (Fig. 3.52) defines characteristics for a single sensor and the associated excitation source. When a microscope used has multiple sensors, multiple **SENSOR** sections will be displayed, one for each sensor.

   i. The **Gain** box defines the sensor gain.

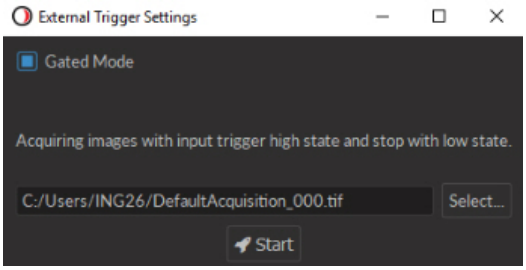
   ii. The **Illuminator power (%)** box defines the power emitted by the excitation light source. The light sources will be activated when the image acquisition is started. The maximum optical power (in mW) depends on the light source model.

c) The **Working Distance Adjustment Slider** appears when an *eFocus Miniature Fluorescence Microscope* is connected to the driver. This slider will adjust the working distance from a central spot (*0 µm*) to the extremes (*± 45 µm*).

d) The **External Trigger** button (Fig. 3.52) opens the **external trigger** window.



(a) *External Trigger Settings Window*



(b) *External Trigger Settings Window-Gated Mode*

Figure 3.53: *External Trigger Settings Windows*

   i. The **Number of images per trig** box (Fig. 3.53a) defines the number of images acquired at each trigger pulse.

   ii. The **File name/location** (Fig. 3.53a) box displays the location where the images are saved as well as their file name.

   iii. The **Select...** (Fig. 3.53a) button allows the selection of the **File name/location**.

   iv. The **Progression bar** (Fig. 3.53a) displays the advancement of the triggered sequence (in %).

   v. The **Gated mode** checkbox (Fig. 3.53a) will change the external trigger to gated mode (Fig. 3.53b). In this mode, the microscope will only aquire images when a high TTL signal is received on the TRIG IN input.

e) The **Save configuration** button (Fig. 3.52) will save all **Microscope settings** and **Image settings** in a **.doric** format file.

f) The **Load configuration** button (Fig. 3.52) will load a selected configuration file.

g) The **Select mask file** button opens a window to select a mask file for the microscope used. This section only appears when a *2-color Fluorescence Microscope* or an *efocus Microscope* is connected. The mask file currently loaded will be shown just above it. For more information on masks, see section 3.4.1.

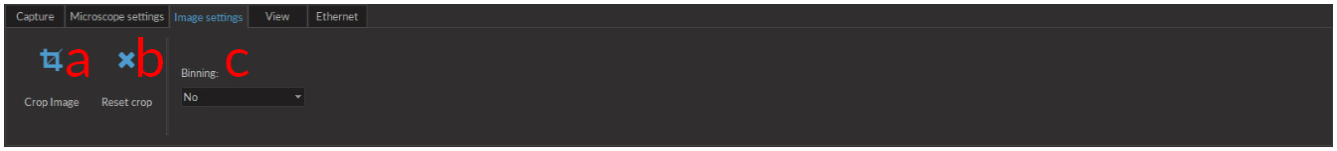


Figure 3.54: *Image Settings Tab*

4. The **Image settings** tab (Fig. 3.48) is used to define certain settings related to the displayed and recorded images.
    a) The **Crop Image** button (Fig. 3.54) allows a square to be drawn onto the image. When a new **Capture** sequence is activated, only the cropped region will be captured.
    b) The **Reset crop** button (Fig. 3.54) resets the cropped image to its original state. The change will only appear when a new **Capture** sequence is activated.
    c) The **Binning** drop-down list (Fig. 3.54) allows the binning of pixels. This reduces the number of pixels for smaller save file sizes.
5. The **View** tab (Fig. 3.48) is used to change viewing parameters of the sensor image. These changes will only appear on the sensor image when a new **Capture** sequence is started. Any adjustments made affect only the displayed image and not the recorded images.

Figure 3.55: *View Tab*

    a) The **Zoom In/Zoom Out** buttons (Fig. 3.55) will increase/decrease the zoom of the sensor image.
    b) The **Reset Zoom** button (Fig. 3.55) will reset the **Zoom factor** to 100%.
    c) The **Zoom Factor** drop-down list (Fig. 3.55) allows the selection of a zoom factor from a pre-set list. The box will also display the current zoom if it was changed using different buttons.
    d) The **Roi shape** drop-down list (Fig. 3.55) allows the selection of the shape used when drawing a **Region Of Interest** onto a sensor image. These shapes include **Freehand**, **Circle**, **Rectangle** and **Square**.
    e) The **SENSOR** section (Fig. 3.55) is used to adjust contrast on a given sensor. When a microscope used has multiple sensors, multiple **SENSOR** sections will be displayed, one for each sensor.
        i. The **Contrast** slider (Fig. 3.55) allows the adjustment of contrast from 0.1 to 5.
        ii. The **Min/Max** sliders (Fig. 3.55) indicate the minimum/maximum number of counts displayed. Should the **Min** be above 0, all pixels with lower count will display a minimal value. Should the **Max** be below 1020, all pixels with a higher count will appear saturated.
        iii. The **Auto contrast** slider button (Fig. 3.55) will active an automatic contrast adjustment algorithm.
        iv. The **Reset** button resets contrast functions to their default settings.
    f) The **Pseudocolor** drop-down list (Fig. 3.55) allow the sensor image color palette to be changed.
    g) The **Show saturation** checkbox (Fig. 3.55) allows all saturation on the sensor image to be displayed in red. This function is only available if no pseudocolor is selected.

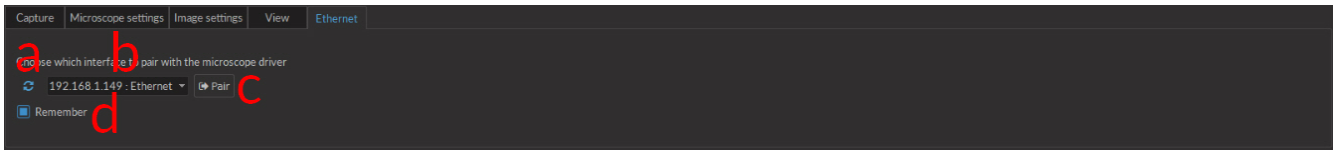


Figure 3.56: *Microscope Ethernet Tab*

6. The **Ethernet** tab (Fig. 3.48) is used to define the ethernet connection used to connect the computer to the microscope driver.
    a) The **Refresh** button (Fig. 3.56) will identify any accessible IP addresses and add them to the drop-down list.
    b) The **Ethernet** drop-down list (Fig. 3.56) includes all IP addresses connected to an ethernet adapter. The proper one must be selected to properly connect the microscope.
    c) The **Pair** button (Fig. 3.56) connects the software to the driver.
    d) The **Remember** checkbox (Fig. 3.56) will keep the chosen IP address so that the chosen microscope driver will be connected automatically next time the software is opened.

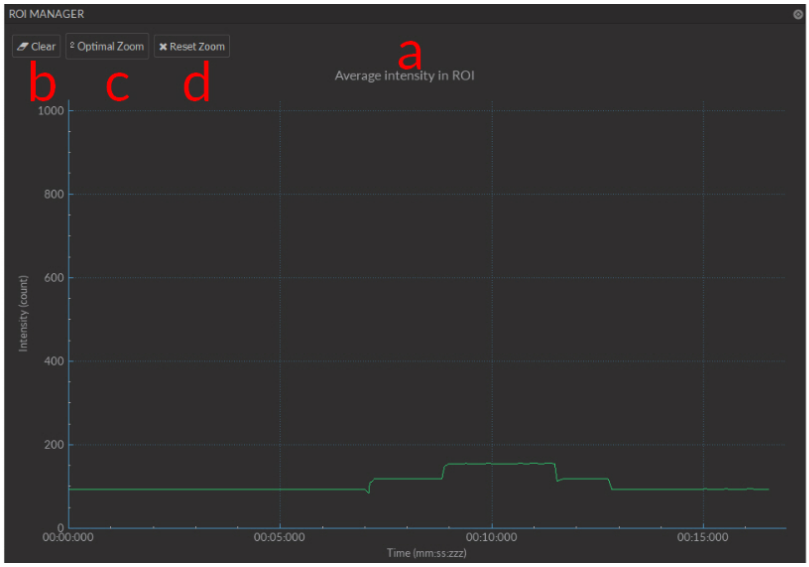


Figure 3.57: *ROI Manager*

7. The **ROI Manager** (Fig. 3.48) displays the live mean pixel intensity from a drawn ROI.
    a) The **Average Intensity in ROI** plot (Fig. 3.57) displays the average intensity over time inside a drawn ROI. *CTRL + mouse wheel* will adjust the x-axis zoom, while *SHIFT + mouse wheel* will adjust the y-axis zoom.
    b) The **Clear** button (Fig. 3.57) will clear any data displayed in the ROI manager and the ROI on the **Image Viewer**.
    c) The **Optimal Zoom** button (Fig. 3.57) sets the zoom factor on the plot to best display all data.
    d) The **Reset Zoom** button (Fig. 3.57) resets the zoom to its default setting.

3.4.1 Mask Installation

For the *2-color fluorescence microscope* and the *eFocus fluorescence microscope* to function properly, a series of **Masks** must be loaded onto the *Neuroscience Studio* at the first use of each microscope body on a given computer. The following section explains how to install said **Masks**.

1. With each microscope is provided a single USB key. The mask file has the name **DoricMaskFile_X00000-00.zip**, where **X00000-00** is replaced by the microscope serial number. Save this file in a secure location, as it is required should the *Neuroscience Studio* be installed on a different computer.
2. Once the system is connected and the microscope in place, go to the **Microscope settings** tab and click **Select Mask File**. This opens a file selection window. Travel to the location of the mask file, select it, and click **OK**.
3. After the file has been selected, the studio will show **Loading Masks** above the **Select Mask File** button. This is replaced by **Masks Loaded** once loading is complete.
4. With the masks installed, the microscope is ready for use.

3.5 Fiberless and Wireless Optogenetically Synchronized Electrophysiology

Optogenetically Synchronized Electrophysiology (OSE) systems require delivery of appropriate optical signals to the point of interest within neural tissue as well as detection and processing of the electrical signals from neural activity. The Wireless OSE module incorporates different functions to control our Doric Fiberless & Wireless OSE system. The core of the system is the Fiberless & Wireless Headstage. This device controls a *Wireless cannula* containing several electrophysiological probes and an LED light source. This cannula is implanted into the brain of an animal and records electrophysiological data while sending pulse sequences of light. This headstage is controlled from the **Electrophysiology Console**, which transfers data between the headstage and the computer.

Figure 3.58: *Electrophysiology console user interface*

3.5.1 Channels

As the electrophysiology console has 4 different channel types. As it shares its basic architecture with our photometry consoles, the 3 first channel types (**Digital I/O**, **Analog Output** and **Analog Input**) are described in section 3.2. The **Antenna** channels are used to control the fiberless and wireless headstage (section 3.5.1).

Figure 3.59: *Electrophysiology channel configuration window*

1. The **Channel Types** (Fig. 3.59) are selected on the left side of the window. Selecting the channel icon will display the parameters of each respective **Channel type**.
2. The **Wireless settings** (Fig. 3.59) are used to select recording and transmission settings.
    a) The **Antenna selection** (Fig. 3.59) box shows the currently selected antenna.
    b) The **Trigger options** (Fig. 3.59) box allows the selection of trigger parameters for data acquisition.
        i. The **Trigger source** can either be **Manual**, or can come from the **Digital I/O** channels.
        ii. The **Trigger mode** can be one of two types. In **Triggered** mode, the measurement sequence starts when a trigger signal is received, and continues even if the trigger signal stops. In **Gated** mode (currently in

development), the measurement sequence when a high TTL signal (>4 V) is detected, and will stop when a low TTL signal (<0.4 V) is detected.

This source can either be **Manual**, or from one of the four **Digital I/O** channels. The **Trigger Mode** can be either **Triggered** or **Gated**.

c) The **Pre-processing** (Fig. 3.59) filters define the high and low-pass frequency cutoff for electrical signal received by the headstage.

3. The **LED options** (Fig. 3.59) are all parameters used to control the light source of the cannula connected to the *Fi-Wi headstage*.

a) The **Channel options** (Fig. 3.59) are used to control the LED mode and current.

i. The **Mode** (Fig. 3.59) allows the selection of the pulse sequence mode. At time of writing, only **Square** mode is available.

ii. The **Maximum current** (Fig. 3.59) defines the current sent to the cannula LED. For proper function of the cannula, the current should always be greater than 10 mA.

iii. The **Baseline** (Fig. 3.59) leaves a small offset to the current sent to the LED. It is reccomended to use a small offset, as a complete shut-down of the LED will induce a spike in the electrical acquisition signal.

b) The **Sequence options** (Fig. 3.59) box is where LED pulse sequence parameters are defined.

i. The **Starting Delay** (Fig. 3.59) sets the delay (in hh:mm:ss:zzz format) before the first pulse.

ii. The **Frequency/Period** (Fig. 3.59) sets the frequency (in Hz) or period (in ms) for the pulse sequence. For example, a signal at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a pulse sequence at 0.5 Hz (frequency) will output one pulse every 2000 ms (period).

iii. The **Time ON/Duty Cycle** (Fig. 3.59) sets the time (in ms) or the duty cycle (in %) for each pulse. The **Time ON** must be lower than (1/frequency)+0.005 ms, while the **Duty cycle** must be below 100 %. These squares will appear red should an impossible **Frequency**/**time ON** be selected.

iv. The **Smoothing** option is used to change the pulse slope in square pulse sequences. The **Edit Edges** button opens the **Smoothing Edge(s)** window (Fig. 3.60).

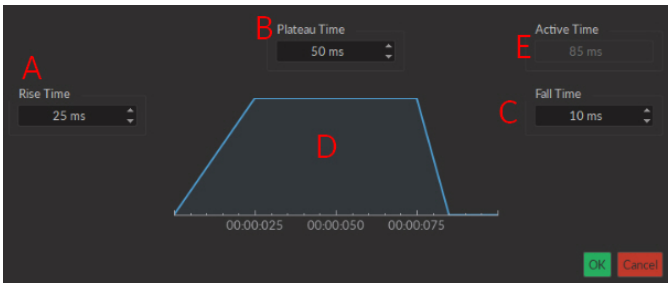


Figure 3.60: *Light Source Smoothing Edge(s) Window*

A. The **Rise Time** box is used to define the duration to rise from 0 to the pulse maximum.

B. The **Plateau Time** box is used to defined the duration the pulse is at its maximum value.

C. The **Fall Time** box is used to define the duration to descend from the pulse maximum to 0.

D. The **Pulse Graph** displays the pulse shape.

E. The **Active Time** box displays the total duration of the pulse. While the **Smoothing** option is active, the **Time ON** is fixed at this value.

(need to include section

v. The **Pulses per sequence** (Fig. 3.59) sets the number of pulses per sequence. If it is set to 0, the pulse will be repeated indefinitely.

vi. The **Number of sequences** (Fig. 3.59) sets the number of times that the sequence will be repeated. If it is set to 0, the sequence will be repeated indefinitely.

vii. The **Delay between sequences** (Fig. 3.59) sets the delay (in hh:mm:ss:zzz format) between each sequence if the **Number of Sequences** is greater than 1.

viii. The **Total Duration** (Fig. 3.59) displays the total time of the experiment. The different values can be *Inf* for infinite, a valid time value or *Err* if the **Time ON** value is greater than 1/frequency.

c) The **Preview** box shows a preview of the pulse sequence.

3.5.2 Control and Settings tabs

The three **Control and settings tabs** are used to manage different parts of the software, and are described in section 3.2.2.

3.5.3 Acquisition View

The **Acquisition View** box displays all information concerning active channel. Each channel chosen using **Add Channel** is displayed on the window, occupying a rectangular box. Each **Channel box** shows a number of basic elements, described in section 3.2.3. Elements uniquely associated with the **Antenna** channel are described here.

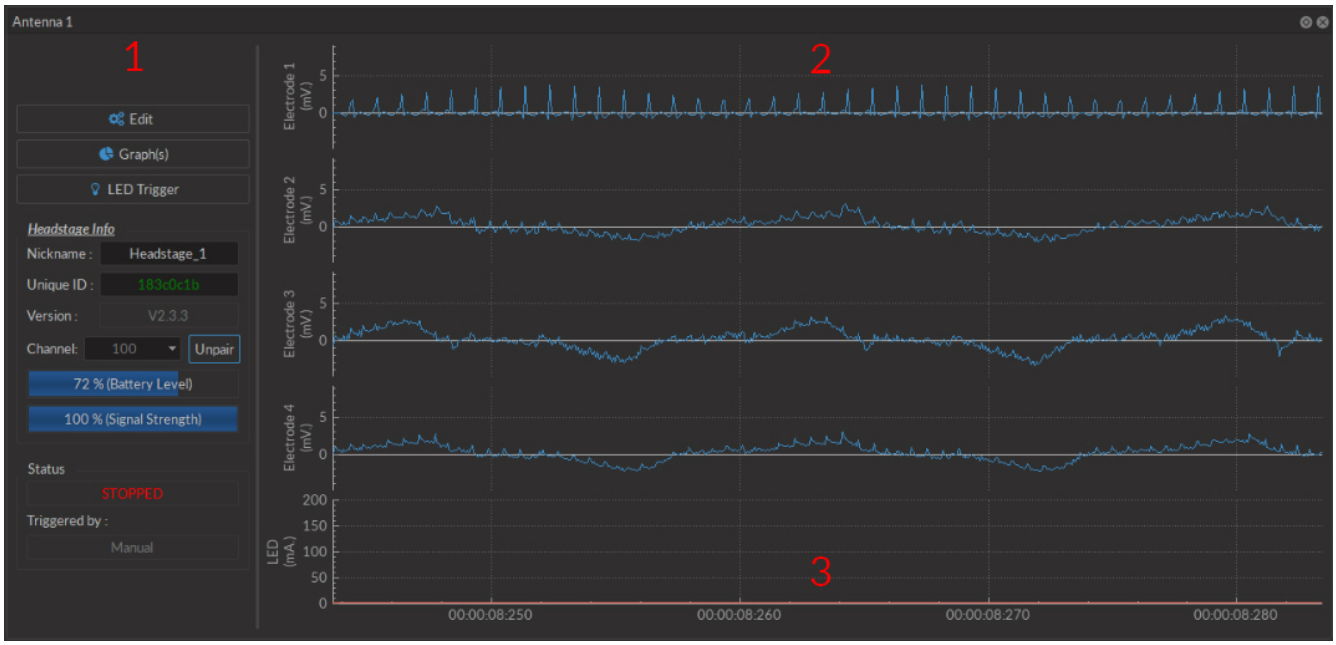
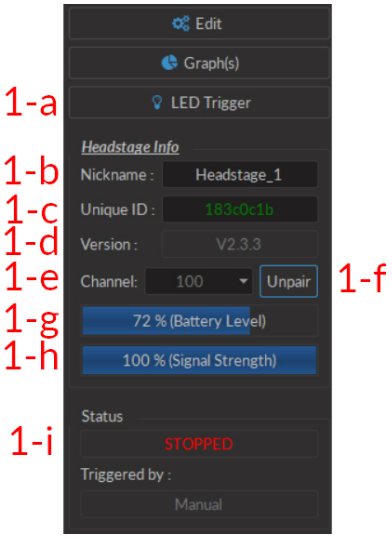
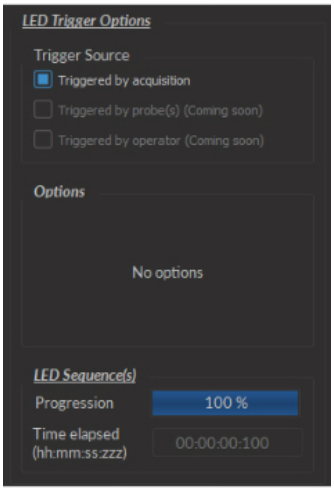


Figure 3.61: *Electrophysiology acquisition view, Antenna box*

1. The **Controls** box (Fig. 3.62a) displays elements to control and monitor the wireless headstage.



(a) *Acquisition View, Controls*



(b) *LED Trigger-Trigged by Acquisition Window*

Figure 3.62: *Acquisition View Interfaces*

a) The **LED trigger** buttons opens the **LED trigger options** window. In this window, the **Triggered By Acquisition** option is available; other options are still in beta, and will be made available at a later date. Sequences can be activated/shut down while this window is open.
    - The **LED Trigged by acquisition** (Fig. 3.62b) mode activates the LED when an acquisition sequence is started. This includes the moment the **Headstage** is initially activated, and when a recording session is started using one of the **Ephys Trigger Options**.

b) The **Nickname** box allows the user to change the name used for the connected headstage.

c) The **Unique ID** box displays the unique ID sequence associated with the headstage currently in use.

d) The **Version** box displays the headstage firmware version.

e) The **Channel** drop-down list shows the channel currently in use by the headstage. When using two headstages, the channel must be different for each headstage.

f) The **Pair** button is used to pair an active headstage to a console. When a headstage is paired, it becomes the **Unpair** button, which unpairs the active headstage associated with the given antenna.

g) The **Battery Level** bar displays the headstage battery level, in %, at all times.

h) The **Signal strength** bar displays the signal strength. If the signal strength is acceptable (100-76%) the bar appears blue. If the signal strength is low (75-50%), it will appear yellow. If the signal strength is critically low (<50%), it will appear red.

i) The **Status** bar displays acquisition status. **STOPPED** is displayed when the acquisition is inactive, and **STARTED** when acquisition is active.

2. The **Electrode Traces** (Fig. 3.61) displays the signal detected by the cannula electrodes, in numbered order.

3. The **LED Trace** (Fig. 3.61) displays the current being sent to the cannula LED.

3.6 Optogenetics TTL Pulse Generator

The *Optogenetics TTL Pulse Generator* (OTPG; 4 or 8 channels) are controlled from the *Doric Neuroscience Studio*. Various pulses train sequences can be designed for any experiment. Each channel can be used to generate pulses with relative phases that remain constant. Channels can be synchronized or started at the same time or independently. Channels 1 to 4 (OPTG 4) and channels 5 to 8 (OTPG 8) can be used as inputs.

The *OTPG* user interface (Fig. 3.63) is split into two main sections. This includes the **Controls & settings** and the **Acquisition view**. From these sections, the **Channel(s) configuration** window can be accessed to add and configure channels.

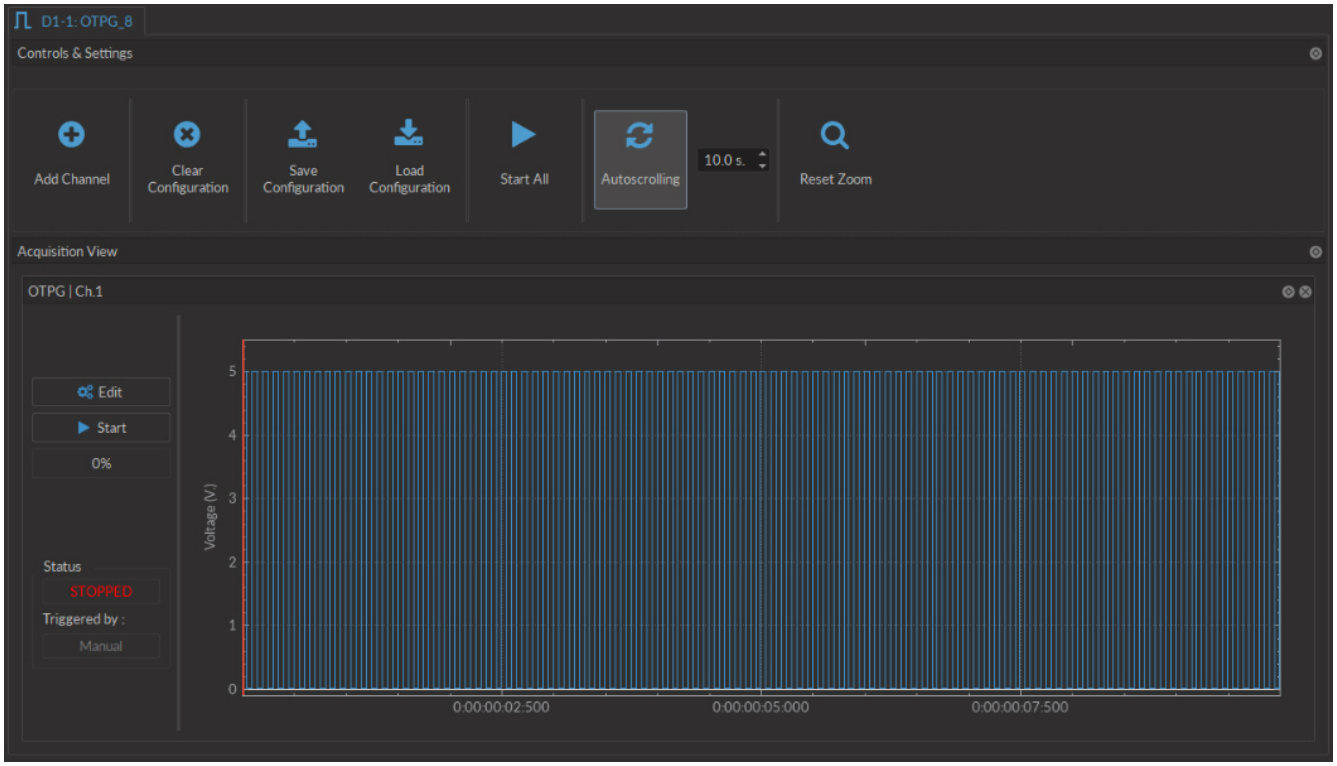


Figure 3.63: *Optogenetics TTL Pulse Generator Interface*

3.6.1 Channel configuration

Figure 3.64: *Channels Configuration, Continuous Wave Interface*

The **Channels configuration** window is used to configure each channel. The window can be accessed by either using the **Add channel** or **Edit** buttons. This window is separated into multiple boxes, defined here.

1. The **Channel Options** (Fig. 3.64) 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.

    - The **CW(Continuous Wave)** channel mode allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

        i. The **Starting Delay** (Fig. 3.64) defines the time between the activation of the pulse sequence and the beginning of the signal.

        ii. The **Time ON** (Fig. 3.64) 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.

        iii. The **Total Duration** (Fig. 3.64) 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**.

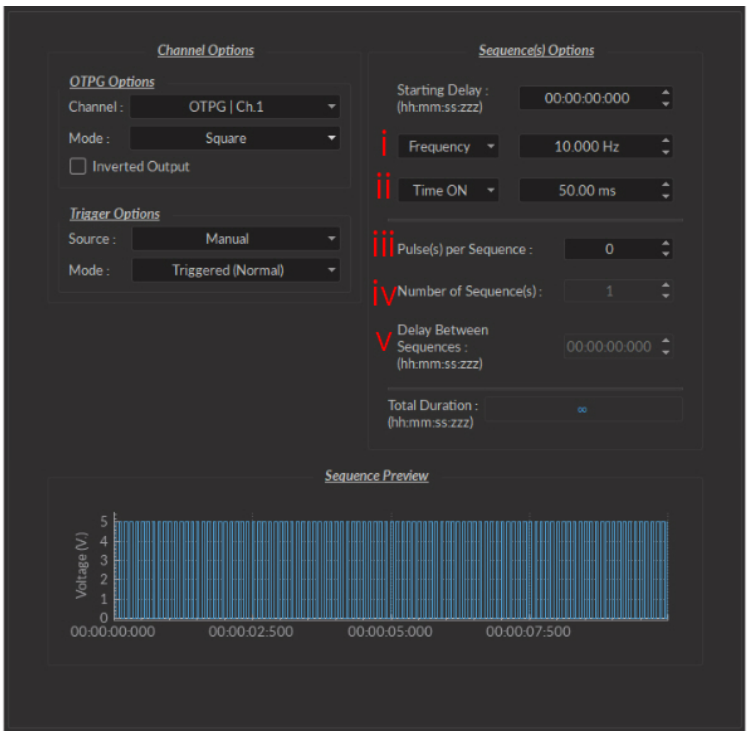


Figure 3.65: *Channels Configuration, Square Interface*

- The **Square** channel mode allows the creation of a square TTL pulse sequence. This includes all sequence options as the **CW** mode, with the following additions.
  i. The **Frequency** (Fig. 3.65) 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).
  ii. The **Time ON** (Fig. 3.65) defines the length of a single pulse. This time can also be converted to a **Duty Cycle**, which represents the % of the period the pulse duration corresponds to.
  iii. The **Pulse(s) per sequence** (Fig. 3.65) set the number of pulses per sequence. If it is set to 0, the number of pulses will be infinite.
  iv. The **Number of sequence(s)** (Fig. 3.65) sets the number of times that the sequence will be repeated.
  v. The **Delay between sequences** (Fig. 3.65) sets the delay between each sequence.

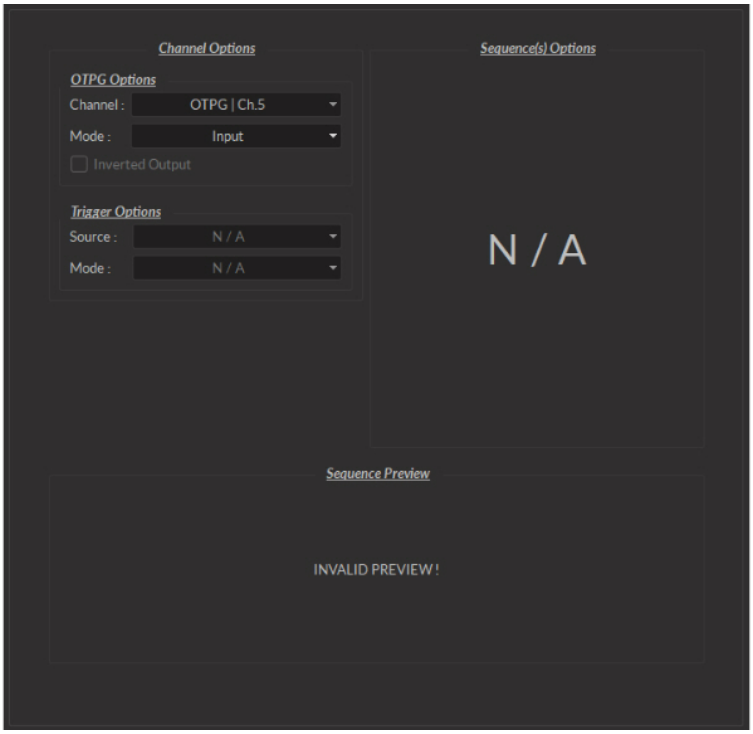


Figure 3.66: *Channels Configuration, Input Interface*

- The **Input** mode (Fig 3.66) records a signal as long as there is a high TTL signal on the chosen console channel. 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.

c) The **Inverted Output** checkbox changes the signal output. When selected, the **ON** TTL signal will send 0 V, while the **OFF** TTL signal will send 5 V.

2. The **Trigger Options** (Fig. 3.64) 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 channel.

   b) The **Mode** defines how the trigger activates a sequence..

   - In **Triggered** mode, the sequence is started manually or by a trigger source from an 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. If a pulse is cut, a new one will start at the next activation signal.

3. The **Sequence Options** (Fig. 3.64) 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/**Frequency**), the color of the option boxes will turn **RED**.

4. The **Sequence Preview** (Fig. 3.64) section allows visualization of the output sequence that will play by selecting the channel in the graph.

5. The **Apply** button (Fig. 3.64) will generate the defined channel OR update an already configured channel with any changes.

3.6.2 Control and Settings

The **Control and Settings** box is used to manage the different parts of the software module.

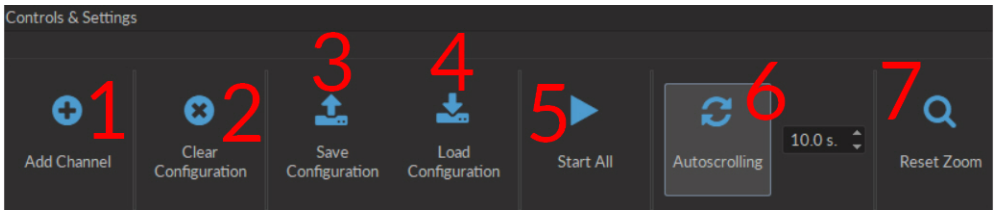


Figure 3.67: *Control & Settings Box*

1. The **Add Channel** button (Fig. 3.67) opens the **Channels configuration** window. This window is detailed in section 3.6.1.
2. The **Clear configuration** button (Fig. 3.67) resets the acquisition view and all other parameters set. Any configurations already set will be lost.
3. The **Save configuration** button (Fig. 3.67) allows an OTPG configuration to be saved in the **.doric** format.
4. The **Load configuration** button (Fig. 3.67) allows an OTPG configuration in **.doric** format to be loaded.
5. The **Start All** button (Fig. 3.67) activates all prepared channels.
6. The **Autoscrolling** button (Fig. 3.67), when clicked, makes the graphs scroll as new data appears. The duration (in s) kept on display is defined in the box beside the button.
7. The **Reset Zoom** button (Fig. 3.67) resets the horizontal axis of all graphs displayed in the **Acquisition View** to the duration chosen in the **Autoscrolling** box.

3.6.3 Acquisition View

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

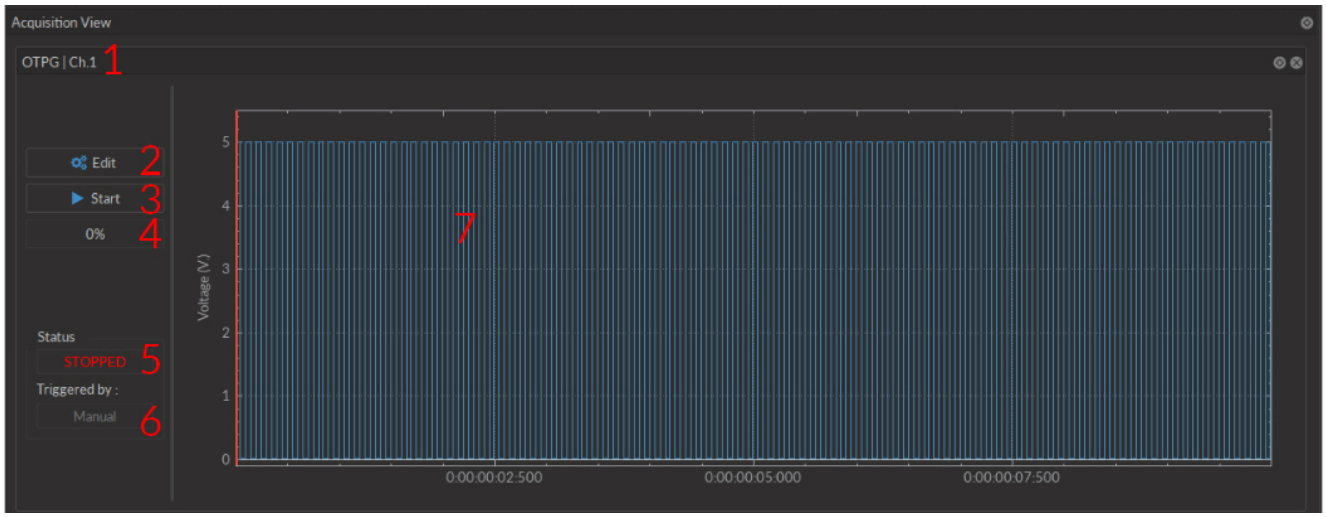


Figure 3.68: *Acquisition View Box*

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

1. The **Channel name** is located on the upper left of the **Channel box** (Fig. 3.68), identifying the type of channel and it's number, corresponding to that on the *OTPG*.

2. The **Edit** button (Fig. 3.68) allows the editing of channel parameters, opening the **Channel configuration** window. For further information, see section 3.6.1.
3. The **Start** button (Fig. 3.68) activates the chosen channel without activating any other channel. The button is Green (Fig. 3.68 when inactive, Red (Fig. 3.70) when active and Gray (Fig. 3.69) when disabled.
4. The **Progress Bar** (Fig. 3.68) will indicate the progress of the chosen sequence. Should the sequence be infinite, a scrolling blue bar (Fig. 3.70) is displayed.

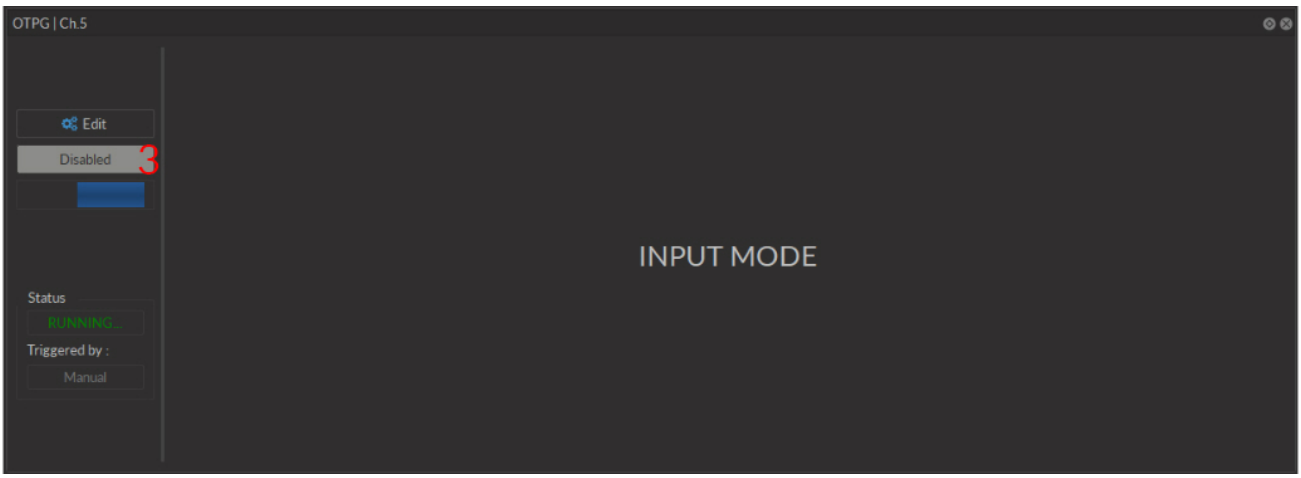


Figure 3.69: *Acquisition View Box, Input Mode*

5. The **Status** box (Fig. 3.68) shows whether the channel is active, displaying **STOPPED** when inactive (Fig. 3.68) and **RUNNING...** (Fig. 3.70) when active.

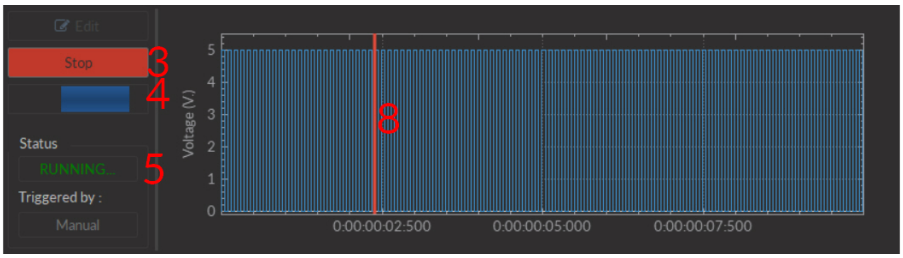


Figure 3.70: *Acquisition View Box, Square Mode*

6. The **Triggered By** box (Fig. 3.68) shows the current trigger source of the channel sequence.
7. The **Sequence Preview** box (Fig. 3.68) shows a graphed preview of the pulse sequence.
8. The **Position Marker** (Fig. 3.69) is a red line on the **Sequence Preview** that indicates the position in time when the sequence is active.

4

Analysis Modules

The analysis modules can be found under the Analysis menu.

4.1 Image Analyser

This module provides an easy way to extract relevant data from the images acquired by the Doric miniature fluorescence microscopes. The software loads images in .TIF format, implements image processing functions and an export tool to save the fluorescence data 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 microscope images. All the underlying algorithms are implemented from the OpenCV library. In this section, we will describe the different functions available, and how to use them.

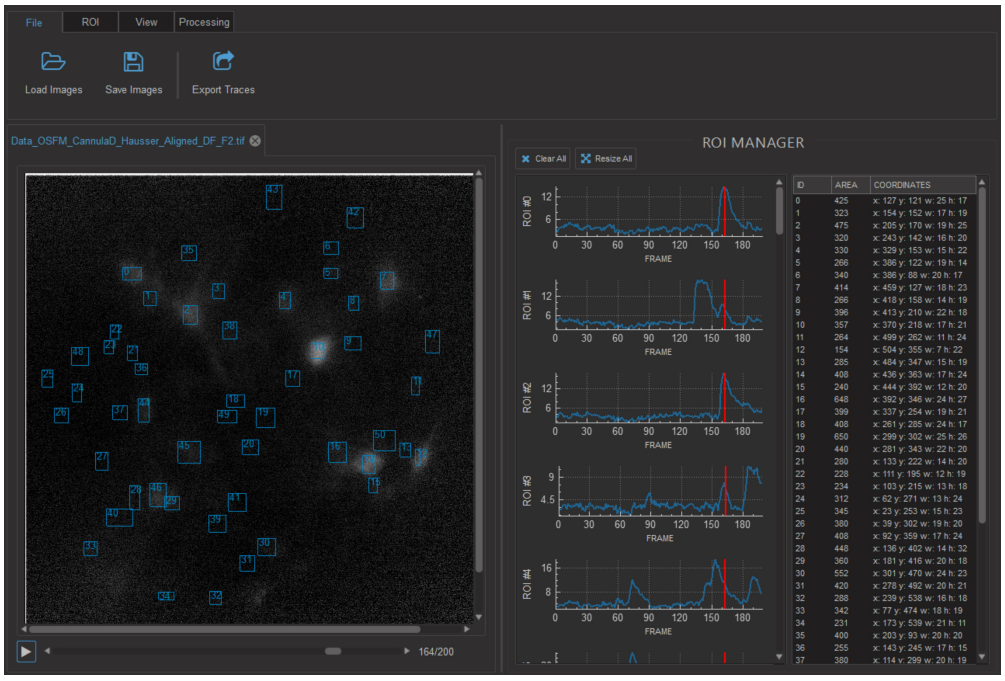


Figure 4.1: *Image Analysis Module Interface*

1. The **Image Viewer** displays the loaded images, allows navigation through the image stack and the drawing of regions of interest (ROIs) by clicking and dragging the mouse over the image. Multiple image sets can be opened, appearing as tabs in the upper left of the image box.
2. The **ROI Manager** displays the different ROI parameters and traces the mean signal intensity for each image.
3. The **Function Toolbar** contains all the buttons and functions accessible.

4.1.1 Function Toolbar

1. The **File tab** (Fig. 4.2) is used to save/load data.

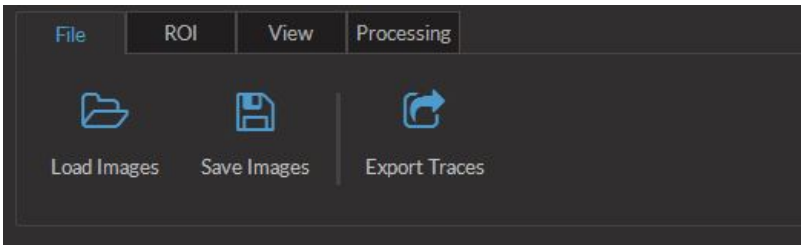


Figure 4.2: *File Tab*

- The **Load Images** function loads a square, 16 bit TIF file or multipage file.
- The **Save Images** function saves the current image tab to a 16 bit TIF multipage file.
- The **Export Traces** function saves the average fluorescence intensity values for each ROI of the current tab to a .CSV or .doric file.

2. The **ROI tab** (Fig. 4.3) is used to save/load data relating to regions of interest drawn on an image.

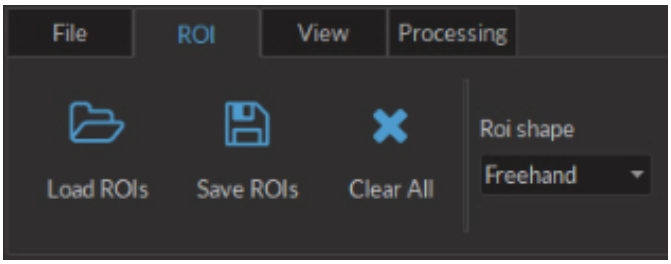


Figure 4.3: *ROI Tab*

- The **Load ROIs** function loads .CSV file containing informations about the saved ROIs.
- The **Save ROIs** function saves the current ROIs information to a .CSV file.
- The **Clear All** button clears all ROIs.
- The **ROI shape** function is a drop-down list that allows the selection of the **ROI** shape. These include **Freehand, Circle, Rectangle** and **Square**.

3. The **View tab** (Fig. 4.4) is used to manipulate the appearance of an image without changing base data.

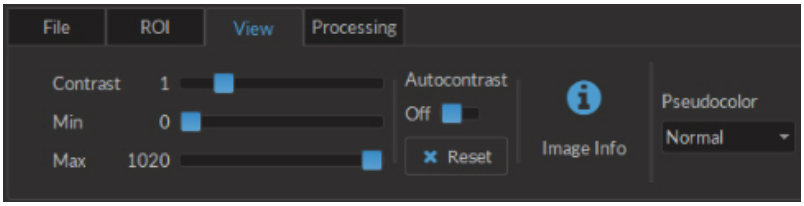


Figure 4.4: *View Tab*

- The **Contrast** function applies a different luminance response curve (gamma). See section 4.1.3 for details.
- The **Min** function applies a lower threshold with the cut-off value defined by the slider. See section 4.1.3 for details.
- The **Max** function applies an upper threshold with the cut-off value defined by the slider. See section 4.1.3 for details.

- The **Autocontrast** function directly applies the equalizeHist function of the OpenCV library.
- The **Reset** function returns the contrast and range values to their default.
- The **Image Info** button displays the image information window.
- The **Pseudocolor** function is a drop-down list for selecting alternate coloring schemes for the images presented.

4. The **Processing tab** (Fig. 4.5) is used to process the image data.

Figure 4.5: *Processing Tab*

- The **Align Images** function aligns the image stack to the user-defined key frame. See section 4.1.3 for computational details. Selecting this button will open the **Align Images** window (Fig. 4.6). By selecting the **Save Alignement Values** checkbox, the image alignement values will be preserved when saving the processed images. There are 4 different methods available.

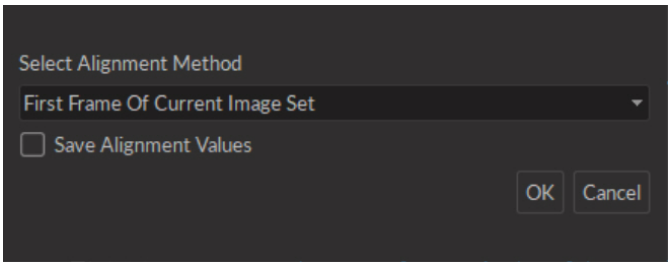


Figure 4.6: *Align Images Window*

  - The **First Frame Of Current Image Set** method uses the first image in the set to align the rest.
  - The **Select Frame From Current Image Set** method allows the selection of a single image in the set to use for alignment of all other frames.
  - The **Select Other Image Set And Frame** method aligns the current set using data from a different image set.
  - The **Select From Alignment Value File** method uses a previously-defined alignment for another image set. This method is most valuable when trying to align images from the *2-color fluorescence microscope*, to align one color channel using the data from the other.
- The **Remove Background** function removes the average value of a selected ROI from all images in the stack.
- The $\Delta F/F_0$ function calculates the normalized fluorescence variation of the images and displays the results in a new tab. When selected, the See section 4.1.3 for details.
- The **Find Cells** function detects the cells and creates the ROI automatically. See section 4.1.3 for details.
- The **Stack Projection** function projects all movie frames to a single frame using the method selected in the Settings dialog. See section 4.1.3 for details.
- The **Batch Processing** function opens the **Batch Processing Window** (Fig. 4.7). This allows the processing of large datasets in sequential order, without needing to activate each individual function.

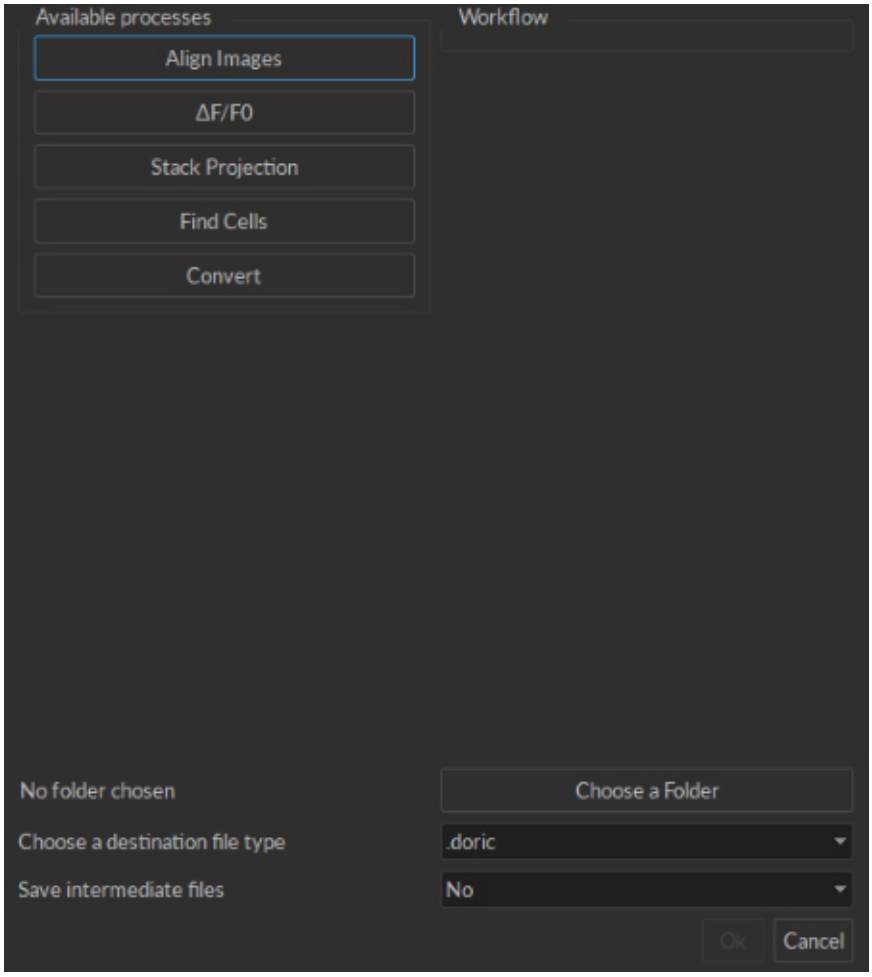


Figure 4.7: *Batch Processing Window*

a) The **Available processes** box lists all processes available. Processes on the list will be greyed out if the work-flow order prevents them from being used. Each process has a number of parameters that are identical to those used outside of batch processing.
   - The **Align Images** process aligns the image stack to the user-defined key frame. See section 4.1.3 for computational details.
   - The Δ**F/F**$_0$ process calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 4.1.3 for details.
   - The **Stack Projection** process projects all image frames to a single frame using the method selected in the Settings dialog. See section 4.1.3 for details.
   - The **Find Cells** process detects the cells and creates the ROI automatically. See section 4.1.3 for details.
   - The **Convert** process is used to convert an image stack to **.doric** or **.tif** format.

b) The **Workflow** box displays the order in which image processing actions will be taken.

c) The **Choose a Folder** button allows the selection of a folder to save batch processing results.

d) The **File Type** list is used to defined the file extension used when the images are saved.

e) The **Save intermediate files** option will save intermediary files in the image processing process alongside the completed files.

4.1.2 ROI Manager

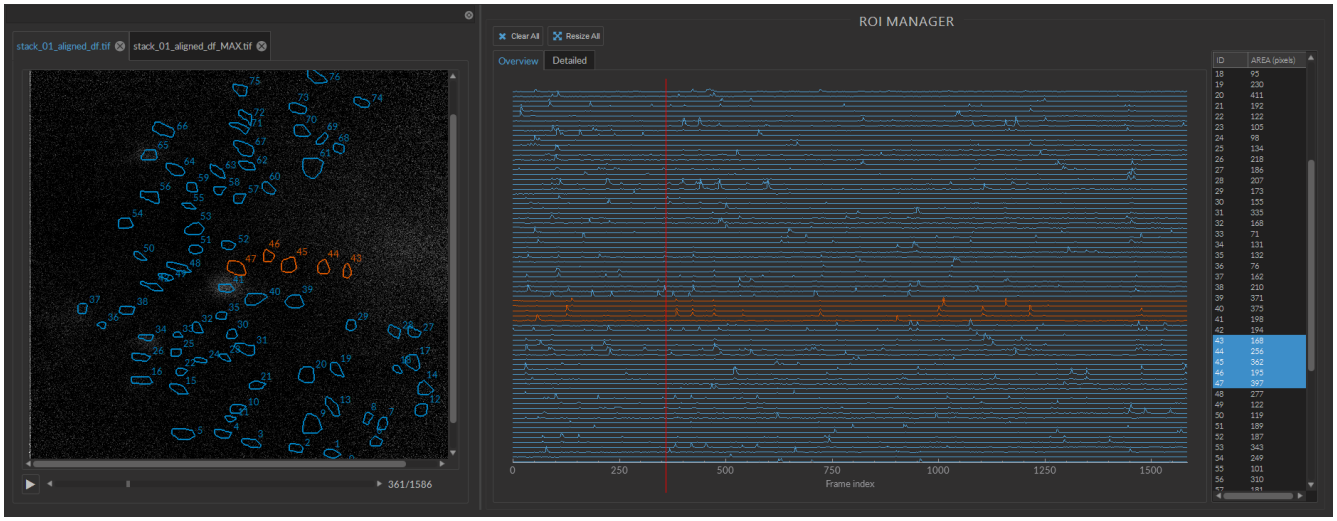
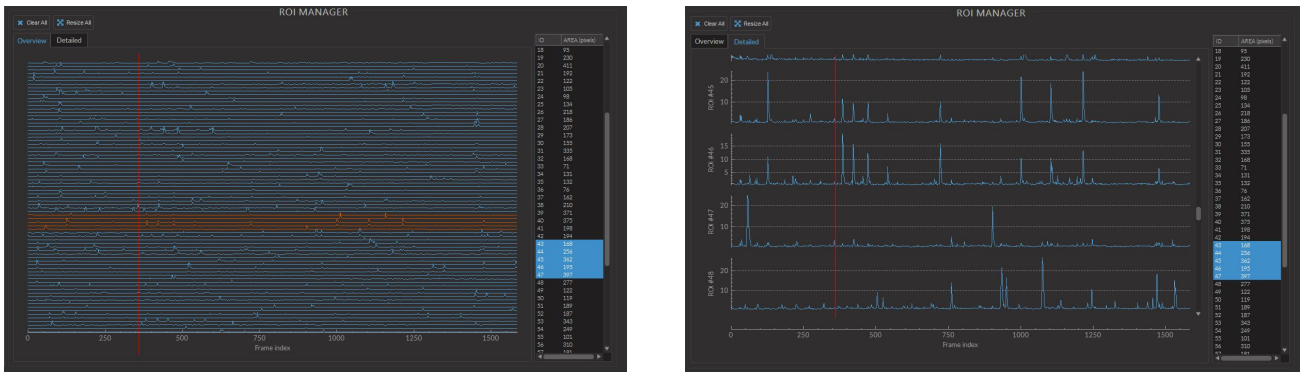


Figure 4.8: *ROI Manager*

The ROI manager extracts average intensity of a defined section of the image over an entire image stack. There is no limit to the number of ROI allowed per image stack.

1. The **Image Viewer** contains the image stack and the ROI, numbered according to the order they where set. The ROI can be saved independently from the image stack on the ROI toolbar. The ROI are drawn directly on the *Image Viewer* in a *freehand* manner.
2. The **Intensity Plot** panel shows the plot of average intensity as a function of the frame index. The Y-axis represent the average count of all the pixels of the ROI. It is separated in *Overview* and *Detailed* tabs.
   - The **Overview** tab displays all the traces on the same graph, on the same scale (see Fig. 4.9a).
   - The **Detailed** tab displays each trace on a separate graph, allowing for precise intensity measurements (see Fig. 4.9b).
3. The **ROI Data** list shows the parameters defining each ROI. Selected items will be displayed in orange on the Image Viewer and in the Overview graph.
   - The **ID** shows the order of the ROI (starting at 0).
   - The **Area** shows the area (in pixels) contained in the ROI.



(a) *Overview Graph* (b) *Detailed Graph*

Figure 4.9: *ROI Manager Graph Tabs*

4.1.3 Algorithms

Contrast

The contrast adjustment applies the following operation to each pixel of the image: $V_{out} = AV_{in}^{\gamma}$, where V_{out} is the corrected pixel value, $A = 1$, V_{in} is the initial pixel value, and γ is the value as selected by the contrast slider.

Min and Max ranges

When the values of the display range are other than the default $min = 0$ and $max = 1020$, the following operation is applied to each pixel: $V_{out} = 1020 * (V_{in} - min)/(max - min)$, where V_{out} is the corrected pixel value, V_{in} is the initial pixel value, min and max are respectively the minimum and maximum slider values.

Image Alignment

The algorithm is inspired from Manuel Guizar-Sicairos, Samuel T. Thurman, and James R. Fienup, *Efficient subpixel image registration algorithms*, Opt. Lett. 33, 156-158 (2008). The basic idea is to obtain an initial estimate of the crosscorrelation peak by a Fourier transform and then refine the shift estimation by upsampling the Fourier transform only in a small neighborhood of that estimate by means of a matrix-multiply Fourier transform. With this procedure, all the image points are used to compute the upsampled crosscorrelation. In order to increase the precision of the algorithm, we use the laplacian of the images as inputs, instead of using the raw images. Briefly, the algorithm applies the following steps:

1. Calculate gaussian blur of the reference image with window of size 39 to smooth high frequency noise.
2. Calculate the laplacian of the blurred reference image.
3. Use the absolute values as the final reference image.
4. Reproduce steps 1 to 4 for the following image.
5. Calculate the 2D Fourier transform of the reference and the target image.
6. Multiply both images.
7. Calculate the inverse Fourier transform of the product image.
8. Get the position of the maximum correlation peak.
9. Create an upsample array around the maximum correlation peak to refine the shift calculations.
10. Calculate the Fourier transform of the larger array.
11. Do the matrix multiplication.
12. Locate the maximum correlation and map it back to the original space.

ΔF/F$_0$

The algorithm calculates a standard ΔF/F$_0$ with F_0 corresponding to the temporal average intensity, with an optional preprocessing step to remove the illumination variation artefacts. In order to properly calculate the $\Delta F/F_0$, the algorithm uses a dark frame to account for the sensor electronic offset. Calculating the $\Delta F/F_0$ without subtracting the offset will lead to artificially lower values. To record a dark frame, set the microscope driver to the desired exposure and gain, the LED power to zero and take a snapshot. Before calculating the F_0, the average temporal variations can be compensated to get a flat temporal average profile (Fig. 4.10). Keep in mind that removing the average temporal profile can also remove global activity patterns.

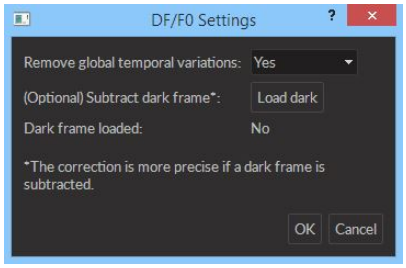


Figure 4.10: $\Delta F/F_0$ *Settings*

Briefly, the algorithm applies the following steps:

1. Calculate the average image intensity as a function of time (C).
2. If the global variation removal option is selected, apply the following correction to each image: $I_{out} = (I_{in} - I_{dark}) * (mean(C - I_{dark})/(C - I_{dark}))$ where I_{out} is the LED illumination corrected image, I_{in} the input image and C is the average temporal trace.
3. Calculate F_0 as the average projection of the movie.
4. Calculate the relative change $R(t)$ of fluorescence signal $R(t) = (F(t) - F_0)/F_0$.

Find Cells

The algorithm is inspired by Eran A. Mukamel, Axel Nimmerjahn and Mark J. Schnitzer, *Automated analysis of cellular signals from large-scale calcium imaging data*, Neuron 63(6), 747-760 (2009). The basic idea is to use a principal component analysis (PCA) as input of an independent component analysis (ICA) to separate the different temporal signals contained in the movie. This method is used as a starting point to determine the position of the different active cells. It is coupled with a segmentation routine optimized for reducing the false positives. The *Find Cells* algorithm uses user-defined boundaries shown in Fig. 4.11. The first parameter is an estimate of the number of cell present in the movie. By design, it must be lower than the number of frames minus five. The next parameters are the smallest and biggest object diameter in microns. These values are used to filtered the object found by the PCA/ICA.

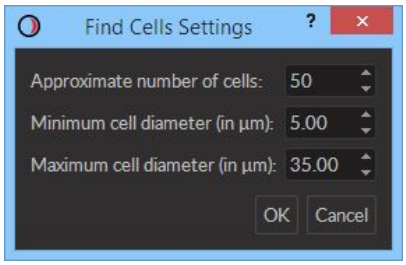


Figure 4.11: *Find Cells Settings*

Briefly, the algorithm applies the following steps:

1. Calculate and remove the spatiotemporal average from the movie, as the PCA/ICA algorithm requires zero-mean data.
2. Run OpenCV PCA algorithm on the centered data.
3. Normalize data by standard variation.
4. Calculate ICA with PCA as input data.
5. Apply segmentation to each ICA found.
6. Filter contours found at the previous step using user-defined boundaries.

Stack Projection

This function can be used to help for ROI drawing. It calculates a temporal projection using the user-defined method (see Fig. 4.12).

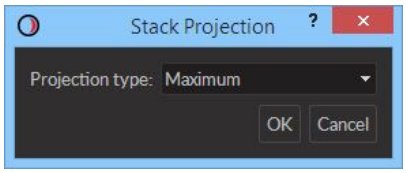


Figure 4.12: *Stack Projection Settings*

Maximum: the output is the maximum value found in all frames for each pixel.

Average: the output is the mean value of all frames for each pixel.

Sum: the output is the sum of all frames for each pixel.

Minimum: the output is the minimum value found in all frames for each pixel.

4.2 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.

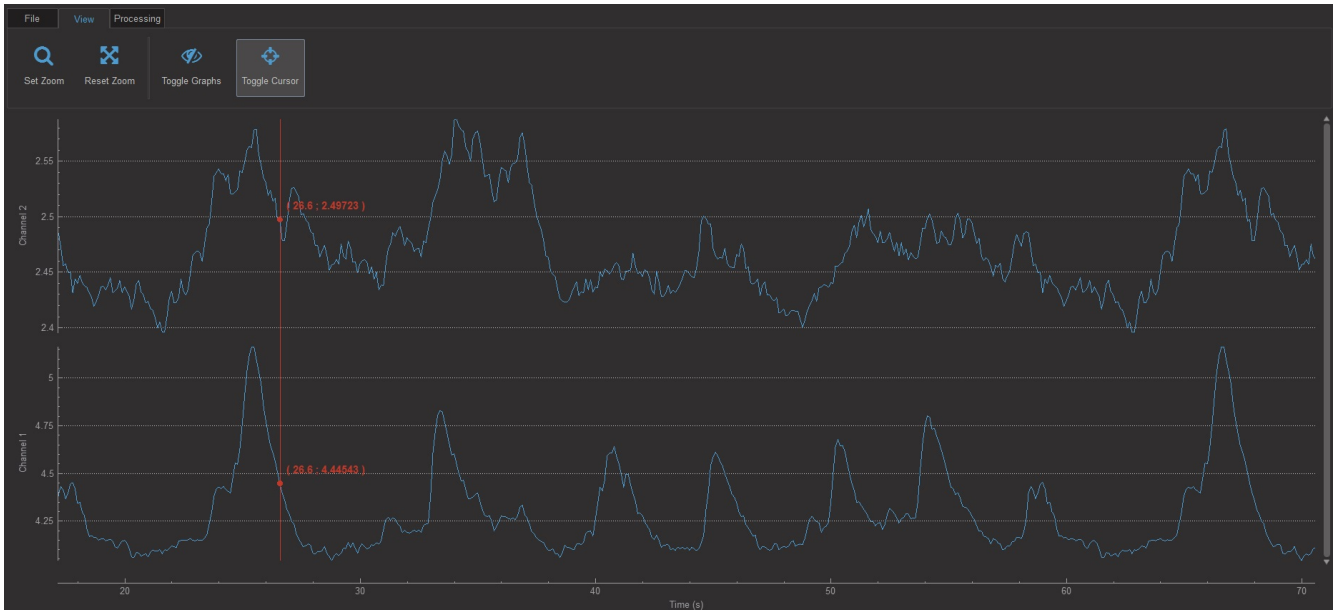


Figure 4.13: *Photometry Analysis Module Interface*

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.

4.2.1 Function Toolbar

1. The **File Tab** (Fig. 4.14) is used to load/save data,

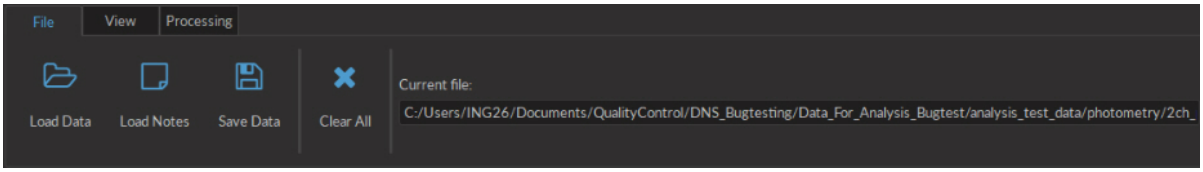


Figure 4.14: *File tab*

   - 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. 4.15) is used to manipulate the appearance of the displayed data.

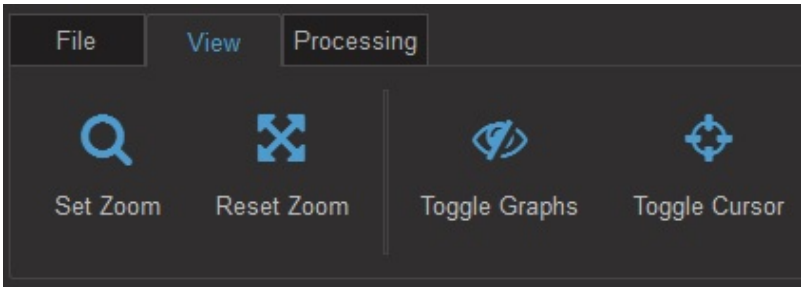


Figure 4.15: *View Tab*

- The **Set Zoom** button opens the **Set custom display range** window (Fig. 4.16). 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.

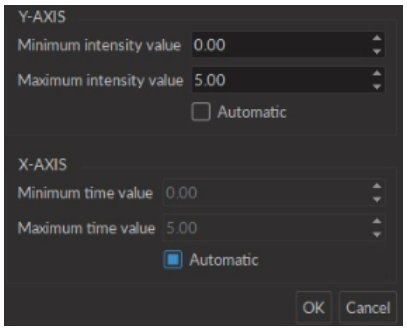


Figure 4.16: *Set Custom Display Range Window*

- 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. 4.17). The list in the window is used to toggle current graphs on or off.

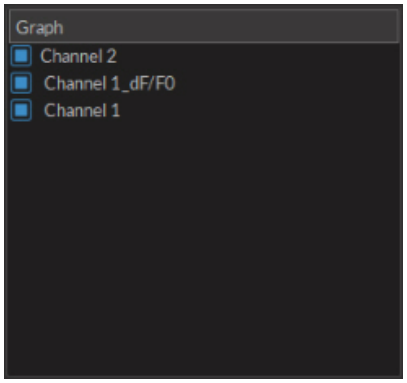


Figure 4.17: *Show/Hide Graph(s) Window*

- The **Toggle cursor** button adds a cursor to the graphs that shows local coordinates of time and intensity, as displayed in red on figure 4.13.

3. The **Processing tab** is used to control the various processing functions.

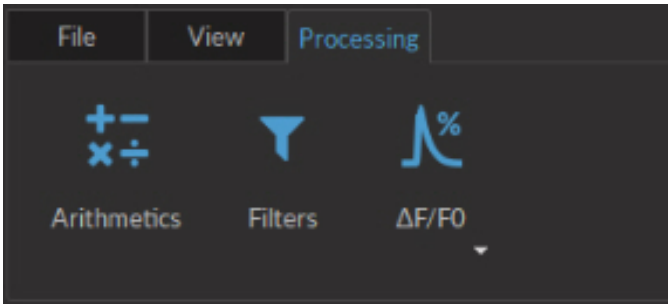


Figure 4.18: *Processing Tab*

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

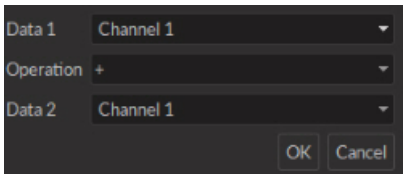


Figure 4.19: *Arithmetics Window*

- The **Filter** function opens the **Filter** (Fig. 4.20) window uses a butterworth filter to remove unwanted frequencies from the data. These filters can be **Bandpass, Bandstop, Low-pass** or **High-pass**.

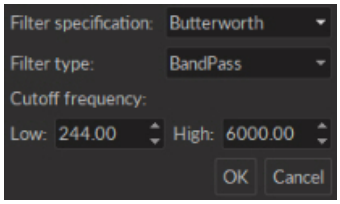


Figure 4.20: *Filters Window*

- The Δ**F/F$_0$** function opens the **Select Which Traces to Process** window (Fig. 4.21). It is used to calculate the normalized fluorescence variation of the data and displays the results in a new graph. See section 4.2.2 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.
  - **F$_0$ calculation method** shows the method used to evaluate F_0, 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.

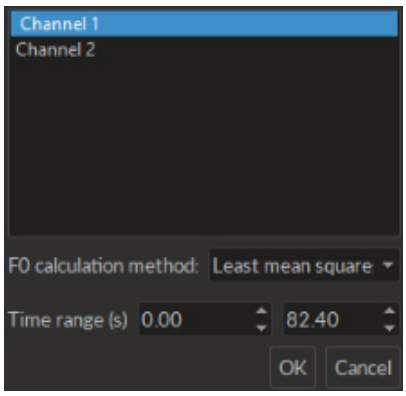


Figure 4.21: $\Delta F/F_0$ *Options*

4.2.2 Algorithms

ΔF/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.

4.3 Behavioral Tracking Analyser

This module allows the observation of behavior video with traces from experimental measurements. Video data is taken in **.avi** format, while trace data is received in **.csv** format.

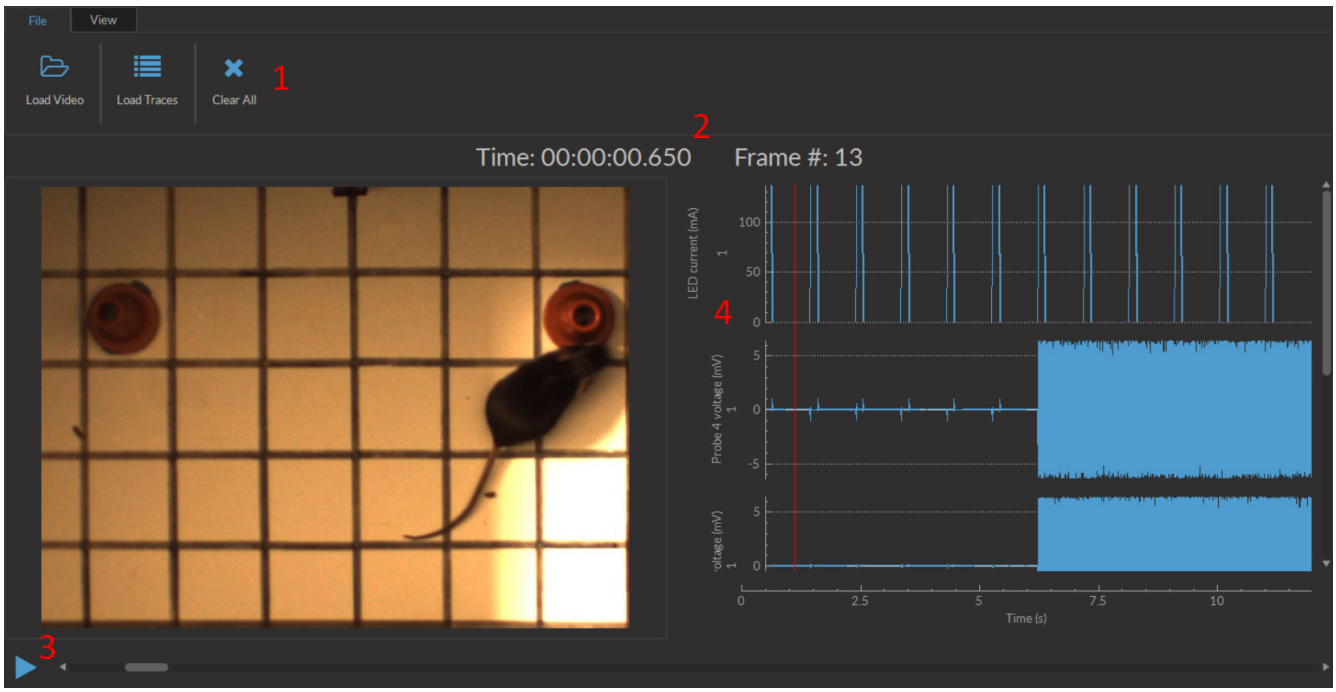


Figure 4.22: *Behavioral Tracking Analysis Module Interface*

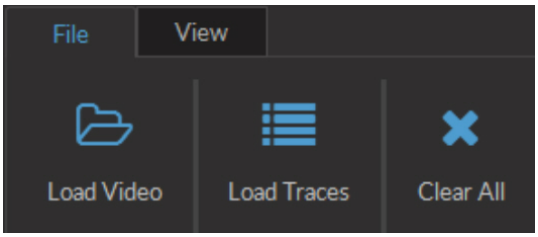
The interface can be separated into 4 major sections (Fig 4.22).

1. The **Tabs** are used to access the functions of the module.
2. The **Time** counters show the timestamp of a given frame in the video. As video is taken at a low frequency (50 Hz), while photometry data can be taken at very high frequency (>10 kHz), the timestamp displayed is that of the data point nearest to that of the frame.
3. The **Video** box is used to show video and control the frames displayed. The **Play** button on the bottom left runs the video. The scrollbar beside it can be used to choose a frame while the video is paused.
4. The **Traces** box shows the various traces associated with the video. The red bar over the traces corresponds to the timestamp of the associated frame of the video.

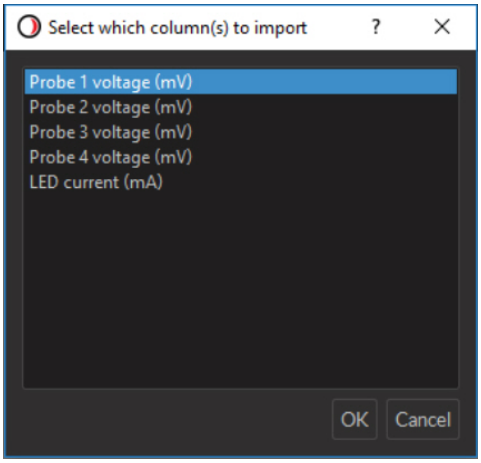
4.3.1 Tabs

Two tabs are found in this module.

1. The **File** tab (Fig. 4.23a) is used to load and remove data. The **Load Video** buttons allows the loading of **.avi** video files. The **Load Traces** button allows you to choose a **.csv** file and then opens the trace selection window (Fig. 4.23b). From this window, the desired traces can be selected. The **Clear All** button clear all experimental data from the module.
2. The **View** tab (Fig. 4.24) allows the modification of the video display. The **Zoom In/Zoom Out** buttons zoom the video display in and out. The **Reset Zoom** button resets the zoom to **100%**. The **Zoom Factor** drop-down list allows the choice of a specific zoom factor, from 10% to 500%.



(a) *Behavioral Tracking Analysis Module, File Tab View*



(b) *Behavioral Tracking Analysis Module, Trace Import Window*

Figure 4.23: *Behavioral Tracking Analysis Module, File Tab*

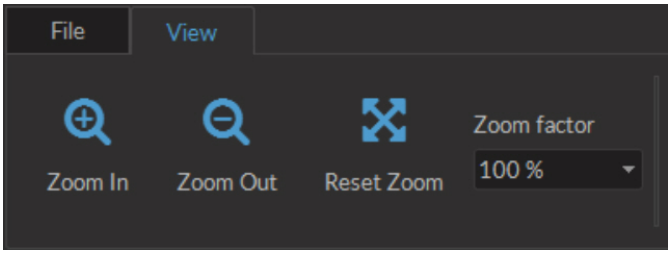


Figure 4.24: *Behavioral Tracking Analysis Module, View Tab*

4.4 Optrode Simulator

This module allows the simulation of the distribution function of light inside tissues when using an optrode. The software simulates the distribution for many types of fibers and conditions, also allowing the placement of electrodes within the model. This software is meant to ease the use of opto-electric probes of all kinds.

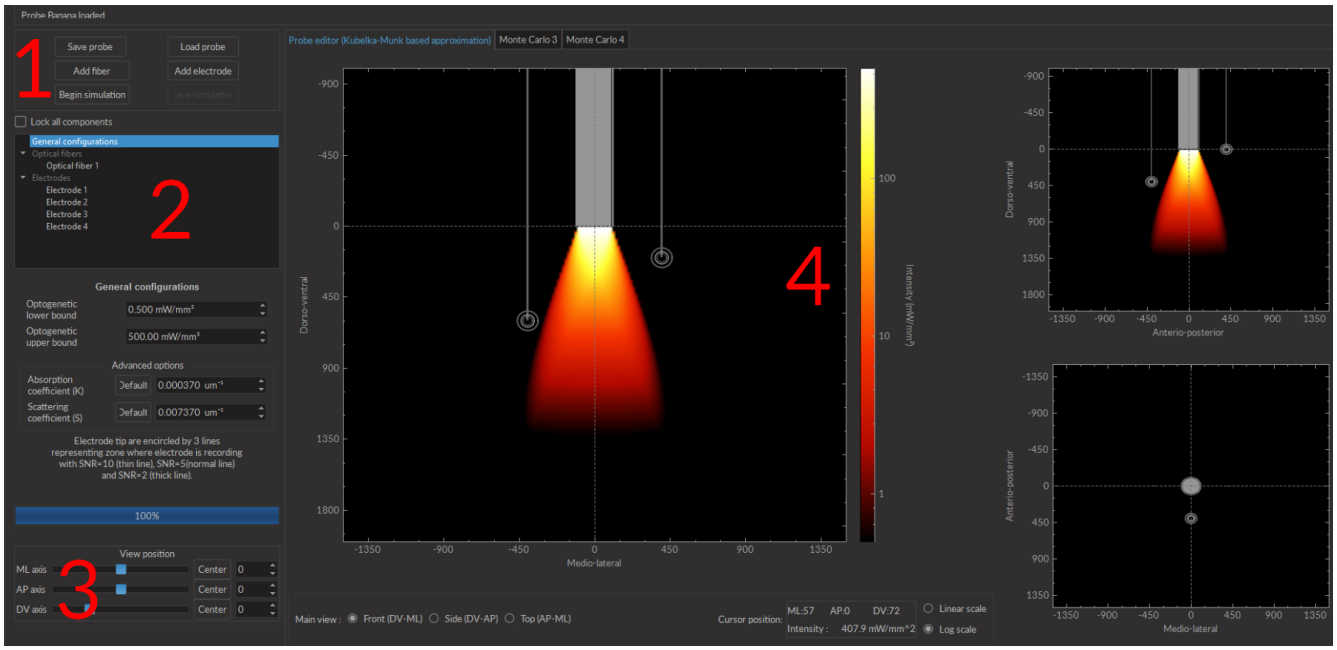


Figure 4.25: *Optrode Simulator*

The Optrode Simulator module (Fig. 4.25) can be separated into four sections.

1. The **Control** window the saving and loading of simulation configurations, the addition of fibers and electrode, as well as performing and saving simulations.
2. The **Configuration** box shows all the electrodes and fibers in the simulation, and allows access to their specifications.
3. The **View Position** window allows the positioning of simulation elements to be changed in 3-dimensional space.
4. The **Plot** box shows the relative position in space of each element of the simulation, as well as the distribution of light within the simulated space.

4.4.1 Control Box

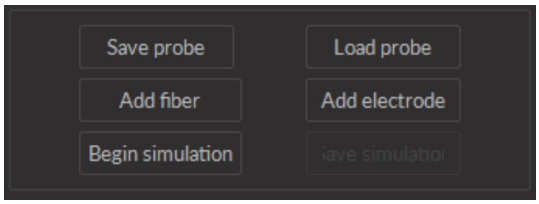


Figure 4.26: *Control Box*

The **Control Box** has 6 buttons used to control the simulation.

- The **Save Probe** button saves the parameters of a single element (fiber or electrode) from the configuration box in a **.json** format file.

- The **Load Probe** button loads the parameters of a single element (fiber or electrode) from a **.json** format file.
- The **Add Fiber** button adds a single new fiber to the **Configuration Box**.
- The **Add Electrode** button adds a single new electrode to the **Configuration Box**.
- The **Begin Simulation** button starts the simulation of the diffusion of light in the simulated medium according to model based on the Monte-Carlo method. When complete, a second tab will appear in the **Plot** box.
- The **Save Simulation** button saves the data obtained from a simulation in a **.csv** text file.

4.4.2 Configuration Box

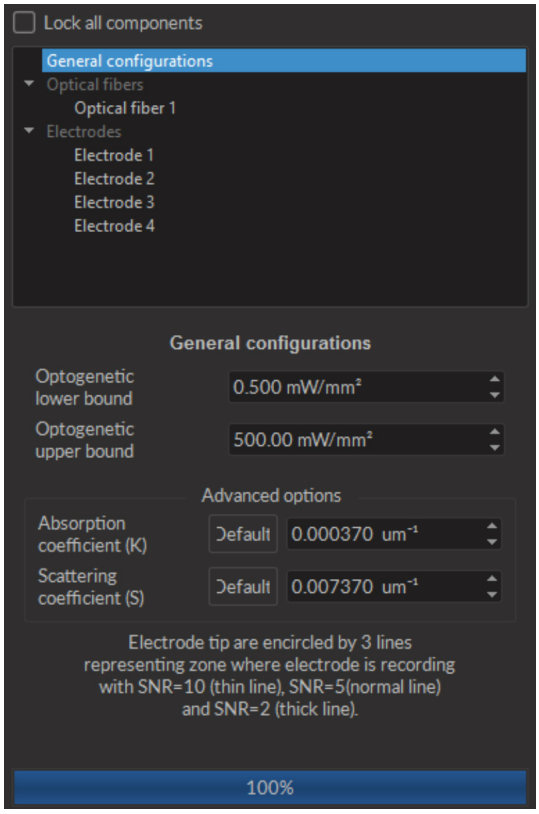


Figure 4.27: *Configuration Box*

1. The **Lock all Components** checkbox locks the movement of components used in the simulation so that they move together rather than separately.
2. The **Element** window shows a list of all parameters currently in use in the simulation. If several electrodes and optical fibers are used, it is possible to hide the full list by clicking on the arrow to the left. By clicking on a name in that list, the associated parameters can be accessed.
3. The **General configuration** section (Fig. 4.27) allows the definition of general elements that will affect the whole simulation.
    - The **Optogenetic upper/lower bound** defines the values of optical power density shown on the plot. Any value of power density above the lower bound and below the upper bound will be shown on the plot of the light distribution.
    - The **Absorption Coefficient** parameter allows the choice of the absorption coefficient of the space simulated. The default values presented are for brain tissues with light at a wavelength of 500 nm.
    - The **Scattering Coefficient** parameter allows the choice of the scattering coefficient of the space simulated. The default values presented are for brain tissues with light at a wavelength of 500 nm.

- In this section, the meaning of the concentric circles appearing on the plot around an electrode is described. The thinner the circle, the lower the SNR of the signal received in that region. From the inner circle to the electrode, SNR=10; from the middle circle to the inner circle, SNR=5; and from the outer circle to the middle circle SNR=2.
- The **Progression Bar** shows the progress of a simulation being computed.

4. The **Optical fibers X** (where X is the fiber number) section (Fig. 4.28) presents all the parameters to define the optical fiber used.

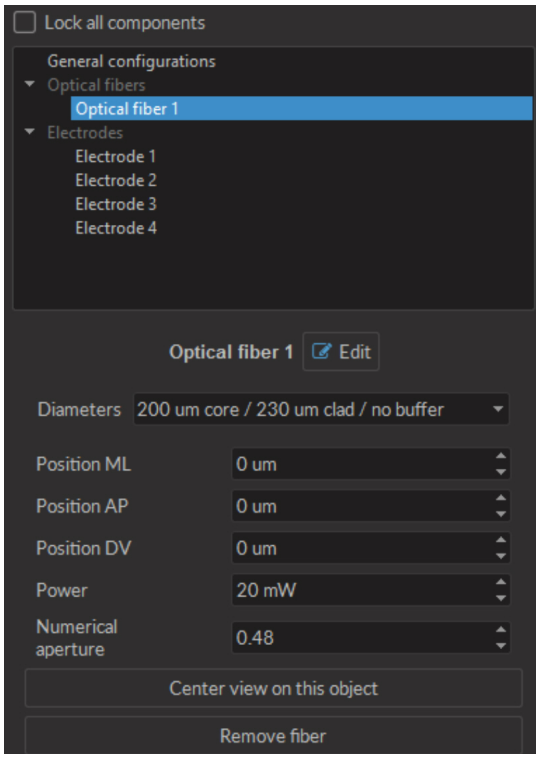


Figure 4.28: *Parameter Window, Optical Fiber X*

- The **Edit** button allows the editing of the fiber's name.
- The **Diameters** parameter allows the choice of the type of fiber used. The drop-down list offers several optical fibers used by Doric Lenses to make optrodes.
- The **Position ML** parameters allows the choice of the position of the fiber along the *Medio-Lateral* axis.
- The **Position AP** parameters allows the choice of the position of the fiber along the *Anterior-Posterior* axis.
- The **Position DV** parameters allows the choice of the position of the fiber along the *Dorso-Ventral* axis.
- The **Power** parameter allows the choice of the optical power (in mW) coming out of the fiber.
- The **Numerical aperture** parameter shows the numerical aperture of the fiber.
- The **Center View on this object** button centers the plot on the given optical fiber.
- The **Remove Fiber** button removes the fiber from the fiber list and the plots.

5. The **Electrode X** section, (where X is the electrode number; Fig. 4.29) presents all the parameters to define the electrode used. Functions are identical to the similarly named ones from the **Optical fiber X** section, with one addition.

- The **Electrode** drop-down list allows the choice of the type of electrode used in the experiment. These are classified according to wire diameter (in μm and impedance (in MΩ) These represent standard electrodes provided by Doric Lenses.

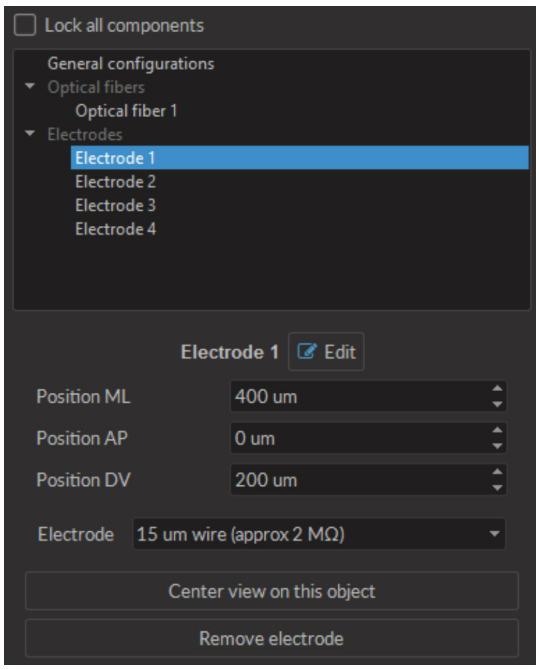


Figure 4.29: *Parameter window, Electrode X*

4.4.3 Plot Window

The **Plot** window (Fig. 4.30) shows views of the simulated space along the *Medio-Lateral*, *Dorso-Ventral* and *Anterior-Posterior* axes. Multiple tabs for different simulations can be used, with the tabs appearing in the upper left of the window. The tabs are named for the model used in the simulation, with the default tab using the Kubelka-Munk model.

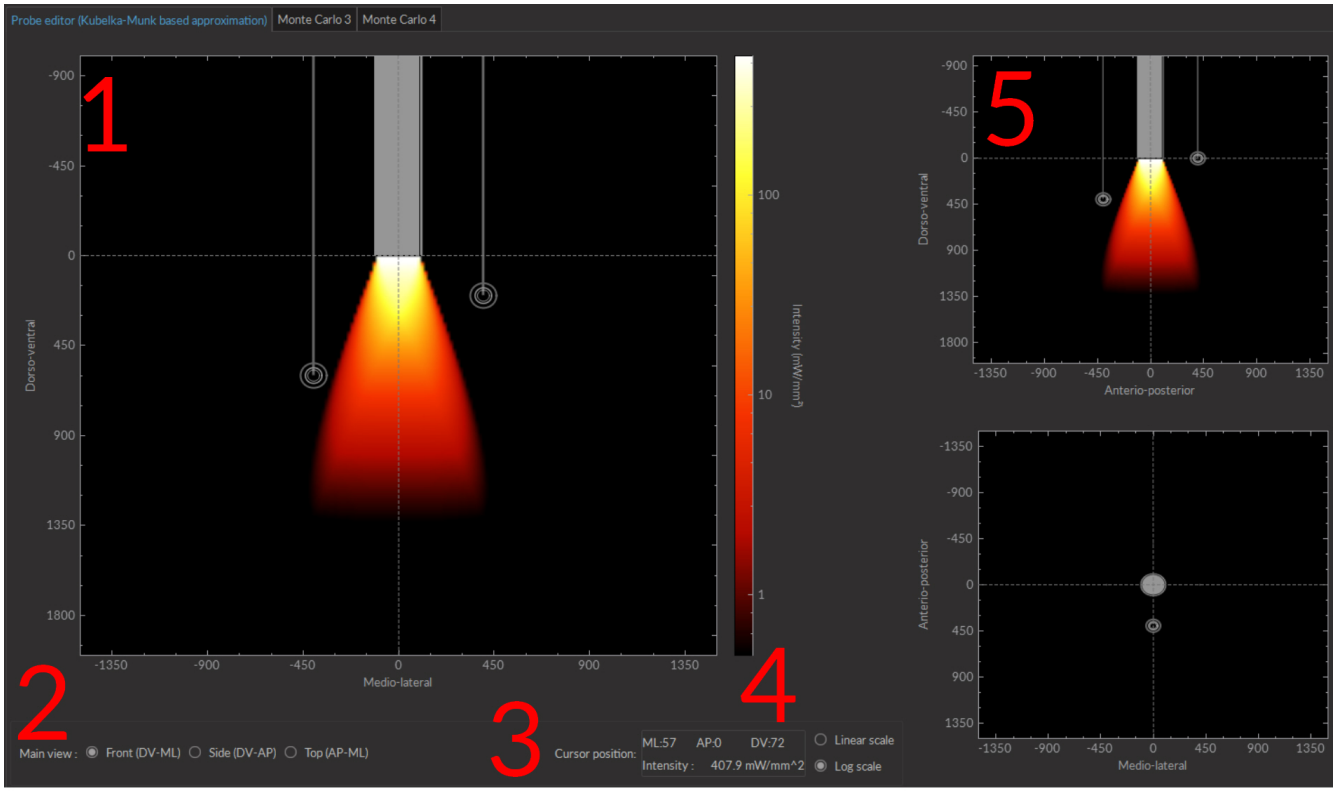


Figure 4.30: *Plot Window*

1. The **Main Plot**, in the left part of the windows, shows a large-scale view of the simulated situation for a given axis.
2. The **Main View** checkboxes in the bottom left allow the primary axis of the main plot to be changed.
3. The **Intensity Scale** (in mW/mm^2), to the right of the plot, displays the colors corresponding to the intensity of light leaving the optrode on the plot. The checkboxes below the scale can also be used to change the scale from **Log Scale** to **Linear Scale**.
4. The **Cursor Position** box shows the location of the cursor on a plot, relative to the three axes. It also displays local optical intensity (in mW/mm^2).
5. The two **Right plots** show scaled down versions of the simulated space along those axes not shown on the main plot.

4.5 Electrophysiology Analysis Module

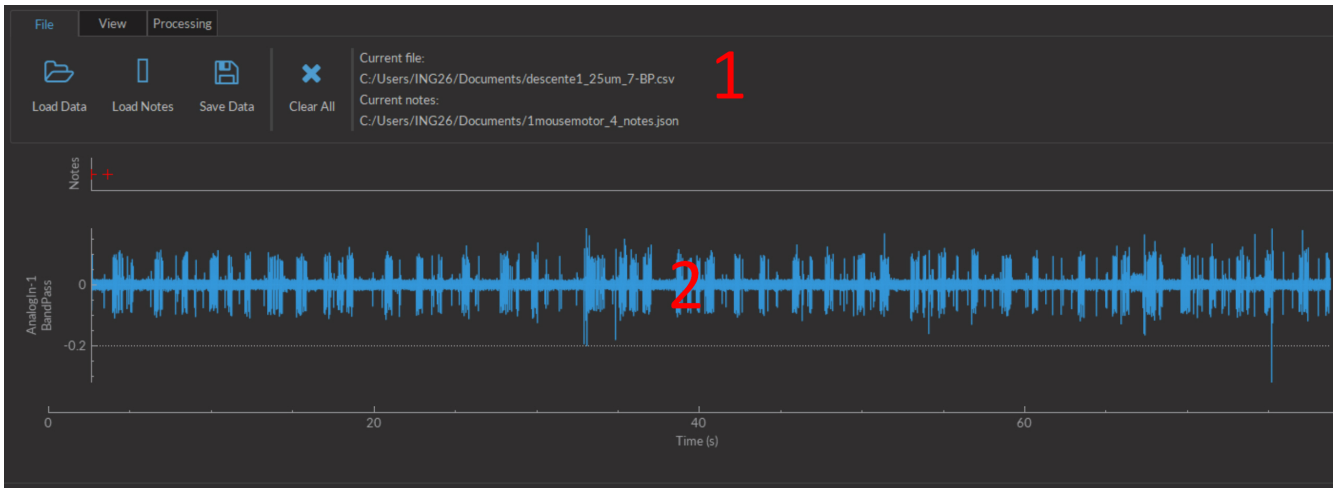


Figure 4.31: *Electrophysiology Analysis Module*

The **Electrophysiology analysis module** (Fig 4.31) is designed to process electrophysiological data. The module is separated into two main sections.

1. The **Control box** contains all controls, separated into the **File**, **View** and **Processing** tabs.
2. The **Graph box** contains all currently displayed graphs as well as timestamped notes.

4.5.1 File

The **File** tab allows the loading and saving of data.

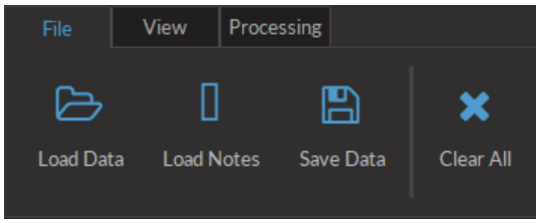


Figure 4.32: *File Tab*

- The **Load data** button allows the loading of a *.csv* file. The file must contain time and signal data.
- The **Load notes** allows the loading of a *.json* file contained timestamp-associated notes. Both the *.csv* and *.json* files must have the same time series.
- The **Save data** button saves graph data in a *.csv* file.
- The **Clear data** button deletes all data currently in the module. This data cannot be recovered, so ensure the data is properly saved before clearing it.
- The **Current File** box displays the files currently loaded into the analysis module.

4.5.2 View

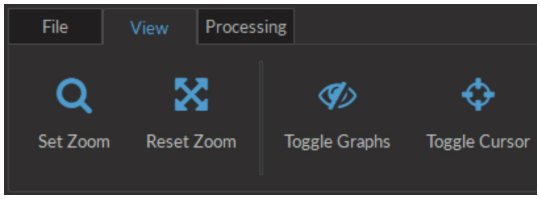


Figure 4.33: *View Tab*

The **View** tab (Fig. 4.33) is used to adjust the view in the **Graphs box**.

- The **Set Zoom** button opens the **Zoom window** (Fig. 4.34). The minimum and maximum values of each axis can be defined. The **Automatic** checkbox chooses axis values to fully display the entire data set.

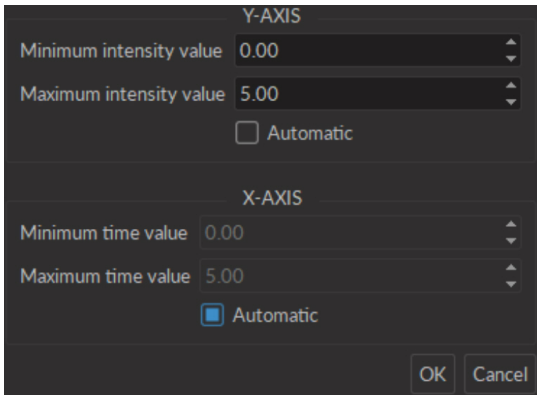


Figure 4.34: *Set Zoom Window*

- The **Reset zoom** button resets the axis values to those defined in **Set zoom**.
- The **Toggle graphs** button opens the **Show/hide graphs** window (Fig. 4.35). Any checked data sets will be displayed in the **Graph box**.

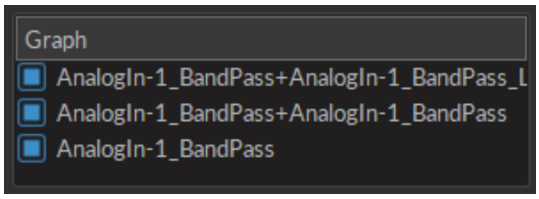


Figure 4.35: *Show/hide Graphs Window*

- The **Toggle cursor** button, when clicked, activates a red cursor in the **Graphs box**. This cursor can be moved to any part of a graph by clicking the location, showing the coordinates of the chosen point. The cursor will be positioned at the same temporal location on each graph.

4.5.3 Processing

The **Processing** tab (Fig. 4.36) is used to process data sets loaded into the module.

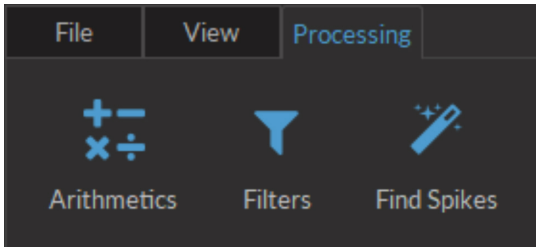


Figure 4.36: *Processing Tab*

- The **Arithmetics** button opens the arithmetic window (Fig. 4.37). From this window simple arithmetic operations (+,-,×,÷) can be performed on any two data sets currently in the module. This includes data sets that have already been processed.

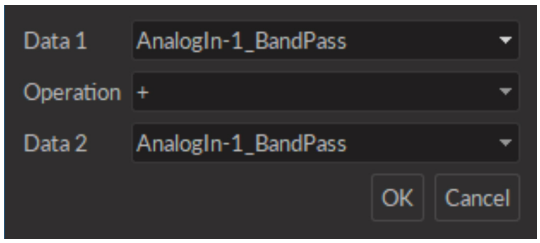


Figure 4.37: *Arithmetic Window*

- The **Filter** button is used to filter the frequency of data sets. After selecting the data set to be filtered, the filter window (Fig. 4.38) is opened. This allows a choice of filter type and variables.

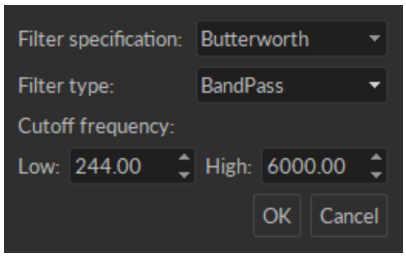


Figure 4.38: *Filter Window*

  - The **Filter specification** allows the selection of a filter model.
  - The **Filter type** defines whether the filter is low-pass, high-pass or bandwidth limited.
  - The **Cuttoff frequency** defines which frequencies are filtered. Which values are accessible depends on the **Filter type**.

- The **Find spikes** button allows the automated identification of spikes in the data set. See section 4.5.4 for more details.

4.5.4 Find Spikes Function

The **Find spikes** function is used to automatically identify and classify spikes in electrophysiological signal. Clicking the **Find spikes** button prompt the selection of a dataset, followed by the **Spike finder settings**(Fig. 4.39) window, showing the following variables.

Spike Finder Settings Window

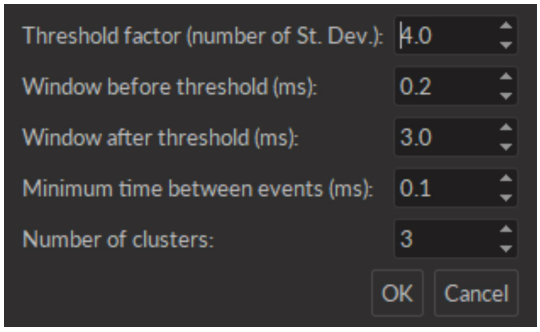
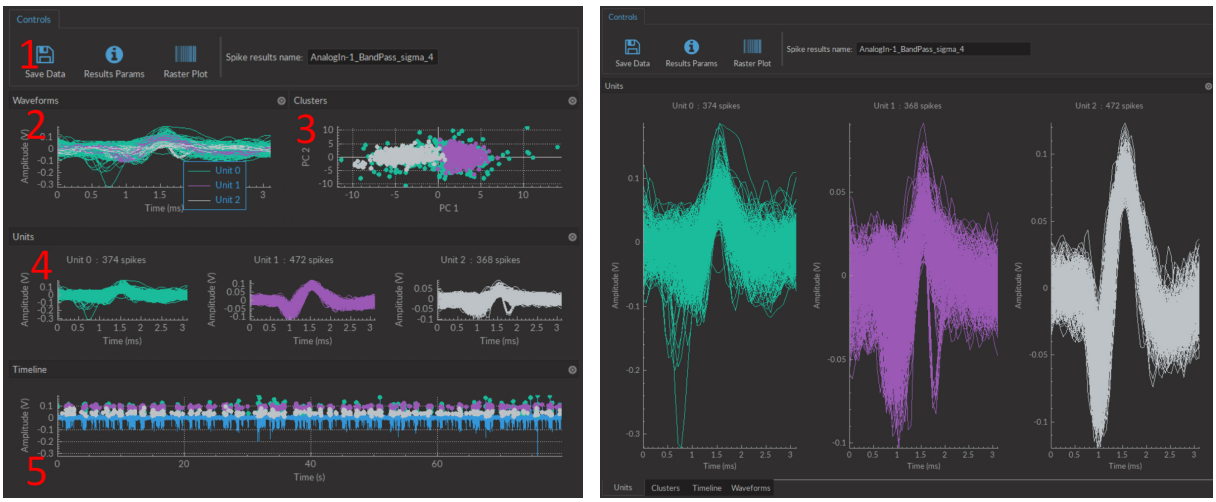


Figure 4.39: *Spike Finder Settings Window*

- The **Threshold factor** defines the number of standard deviations above the average the amplitude must have to be identified as a spike. A low threshold factor will give more false positives, while a high threshold factor can reject good spikes.
- The **Window before/after threshold** defines the time recorded (in ms) before/after the point identified as a spike.
- The **Minimum time between events** defines the minimum time (in ms) between two different spikes.
- The **Number of units** defines the number of different spike waveforms (also called **units**) searched for.

Spike Finder Results Window

The results provided by the spike finder algorithm are displayed in the **Spike finder results window** (Fig. 4.40a). The window is separated into several boxes. Each box can be undocked from the main window. Each box can be clicked and dragged onto each other; this converts each box into a tab (Fig. 4.40b, lower left).



(a) *Spike Finder Results Window, Full display*

(b) *Spike Finder Results Window, Tab Mode*

Figure 4.40: *Spike Finder Results Window*

1. The **Control box** (Fig. 4.41) displays options usable in the window.

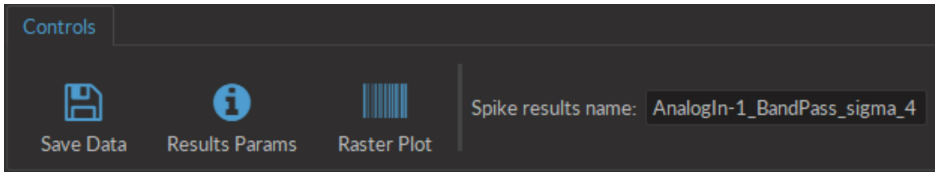


Figure 4.41: *Spike Finder Control Box*

- The **Save data** button records data in this window in the *.HDF5* format. The data can be added to a pre-existing database.
- The **Results Params** button opens a window displaying the **Spike finder settings**. The settings cannot be changed in this window.
- The **Raster Plot** button opens the **Raster plot window** (Fig. 4.42). The window displays the raster plot of all spikes over time, with each color corresponding to a single cluster. This window can be kept active over multiple uses of the **Spike finder**, with all raster plots displayed within.

Figure 4.42: *Raster Plot Window*

- The **Spike results name** shows the filename used to save the spike results.

2. The **Waveform box** (Fig. 4.43) shows a superimposed view of all different spike waveforms recovered. Each different color corresponds to a different unit, as shown on the legend.

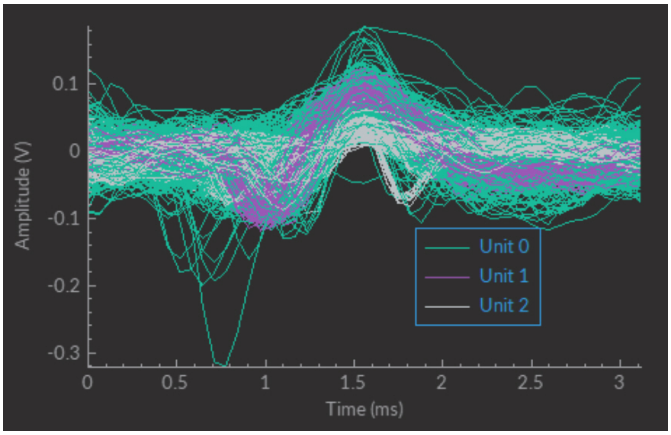


Figure 4.43: *Waveform box*

3. The **Clusters** box shows a scatter plot of all spikes. Each color corresponds to a different unit.

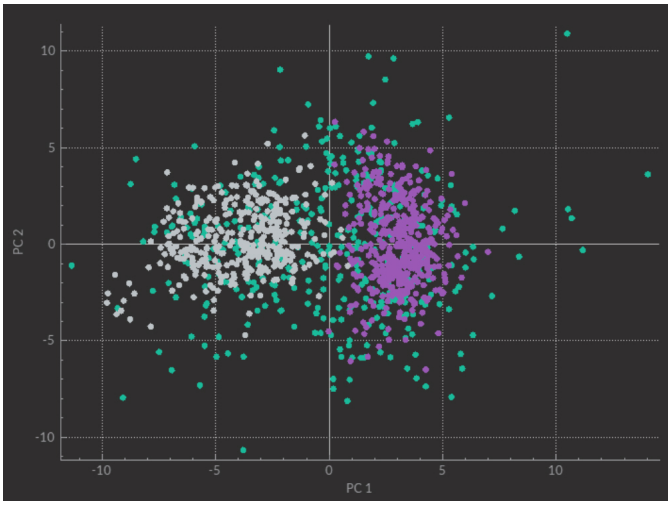


Figure 4.44: *Clusters box*

4. The **Unit box** shows a superimposed view of all different spikes associated with each individual unit.

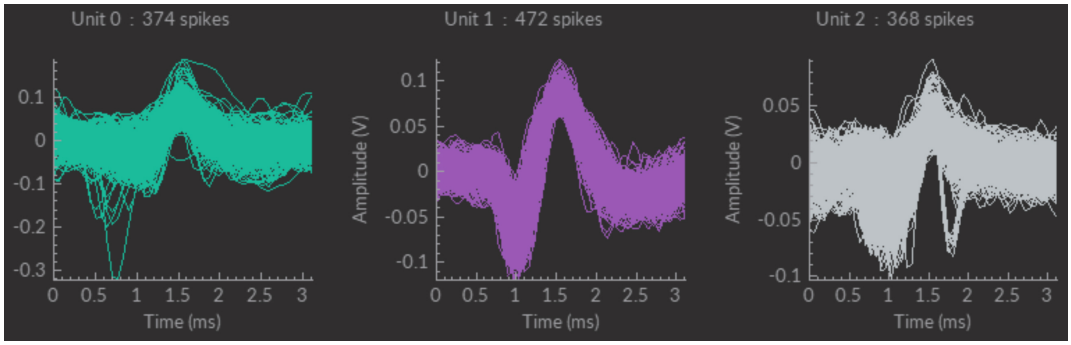


Figure 4.45: *Unit box*

5. The **Timeline box** shows the original graph with each spike identified. Each color indicates a given unit, as defined in the legend of the **Waveform box**.

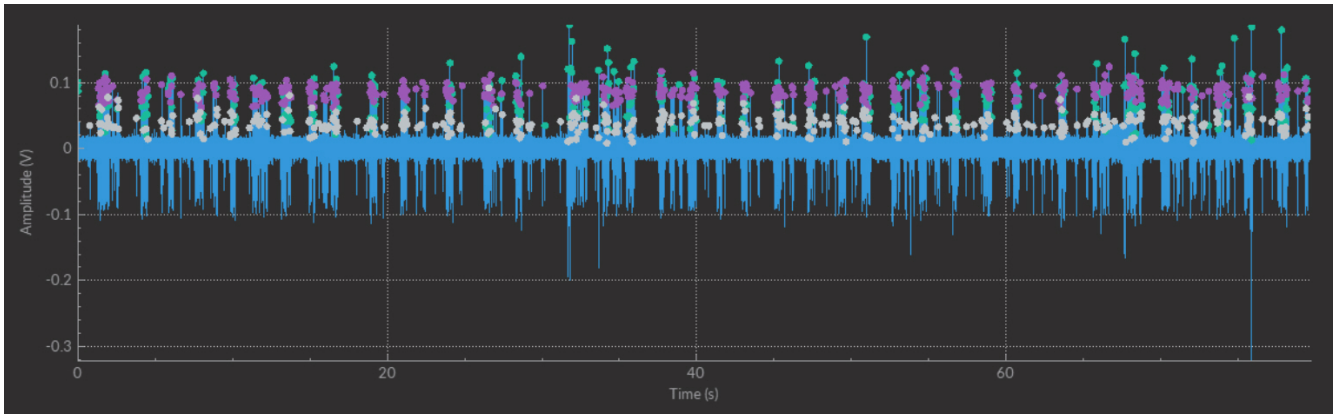


Figure 4.46: *Timeline box*

5

Specifications

Table 5.1: *Doric Neuroscience Studio Hardware Requirements*

SPECIFICATIONS	VALUE	NOTES
Operating System	Windows 7, 8 , 10	64-bit
Memory (Minimum/Recommended)	4 GB/16 GB	
Processor Speed (Minimum/Recommended)	2 Ghz Quad-Core i5/ 3.46 Ghz Eight-core i7	
Hard Drive	500 MB	

Table 5.2: *Doric Neuroscience Studio Module Hardware Requirements*

MODULE	REQUIRED HARDWARE	NOTE
Microscopy	Dedicated Graphics Card	
	i7 or greater CPU	
	Gigabit ethernet card	Do not use a USB to ethernet adapter
Behavior Camera	Power USB3 or Gigabit ethernet port	

6

Support

6.1 Contact us

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

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