doric

Doric Neuroscience Studio

User Manual

Version 6.1.1

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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 ⋆LISER^{TM} Light Sources Drivers.
- Acquisition of the voltage of a chosen light source input BNC.
- Acquisition of data from our Doric Neuroscience 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 10, 11; 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, SSD recommended

Software Installation and Updates

2.1 Installing softwares

2.1.1 Doric Neuroscience Studio

1. **Run** the Doric Neuroscience Studio Installer from the supplied USB key or download the latest version of the software from our Doric Neuroscience Studio webpage. See Table 14.1 for computer requirements.

2. **Select** the language to use during the installation.

Note: If a previous version of **Doric Neuroscience Studio** is already installed, the software will asked and help to uninstalled the previous version before install the new version (see section 2.2.1).

3. Click **Next** in the Information window.

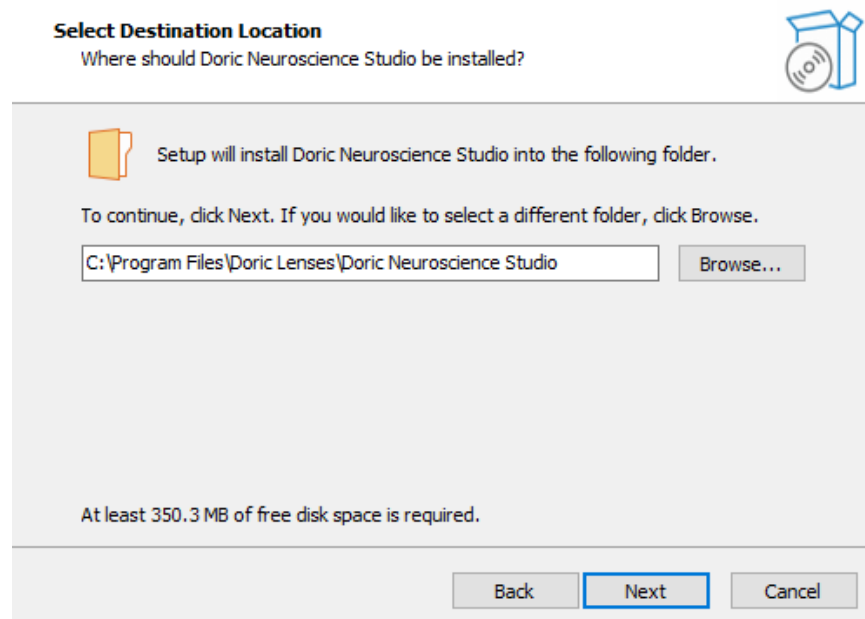4. **Choose** where to install the software (Fig. 2.1) and click **Next**.

Figure 2.1: *Select Destination Location*

5. **Choose** if desired to create a shortcut in the Start Menu folder and click **Next**.

6. Several Options are possible:

   - Select **Create a desktop shortcut** if you want a a shortcut to lunch the software on your desktop (Fig. 2.2).

- Select **Doric Maintenance Tool** to install the Doric service system to update device firmware, more information about update will be provide further in this user manual. We highly recommended to make this installation and to keep the devices and software up to date (Fig. 2.2).
- Select **Device Driver for 33U, 37U, 38U series cameras** to installed the drivers for USB cameras. This is necessary mainly for **Behavior Camera** and **BPFD systems** (Fig. 2.2).
- Select Device Driver for all GigE cameras to installed the drivers for Ethernet cameras (Fig. 2.2).

Click **Next** once the selection is done.
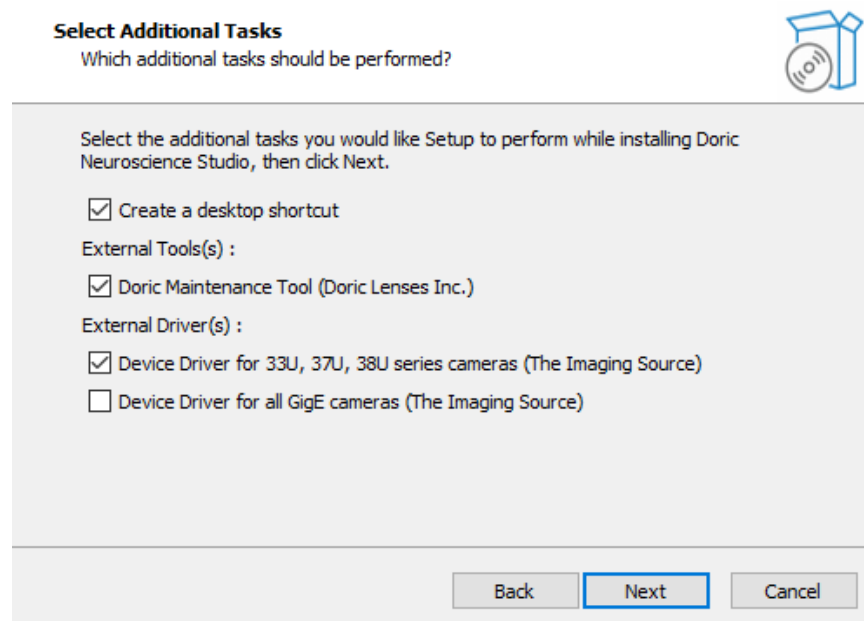
Figure 2.2: *Select complementary installations*

7. When ready, click **Install** to begin the process. This should take a few moments.
   Note: If you have select the installation of **Doric Maintenance Tool** and/or the installation of other driver, there installation will start at the end of the installation of **Doric Neuroscience Studio**, before the next step. See next sections for information about their installation.
   When the installation is done, the message in figure 2.3 will show up.
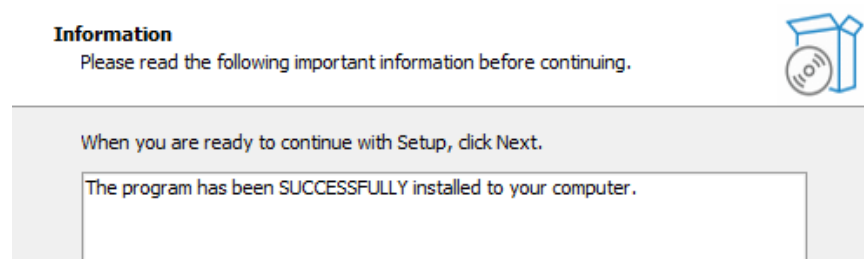
Figure 2.3: *Successful Installation of the Doric Neuroscience Studio*

8. Click **Finish** to exit the setup.

9. The software is ready for use.

2.1.2 Doric Maintenance Tool

If Doric Maintenance Tool had been selected to be install during installation of Doric Neuroscience Studio, the installation begin just after the end of Doric Neuroscience Studio installation.

1. **Select** the language to use during the installation.

Note: If a previous version of **Doric Maintenance Tool** is already installed, the software will asked and help to uninstalled the previous version before install the new version (see section 2.2.1).

2. Click **Next** in the Information window.

3. **Choose** where to install the software (Fig. 2.4) and click **Next**.
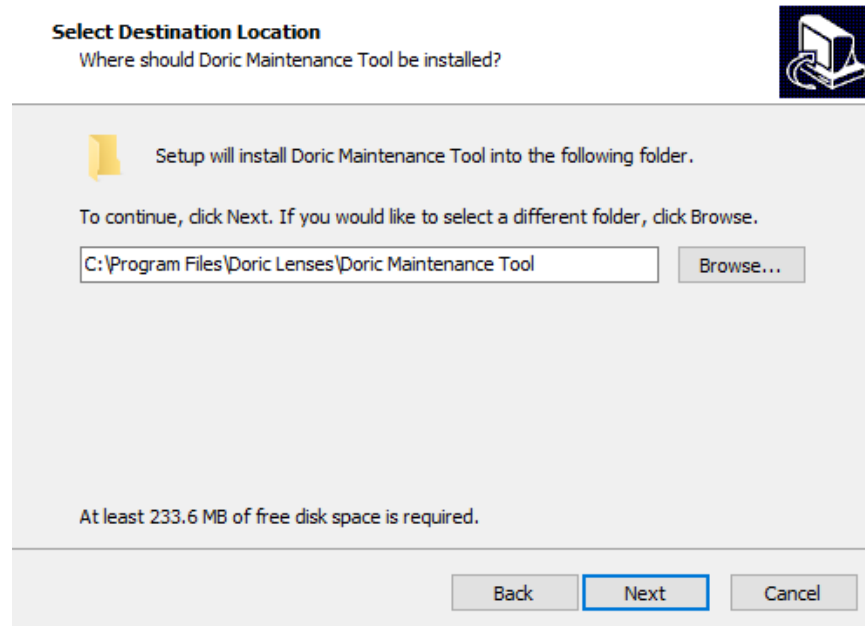
Figure 2.4: *Select Destination Location*

4. **Choose** if desired to create a shortcut in the Start Menu folder and click **Next**.

5. **Unselect Create a desktop shortcut** if you do not want a desktop shortcut for **Doric Maintenance Tool** on your desktop and click **Next**.

6. When ready, click **Install** to begin the process. This should take a few moments.
   When the installation is done, the message in figure 2.5 will show up.
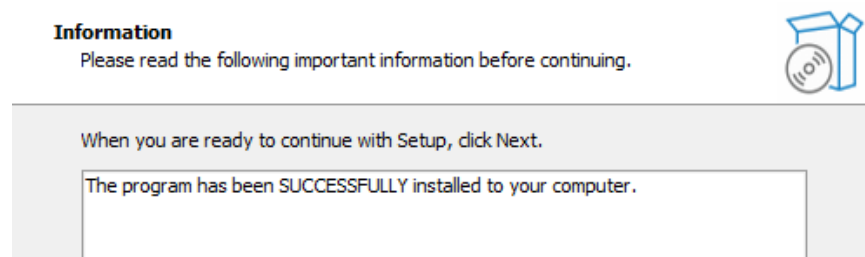
Figure 2.5: *Successful Installation of the Doric Neuroscience Studio*

7. Click **Finish** to exit the setup.

2.1.3   USB Camera Driver

The installation of the Camera Driver is simpler than the others installation.
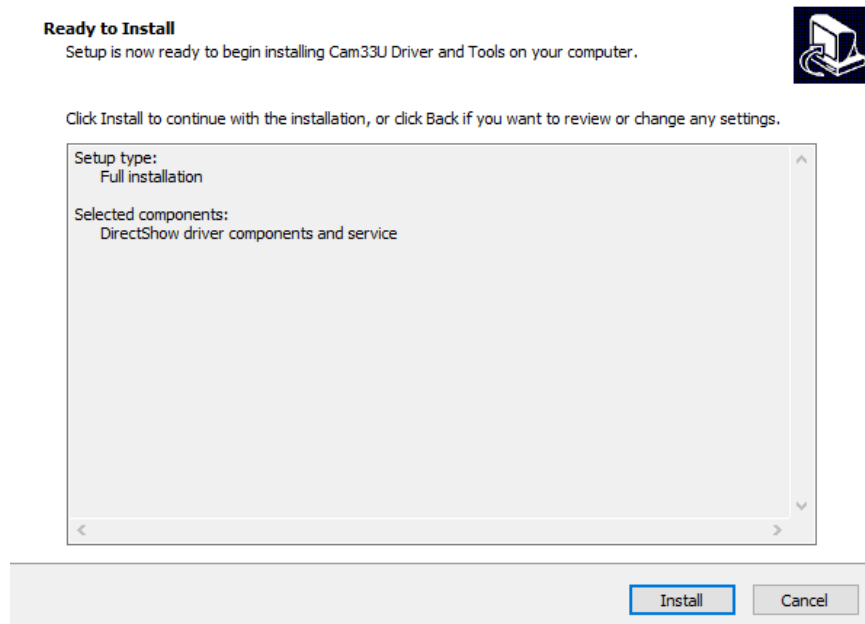
Figure 2.6: *Successful Installation of the Doric Neuroscience Studio*

1. Click **Install** to begin the process (Fig. 2.6). This should take a few moments. When the installation is done, the message in figure 2.9 will show up.
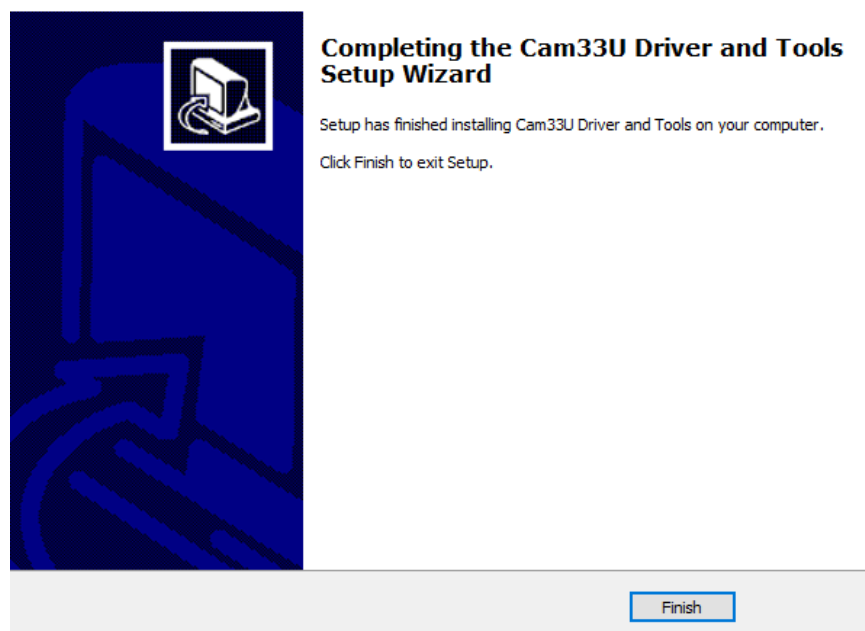
Figure 2.7: *Successful Installation of the Doric Neuroscience Studio*

2. Click **Finish** to exit the setup.

2.1.4 Ethernet Camera Driver

This installation is necessary if you plan to use GigE Ethernet camera for the experiments.

1. Click **Next** in the Welcome window

2. In Select Components windows, it is possible to select different options Fig. 2.8):

- Full installation: all the components will be install.
- Compact installation: to only install the driver.
- Custom installation: to select what will be install during the process.

Note: In the same time as the driver it is possible to install 2 options:

- Kernel-Mode filter driver to enhances the performance. It is recommended to use it if possible.
- IP configuration API files consists of two DLLs:$ipconfig\_api\_x64.dll$ and $ipconfig\_api\_win32.dll$.These DLLs can be imported into a C# program. Using the API, a program can query camera name, serial number, IP adress, firmware version and so on (for more information about this module, see here).

When components had been selected, click on **Next** to start the installation.

3. When the installation is done, the message in figure 2.9 will show up.

4. Click **Finish** to exit the setup.
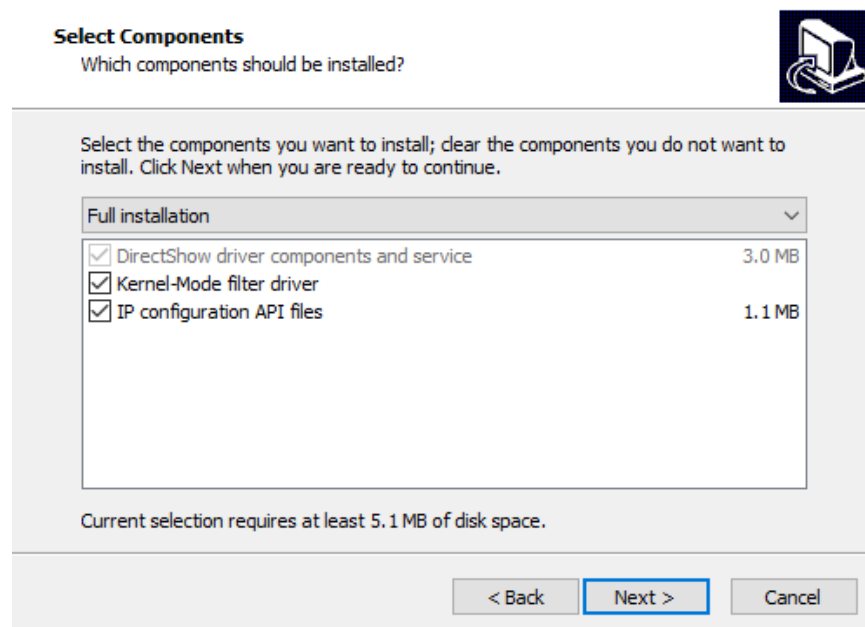

Figure 2.8: *Options selection in Ethernet Camera driver installation*
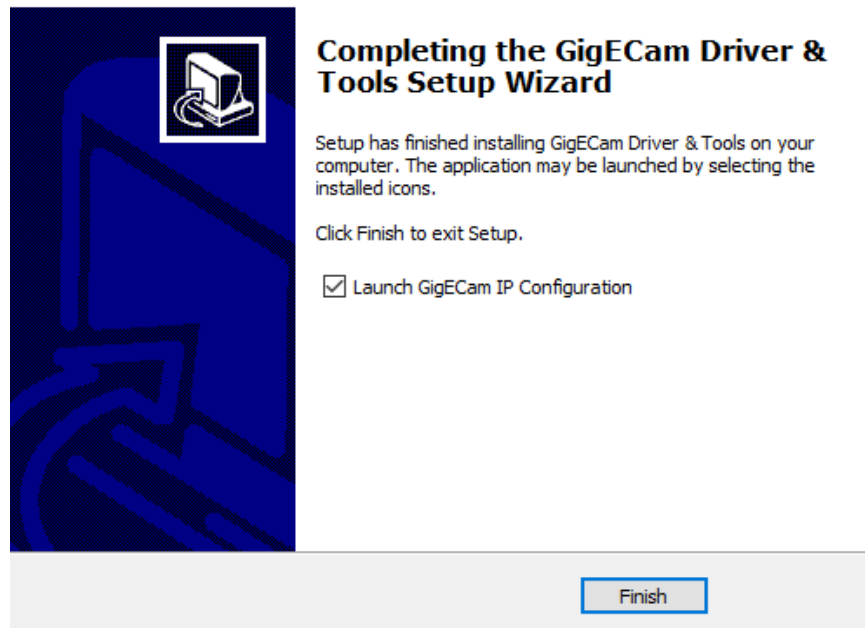
Figure 2.9: *Successful Installation of the Ethernet Camera Driver*

2.2 Updating Software & Firmware

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

1. Make sure to keep the software regularly updated. By selecting **Help — Check for updates** the **Update** window will appear (see Fig.2.10).
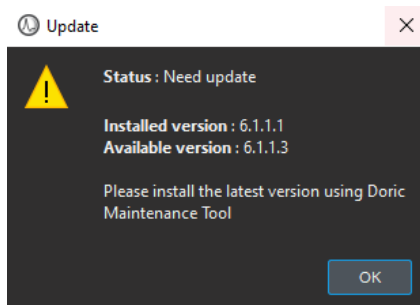
(a) *Update Window: Need update*
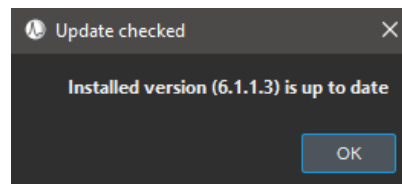
(b) *Update Window: Up to date*

Figure 2.10: *Update Window*

2. Should the installed version be older than the version online, the window **Update** appears (2.10a) asking to install the latest version with **Doric Maintenance Tool**. The procedure is describe just below. Else The window **Update checked** appears if the version is up to date.

2.2.1 Updating Doric Neuroscience Studio and Doric Maintenance Tool

1. Disconnect all Doric devices from the computer before starting the update and close **Doric Neuroscience Studio**.

2. Open Doric Maintenance Tool and Select the tab **Software(s)**.
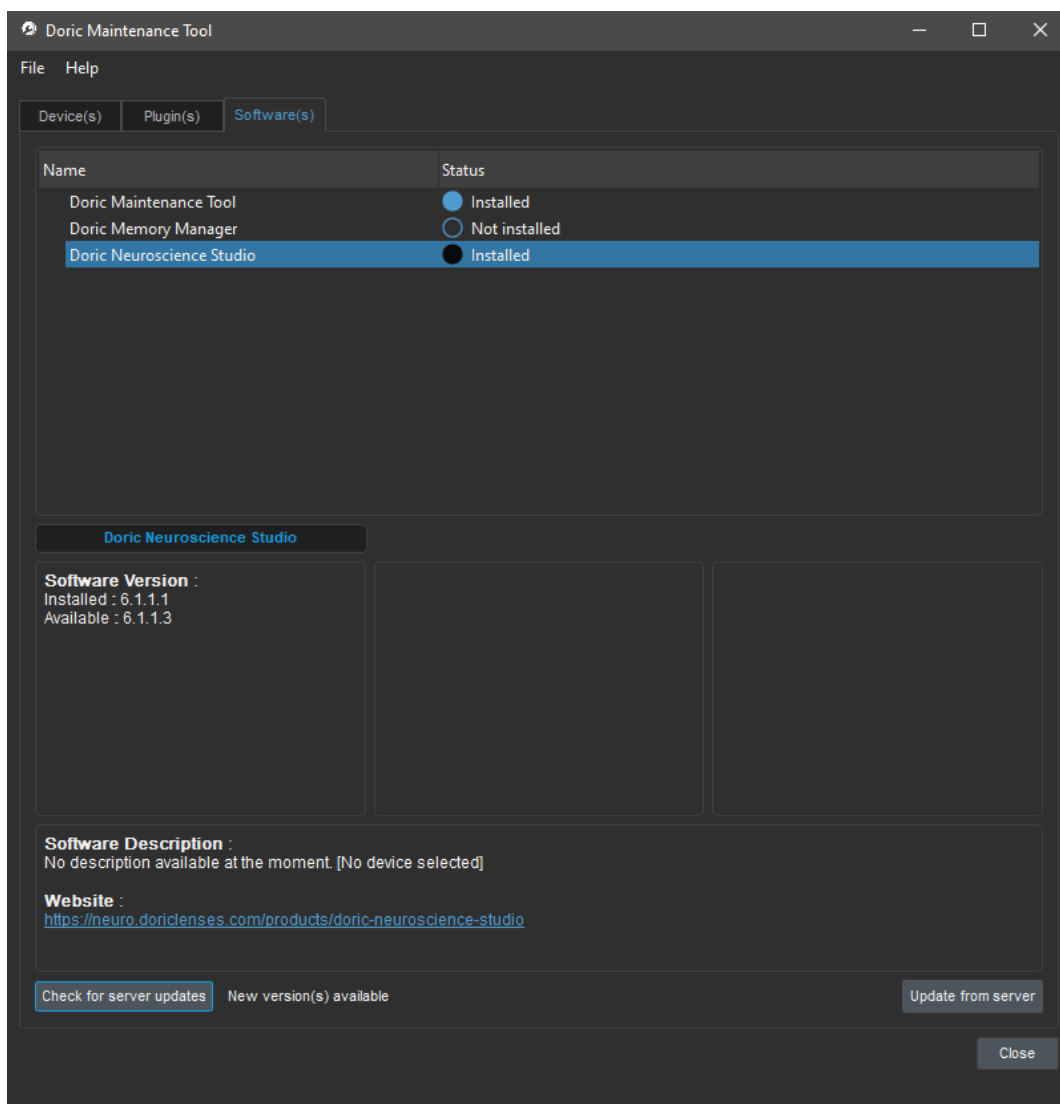
Figure 2.11: *Doric Maintenance Tool - Overview*

3. Select **Doric Neuroscience Studio** and click on **Check for server updates**.

4. The version displayed next to Available will be updated to the latest version available.

5. Click on Update from server to start the update of the version. After a short time of download Doric Maintenance Tool will turned of and the installation will be displayed.

6. **Select** the language to use during the installation.

7. The installer will immediately detect the previous version and present the option of uninstalling it (Fig. 2.11). **Click Yes**.
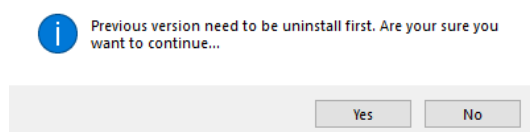
Figure 2.12: *Uninstall Window*

8. When the program asks if you are certain, **Click Yes**.

9. When the previous version is uninstalled, the installation of the new version need to be done like the first installation (see section 2.1).
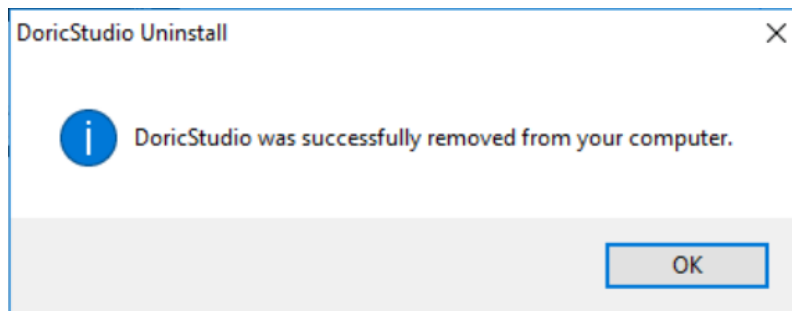
Figure 2.13: *Uninstall Completion*

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

Note: Always update **Doric Maintenance Tool** in the same time as **Doric Neuroscience Studio**.

2.3 Updating Firmware

To update the firmware version, close **Doric Neuroscience Studio**.

1. Open Doric Maintenance Tool. (The software can be install at the same time as **Doric Neuroscience Studio**)

2. Turn On the device.
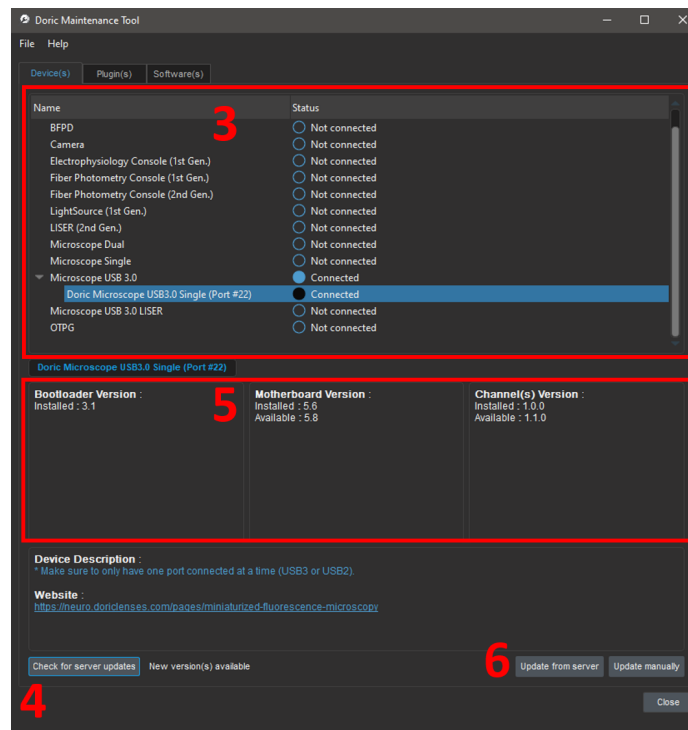
Figure 2.14: *Doric Maintenance Tool home page*

3. In the list under **Name**, select the device to update (it's status need to be **Connected**) (Fig. 2.14).

4. Select **Check for server updates**. **Doric Maintenance Tool** will connect to the server and verify if an update is available (Fig. 2.14).

5. Under **Motherboard Version** and **Channel(s) Version**, the present version and the available versions are displayed (Fig. 2.14).

6. Select **Update from the server** to lunch the update. (In some case an update can be necessary without use the server version. In this case, a representative of Doric Lenses will sent you the update file and you can use **Update manually** in replacement of **Update from server**) (Fig. 2.14).
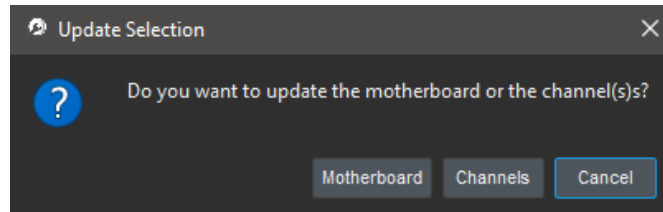
Figure 2.15: *Doric Maintenance Tool Update Selection*

7. A window ask you to choose between **Motherboard** or **Channels** to update (if you need to update both, select one of the both and repeat the process after the end of the first update) (Fig. 2.15).

8. Wait the end of the installation and select **OK**.

9. Wait 10 seconds and turn OFF the device. Turn ON the device and start **Doric Neuroscience Studio**.

Software Organization

When the software is properly installed, devices can be controlled from the computer via the **Doric Neuroscience Studio** as well as making analyses. When the software is launched a **Device Selection** window will appear (Fig. 3.1. These tabs are used to select which device will be used and which devices are compatible with **Doric Neuroscience Studio**. At the each start of the software, every detected device is be displayed in **Available device(s)** (Fig.3.1).
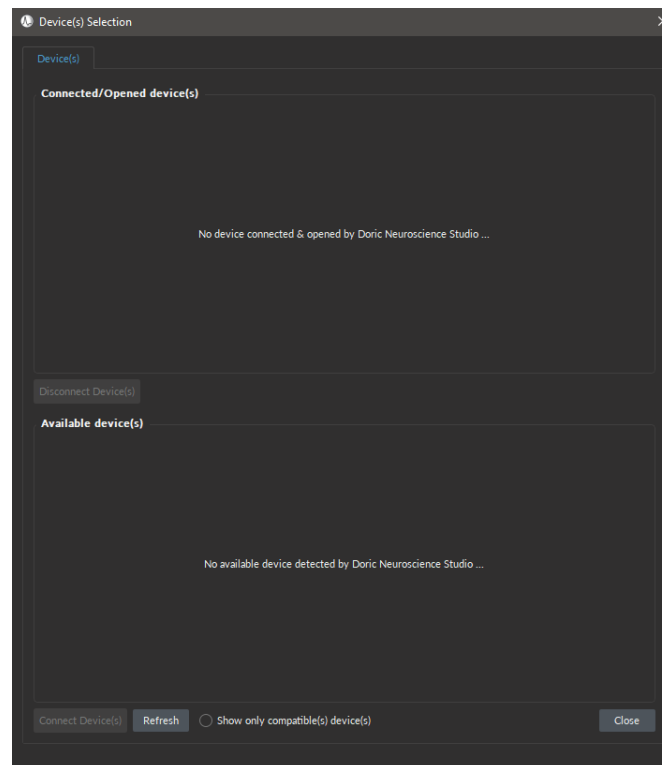
Figure 3.1: *Devices Selection tab*

<u>Notes:</u>

- If the device is not displayed in **Available device(s)** section, select **Refresh** to lunch a new detection process (Fig.3.1).

- If the device detected is not compatible, the message **Device Incompatibility** will be displayed (Fig.3.2a). See section 2.3 for update the firmware.

- If only a part of the device can be used, the message **Device Initialization** will be displayed (Fig. 3.2b).
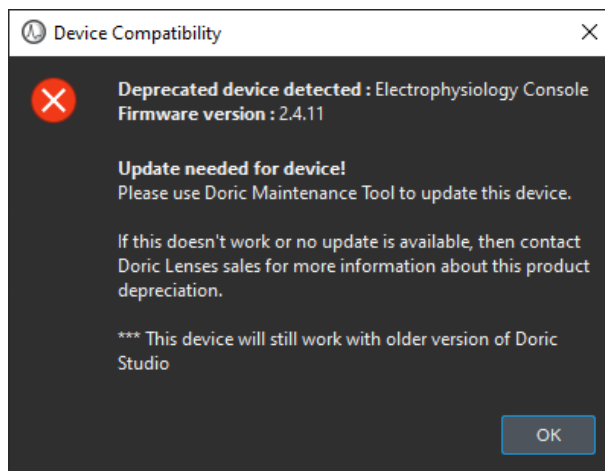
(a) *Device not compatible*
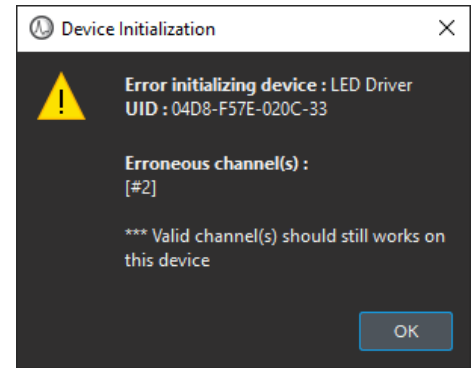
(b) *one (or more) channel is unavailable*

Figure 3.2: *Devices Compatibility/Initialization window*

To connect Doric Neuroscience Studio to a device:

1. Select the device in **Available device(s)** (A multiple selection can be done by maintaining Ctrl and clicking on devices to connect with).

2. Click on **Connect Device(s)** (a quick double click can also be used to connect Doric Neuroscience Software to the device). When the software is connected to the device, it appeared in **Connected/Opened device(s)**.

3. Select **Close** to quit the device selection and access to Doric Neuroscience Studio menus.

3.1   Software Organization

On Figure 3.3, the different menu options available at all times in the studio are shown.
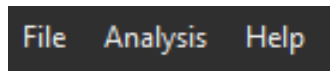
Figure 3.3: *Menu Options*
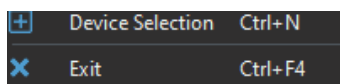
(a) *Files*
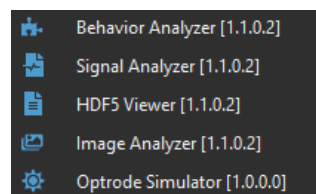
(b) *Analysis*
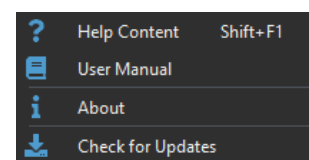
(c) *Help*

Figure 3.4: *Menu Drop-down Lists*

- **File** is used to select a device (see Fig.3.1) or quit the software

- **Analysis** (Fig. 3.4b) is used to open analysis modules. These modules are detailed in Chapter **??**.

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

  – When the **Help Content** buoy is selected (Shift + F1 shortcut), 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. 3.5), which shows the current version of the software.
- The **Check for updates** selection open the window (Fig. 3.6) of the same name. It can be used to check if the software can be updated. This function requires an Internet connection.
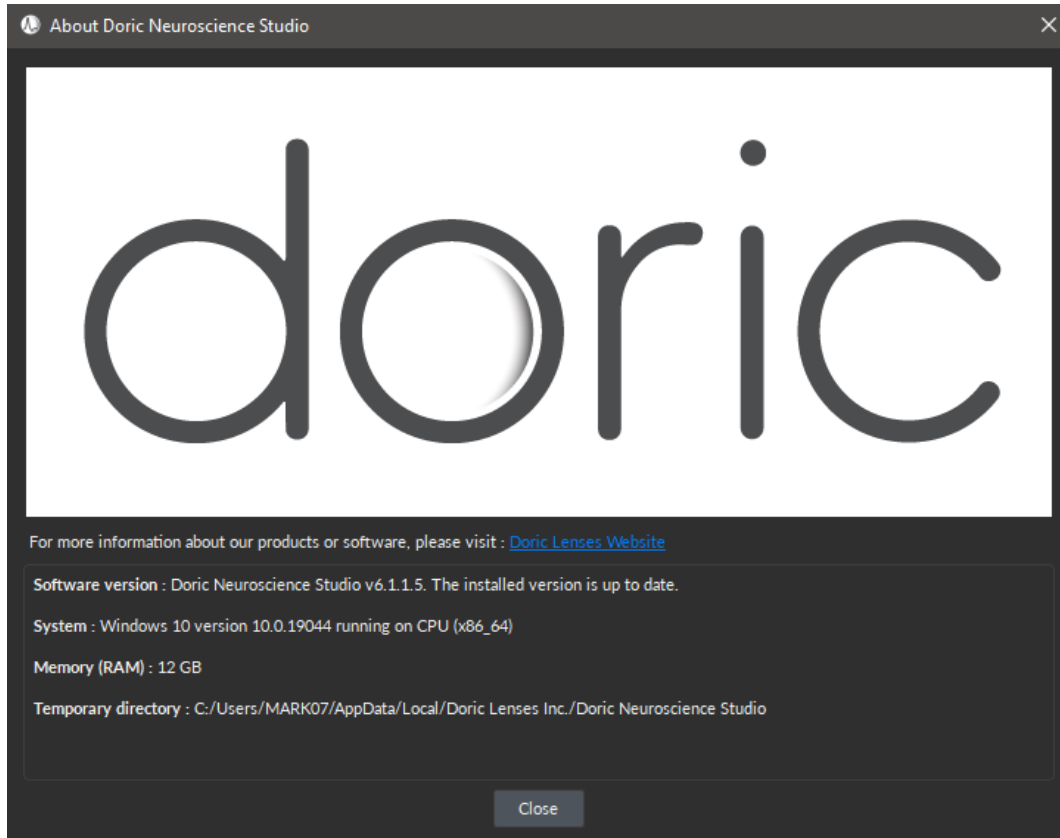
Figure 3.5: *About: information on Doric Neuroscience Studio version*
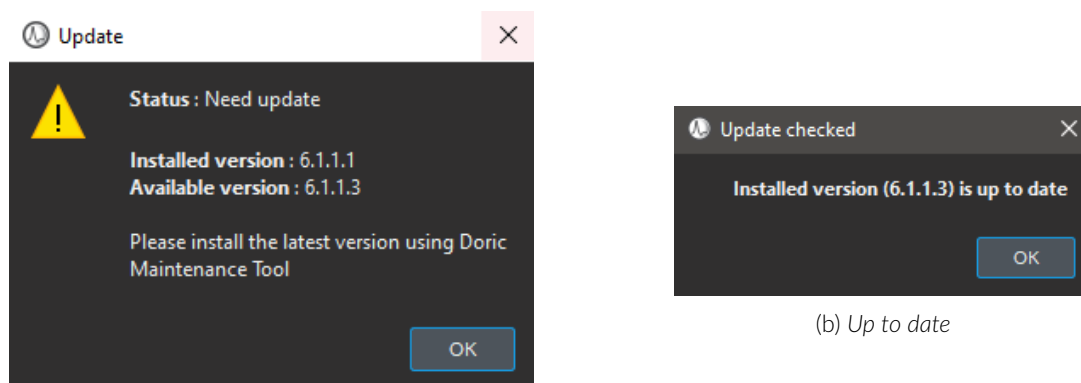
(a) *Need update*

(b) *Up to date*

Figure 3.6: *Check for updates*

3.2   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.

3.3   General Software Features

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

3.3.1   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.

3.3.2   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.

3.3.3   .doric Format

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

Light Sources

Doric Light Sources can be controlled by the Doric Neuroscience Studio. These include *LED Modules*, *Laser Diode Modules* and *⋆LISER*^{TM} *Light Source*[1]. 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** button, will be the first detailed.

4.1 Channel Configuration Window Overview
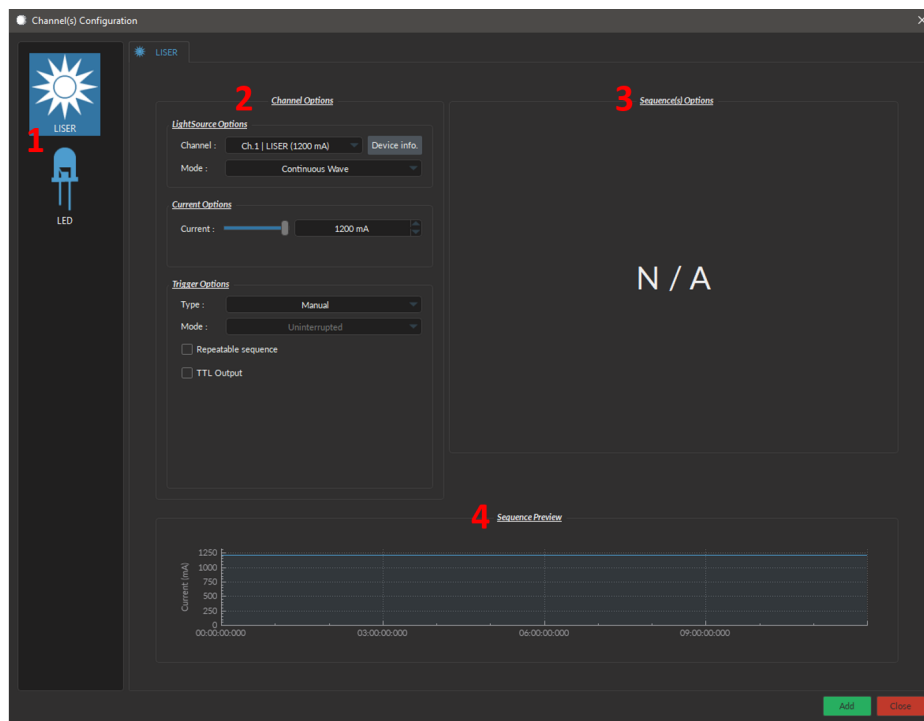
Figure 4.1: *Channels Configuration Main Interface*

The **Channels configuration** window is used to configure each channel. The window can be accessed by using either the **Add Channel** or **Edit** buttons. This window is separated into multiple sections shown in Figure 4.1 that are defined below.

1. The **Channel Types** are displayed on the left side of the window. These include the ⋆**LISER**^{TM} light sources, the **LED** light sources and the **Laser Diode** light sources.

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[1]The ⋆LISER^{TM} Light Source are also known in older models as Ce:YAG Fiber Light Source.

2. The **Channel Options** section allows you to define the Light Source Option, the Current options and the Triggering Options. The different fields of this section are explained in more detail in section 9.1.2.

3. The **Sequence Options** defines the parameters of each pulse sequence for the channel. These parameters are different for each Channel Mode. The different fields for the different Channel Mode are explained in more detail in section 9.1.3.

4. The **Sequence Preview** section shows a visualization of the output sequence that will be generated by the current configuration.

5. The **Add** button will save the current channel configuration and enables a new channel to be configured. The **Close** button will close the window without saving the current channel configuration.

4.1.1 Channel Options Section
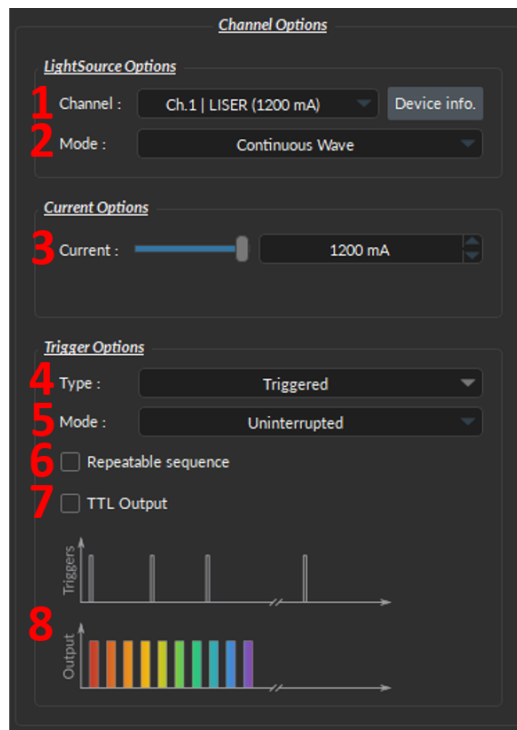
Figure 4.2: *Channel Options of the Channel Configuration Window*

The Channel Option section (Fig. 4.2) is separated in 3 sub-sections, the **LightSource Options** section that defines the channel and its mode, the **Current Options** and the **Trigger Options** section that control the trigger method of the selected channel.

4.1.1.1 LightSource Options

1. The **Channel** field identifies which of the available channels is currently being modified. The Light Source can be changed by selecting a new one from the drop-down list.

2. The **Mode** field identifies the mode used to generate the light. Five modes are available, **Continuous Wave** (fix current), **External TTL** (external digital command), **External Analog** (external analog command), **Square Sequence(s)** (internal digital command), and **Complex Sequences(s)** mode (internal analog command). Each mode enables different options of the Sequence Option section that are explained in more detail in section 9.1.3.

4.1.1.2   Current Options

3. The **Current Options** includes the slider used to control the current sent to the light source.

   - When using some *LED* 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 *CLED* module, 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 to monitor the LED power, its output voltage 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) in low power mode. The **BNC output** of the driver will still relate LED current with a 400 mA/V conversion factor.

4.1.1.3   Trigger Options

4. The **Type** defines the type of trigger that is used to start/stop a sequence. The **Triggered** type can starts and stops a sequence at a rising edge while the **Gated** type can starts the sequence at a rising edge and stops it at a falling edge. A more refined interaction of the trigger with the defined sequence can be set up using the **Mode** field. Not all Trigger Type are available for each combination of Trigger Mode and Repeatability. The different combinations are shown in Figure 4.7.

5. The **Mode** field defines how the trigger activates a sequence. Each mode are not compatible with each combination of trigger type and repeatability. Figure 4.7 shows the different available combinations for the different Trigger Modes. Four Modes are available and are the following:

   - **Uninterrupted**: This mode activates the channel sequence when an input greater than 3.3 V is detected by the BNC input. Following input pulses will be ignored while the sequence is running (Fig. 4.3). When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Fig. 4.3b). This mode is available for *Triggered* pulse only.

   - **Pause**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 4.4). Following input pulses (when *Triggered*, Fig. 4.4a) or falling edge (when *Gated*, Fig. 4.4c) will pause the sequence and the sequence will continue when the next rising edge is received. When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Figs. 4.4b and 4.4d).

   - **Continue**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 4.5). The following input pulse (when *Triggered*, Fig. 4.5a) or a falling edge (when *Gated*, Fig. 4.5c) will turn off the output, but the sequence will continue. The output will be turned back on at the reception of the following rising edge. Triggers only affect the output voltage value. When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Figs. 4.5b and 4.5d).

   - **Restart**: This mode activates the channel sequence when a rising edge higher than 3.3 V is detected on the BNC input. The following input pulse (when *Triggered*, Fig. 4.6a) or falling edge (when *Gated*, Fig. 4.6b) will stop the sequence and the sequence will restart from the beginning when the next rising edge is received. When the sequence is completed, it will restart with the next input pulse.

(a) Triggered Non-Repeatable Sequence

(b) Triggered Repeatable Sequence
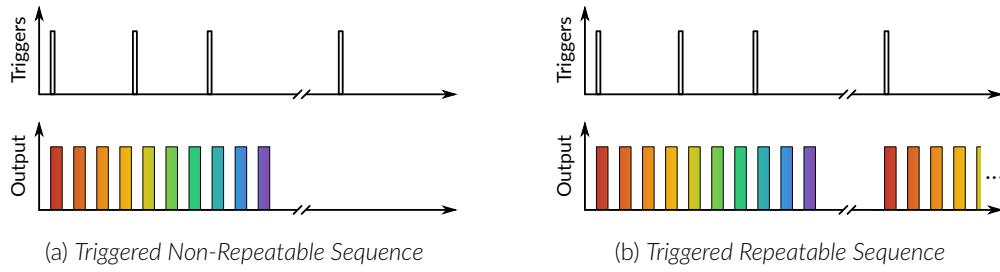
Figure 4.3: *Uninterrupted Sequence Mode*

(a) Triggered Non-Repeatable Sequence

(b) Triggered Repeatable Sequence

(c) Gated Non-Repeatable Sequence

(d) Gated Repeatable Sequence
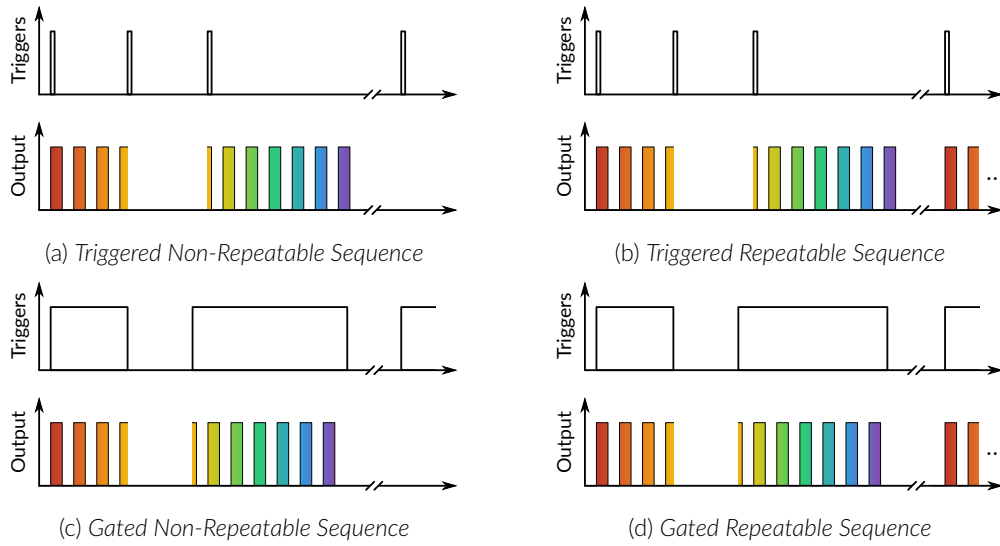
Figure 4.4: *Pause Sequence Mode*

(a) Triggered Non-Repeatable Sequence

(b) Triggered Repeatable Sequence

(c) Gated Non-Repeatable Sequence

(d) Gated Repeatable Sequence
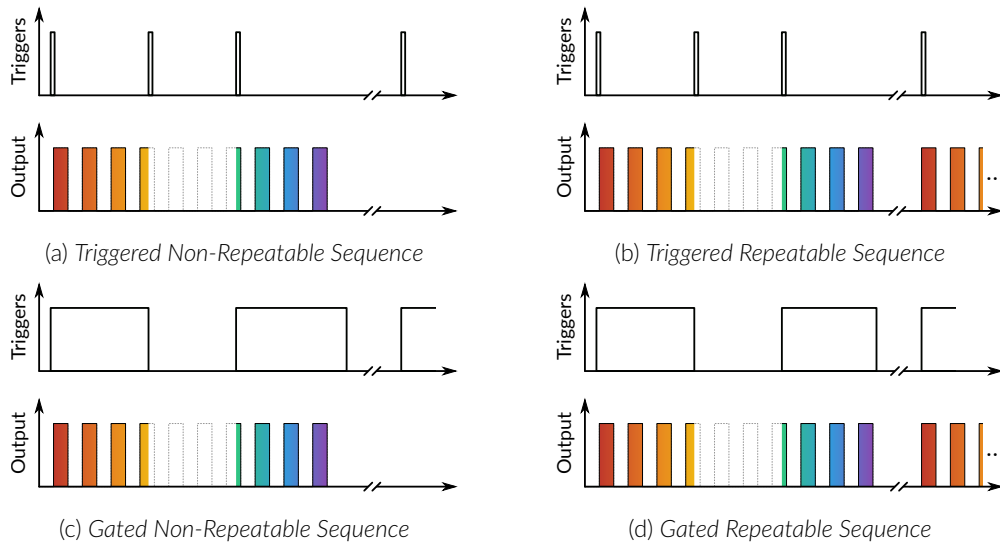
Figure 4.5: *Continue Sequence Mode*

(a) Triggered Repeatable Sequence

(b) Triggered Gated Sequence

Figure 4.6: *Restart Sequence Mode*

6. The **Repeatable sequence** checkbox, when selected, allows a sequence to be repeated. Not all modes and trigger types can be repeated. Please refer to the Figure 4.7 to know the repeatable sequence combinations.

7. The **TTL Output** checkbox, when selected, allows the output BNC channel to 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.

8. The **Sequence Visualisation** shows a graphical representation of the behavior of the selected Trigger Option Type, Mode and Repeatability.

	Triggered		Gated	
	Non-repeatable sequence	Repeatable sequence	Non-repeatable sequence	Repeatable sequence
Uninterrupted	✔	✔		
Pause	✔	✔	✔	✔
Continue	✔	✔	✔	✔
Restart		✔		✔

Figure 4.7: *Trigger options possibilities*

4.1.2   Sequence(s) Options Section

4.1.2.1   Continuous Wave

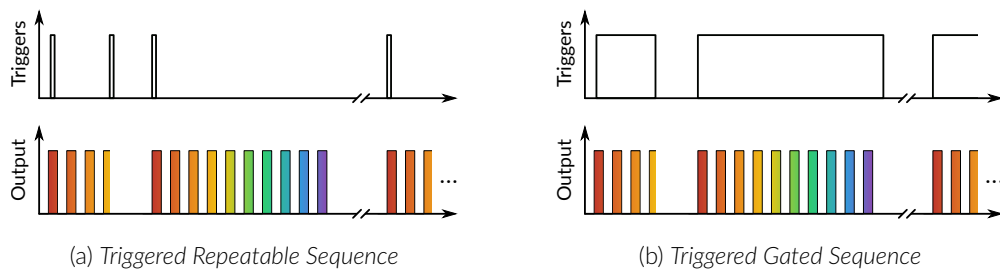The **Continuous Wave** mode is used to set the Light Source to a chosen intensity without variations during experiments.

4.1.2.2   External TTL

The **External TTL** mode is used to drive the Light Source to a chosen intensity when the External TTL signal is high. When the External TTL signal is low, the Light Source is turned OFF.
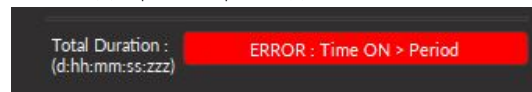
4.1.2.3   External analog

The **External Analog** mode is used to drive the Light Source in function of the analog voltage used as input. The input voltage may varies between 0 V and 5 V and the intensity will follow the variations between 0 mA and the maximum current.

4.1.2.4   Square Sequence(s)

(a) *Square Sequence(s) Mode Interface*

(b) *Exemple of Error*

Figure 4.8: *Sequence Options of the Square Sequence(s) Mode.*

The **Square Sequence(s)** mode allows the creation of a square TTL pulse sequence. The Sequence(s) Options of this mode are shown in Figure 4.8a and are explained below.

1. The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the first light illumination.

2. 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 light illumination at 10 Hz (frequency) will output one pulse every 100 ms (period), whereas a light illumination at 0.5 Hz (frequency) will output one pulse every 2 seconds (period).

3. The **Time ON** 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.

4. The **Smoothing** check box allows to change the pulse slope in square pulse sequences. The **Edit Edges** button opens the **Smoothing Edge(s)** window. An overview of the window opened by **Edit Edges** will be done in the next subsection.

5. 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.

6. The **Number of sequence(s)** sets the number of times that the sequence will be repeated.

7. The **Delay between sequences** sets the delay between each sequence.

8. 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 turn red and display what is the error (see Figure 4.8b).

4.1.2.5 Smoothing Edge(s)

The Smoothing Edge(s) window (Fig. 4.9) allows to change the pulse slopes of the square pulse sequences.
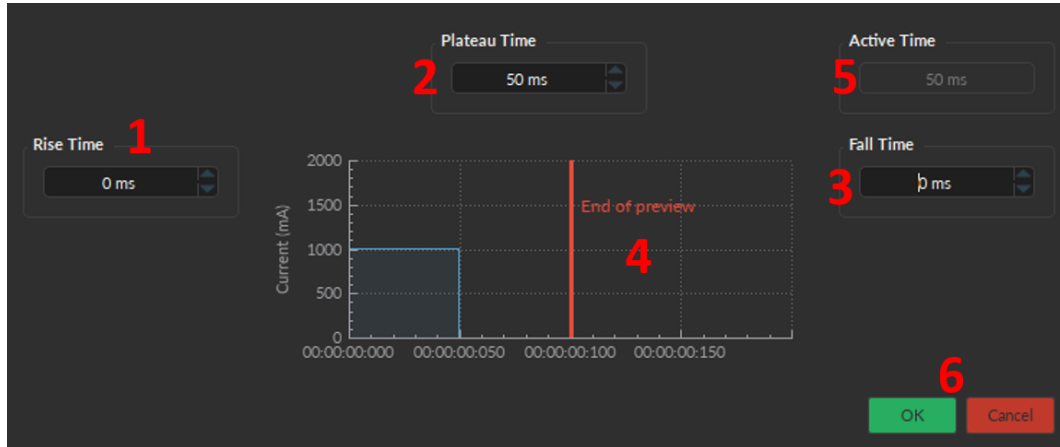
(a) *Overview of the Smoothing Edge(s) window*
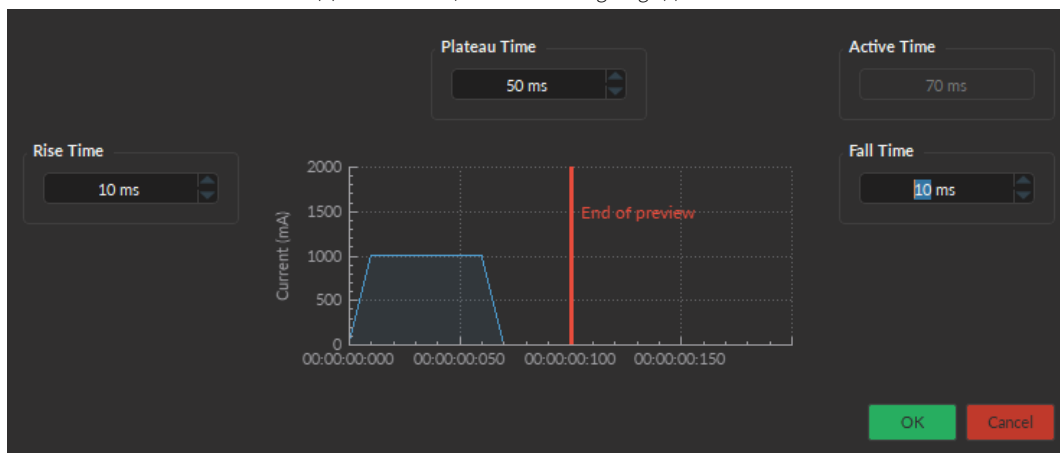
(b) *Exemple of smoothing edges (10ms for rise and fall time)*

Figure 4.9: *Smoothing Edge(s) window*

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

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

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

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

5. 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.

6. The **OK** button save the changes of the shape of the pulses. The **Cancel** button discard the changes. Both buttons close the window.

4.1.2.6 Complex Sequence(s)

If needed, it is possible to define a complex sequence to trigger the light source in the **Complex Sequence(s) Options** (Fig. 4.10).
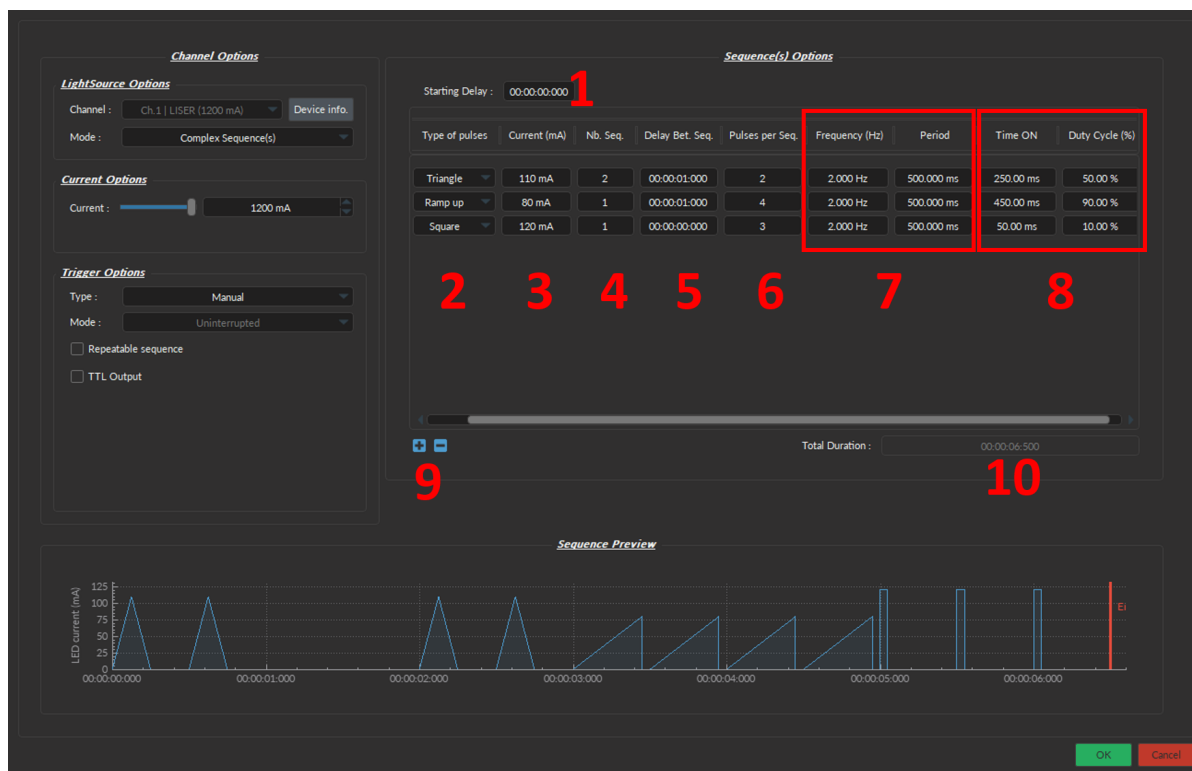

Figure 4.10: *Complex Sequences Window*

1. The **Starting Delay** sets the delay (in hh:mm:ss:zzz format) before the first light illumination.

2. The **Current** sets the maximum current (in mA) for the given sequence.

3. The **Nb. Seq.** sets the number of times that the sequence will be repeated, with a minimum of 1.

4. The **Delay between sequences** sets the delay (in hh:mm:ss:zzz format) between each sequence if **Nb.Seq.** is greater than 1.

5. The **Pulses per Seq.** sets the number of pulses per sequence, with a minimum of 1.

6. The **Frequency**/**Period** 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).

7. The **Time ON**/**Duty Cycle** 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 **Period**+0.005 ms, while the **Duty cycle** must be below 100 %.

8. The **Types of pulses** sets the pulse type. Pulses can be **Square**, triangular (**Triangle**), **Ramp up**, **Ramp down** or **Delay**. The **Delay** pulse type is used to create a delay between different sequence.

9. The **Sequence controls** allow the addition (**+**) or removal (**-**) of sequences to the spreadsheet.

10. The **Total Duration** 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 the **Period**.

4.2   Control ans Settings

The **Control and Settings** box is used to manage the different parts of the software. It contains three tabs, the **Acquisition**, **Configuration**, and **View** Tabs.

4.2.1   Acquisition Tab

Figure 4.11: *Acquisition Tab*

The different buttons of the **Acquisition Tab** are shown in Figure 4.11 and their functions are explained below.

1. The **Start All** button starts all currently configured channels.

2. The **Time Series** button opens the Time Series window (Fig. 4.12). This tool allows all channels to share the same timing.

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

4. The **Ce:YAG Temp.** indicator displays the temperature of the Ce:YAG crystal base in real time. This indicator will only appear when a *★LISER*^{TM} 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.

4.2.1.1 Time Series
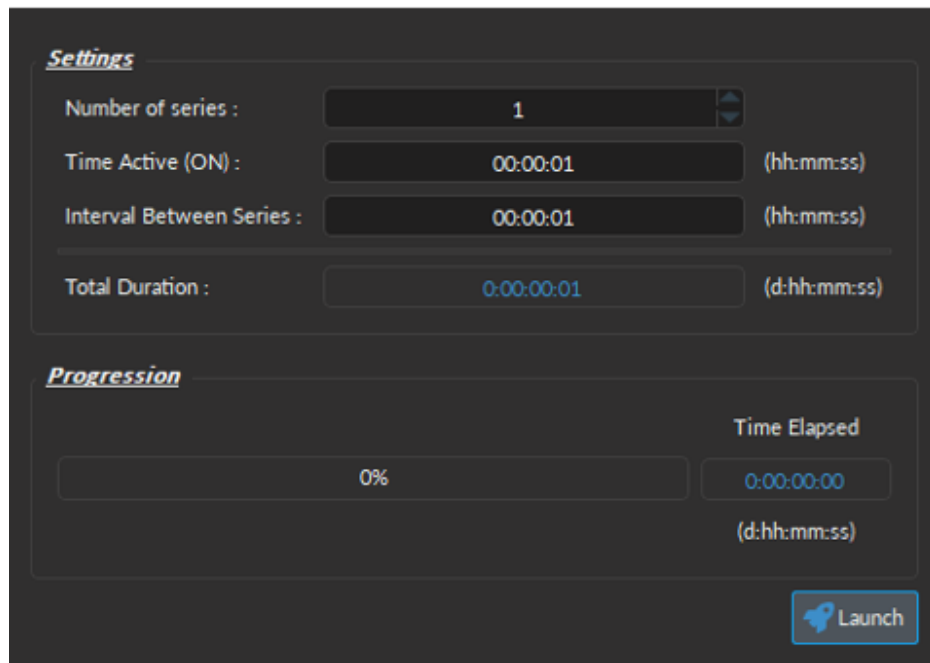
Figure 4.12: *Time Series Window*

- The **Number of series** sets the number of times that the sequence will be repeated, with a minimum of 1.

- The **Time Active (ON)** sets the duration of each series in hh:mm:ss format. The **Time series** can be used in combination with a sequence such as the Square Sequence(s) or the Complex Sequence(s) Mode. If the **Time Active** duration is shorter than the sequence time length, the sequence will stop at the end of the **Time Active** time length.

- The **Interval between series** sets the duration between each series in hh:mm:ss format.

- The **Total Duration** displays the total duration of the sequence in d:hh:mm:ss format.

- The **Progression** bar displays the progression of the sequence in %, while the **Time Elapsed** counter displays the progression in d:hh:mm:ss format.

- The **Launch** button starts the sequence.
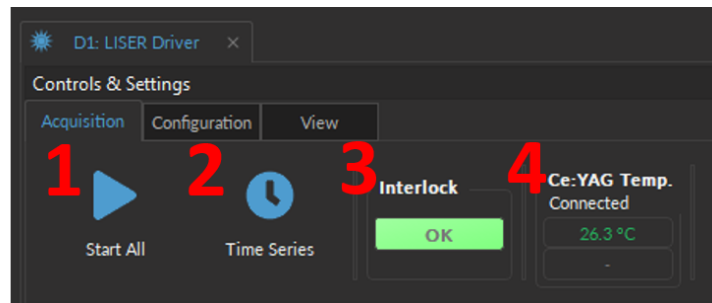
4.2.2 Configuration Tab
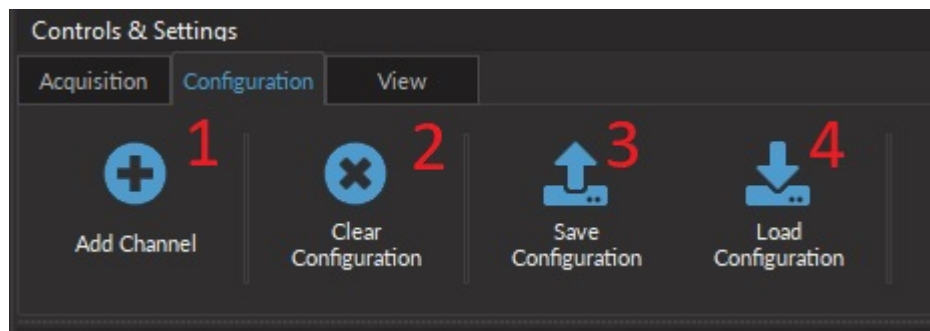
Figure 4.13: *Configuration Tab*

The different buttons of the **Configuration Tab** are shown in Figure 4.13 and their functions are explained below.

1. The **Add Channel** button opens the **Channels Configuration** window to setup the channels. This window is detailed in section 4.1.

2. The **Clear Configuration** button resets the acquisition view and all other parameters set. Any configurations already set will be lost.

3. The **Save Configuration** button is used to save the Light Source configuration in a **.doric** format.

4. The **Load Configuration** button allows a Light Source configuration in **.doric** format to be loaded. Recorded data files also contains the configuration used during the experiment and this configuration can be loaded using this button.

4.2.3   View Tab
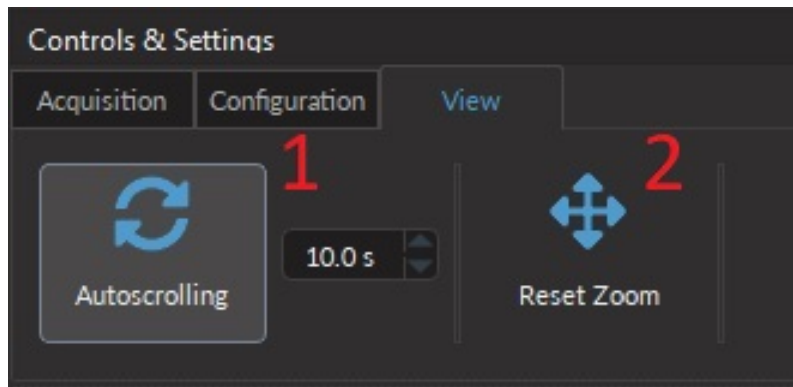
Figure 4.14: *View Tab*

The different buttons and fields of the **View Tab** are shown in Figure 4.14.

1. The **Autoscrolling** button, when clicked, makes the graphs scroll as new data appears. The duration (in seconds) kept on display is controlled by the field beside the button.

2. The **Reset Zoom** button resets the horizontal axis of all graphs displayed in the **Acquisition View** to the duration chosen in the **Autoscrolling** field.

4.3   Acquisition View

4.3.1   Acquisition View Overview
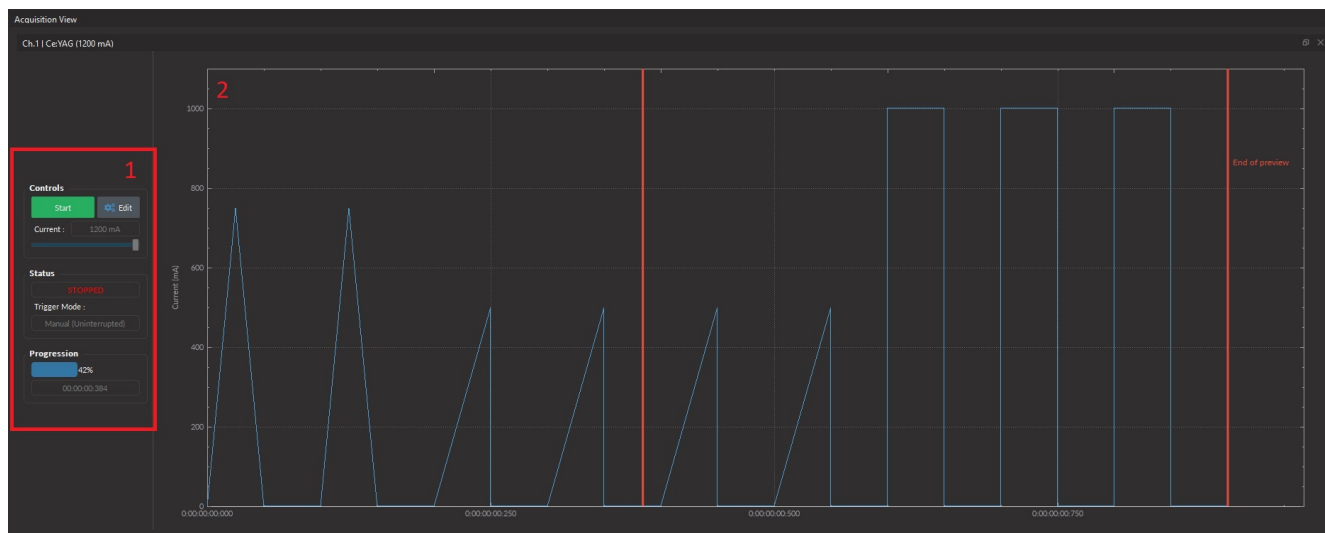
Figure 4.15: *Experiment View*

The Acquisition View (fig: 4.15) is composed of two sections:

1. The **Controls View** displays all elements to control/configure the channel. An overview this part will be done in section 4.3.2.

2. The **Graph View** displays a preview of the pulse sequence for Light Source Channels.

4.3.2   Acquisition View Control

Figure 4.16: *Control of the Acquisition View*

The different buttons of the **Control of the Acquisition View** are shown in Figure 4.16 and their functions are explained below.

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

2. The **Edit** button opens the **Channel configuration** window to edit the pulse sequence. This button is only accessible when the channel is deactivated and an overview of the **Channel Configuration** window is done in section 4.1.

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

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

5. The **Status** box displays the status **Light source**. The **Status** will display RUNNING... when active and STOPPED when deactivated.

6. The **Trigger Mode** of the light source is displayed in this box. For more information on the different Trigger options, see section 4.1.1.3.

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

Fiber Photometry

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.
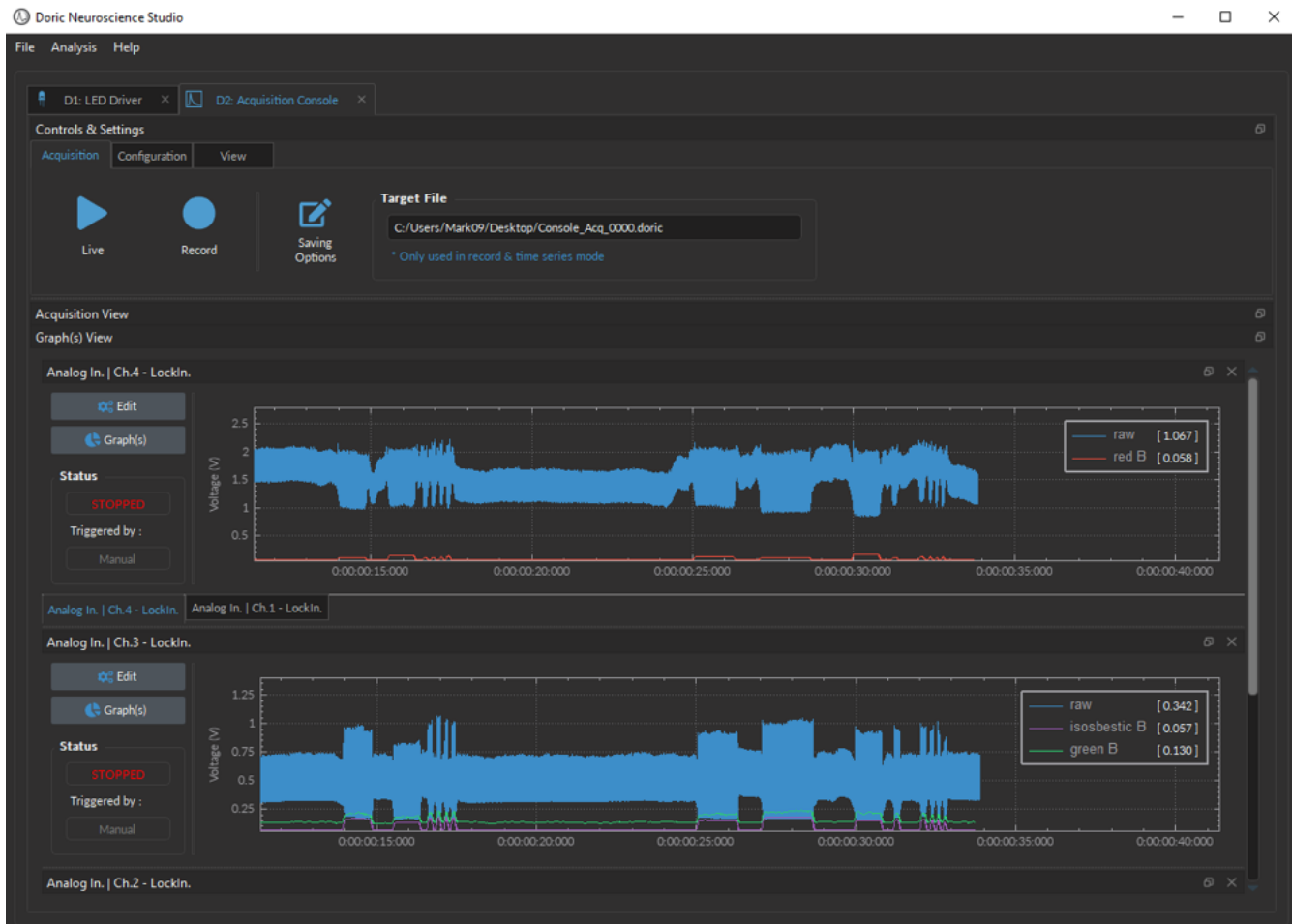
Figure 5.1: *Acquisition Console interface*

5.1 Device Selection Window

Once *Doric Neuroscience Studio* is opened, the *Device Selection* window should automatically pop up, if the device is turned ON and properly connected to the computer with USB port (as in Fig. 5.2).

To add a device to the studio, **double click** on the device of choice in the *Available device(s)* sections (bottom half of window). If the device in question does not show up, double-check that it is indeed turned ON and the two ends of the USB cord are properly connected within the USB port. Then click *Refresh*. When properly connected to the system, the device will appear in the *Connected/Opened device(s)* section of the Window (see the green checkmark in Fig. 5.2).
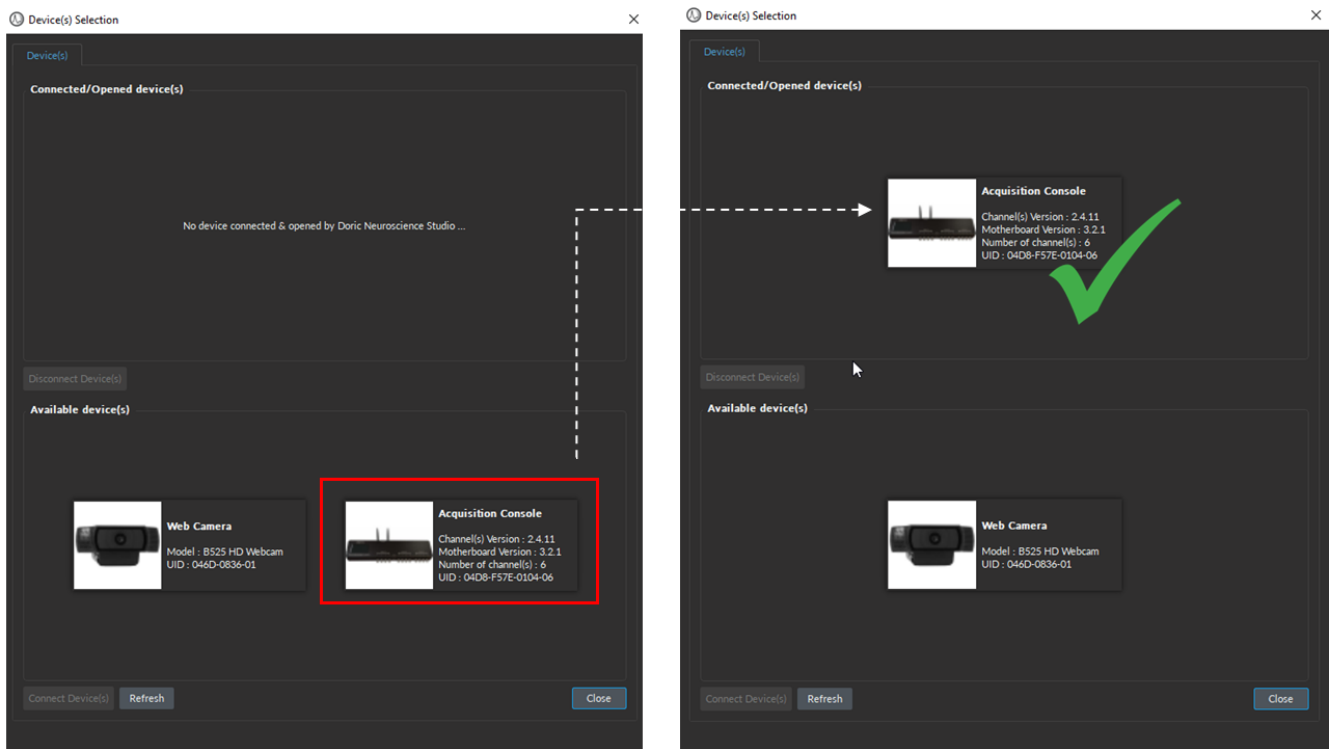
Figure 5.2: *Double click on the device of choice to connect it to DNS*

NOTE: If you have switched to DNS v6, older devices will require a firmware update to be recognized by the new version of the software. This update can be easily done using *Doric Maintenance Tools (DMT)* application and must be done one by one for each device. Further instructions can be found **HERE**.

Manually opening the *Device(s) Selection* window:
To manually open the *Device(s) Selection* window, select the *File*, then *Device Selection* (as per Fig. 5.3) or use the hot key: *Ctrl+N*.
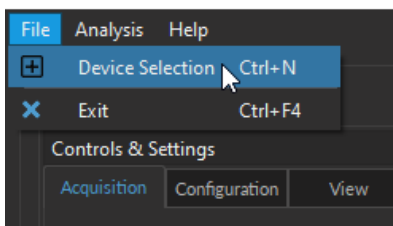
Figure 5.3: *Open Device Selection Window*

5.2 Overview

The **Acquisition Console** interface of *Doric Neuroscience Studio* software is split into two sections: **(1) Control and settings tabs** (Section 5.3) are used to manage different elements of the software (Acquisition, Configuration, and View); **(2) Acquisition view** (Section 5.5) displays the input and output traces for visualization.
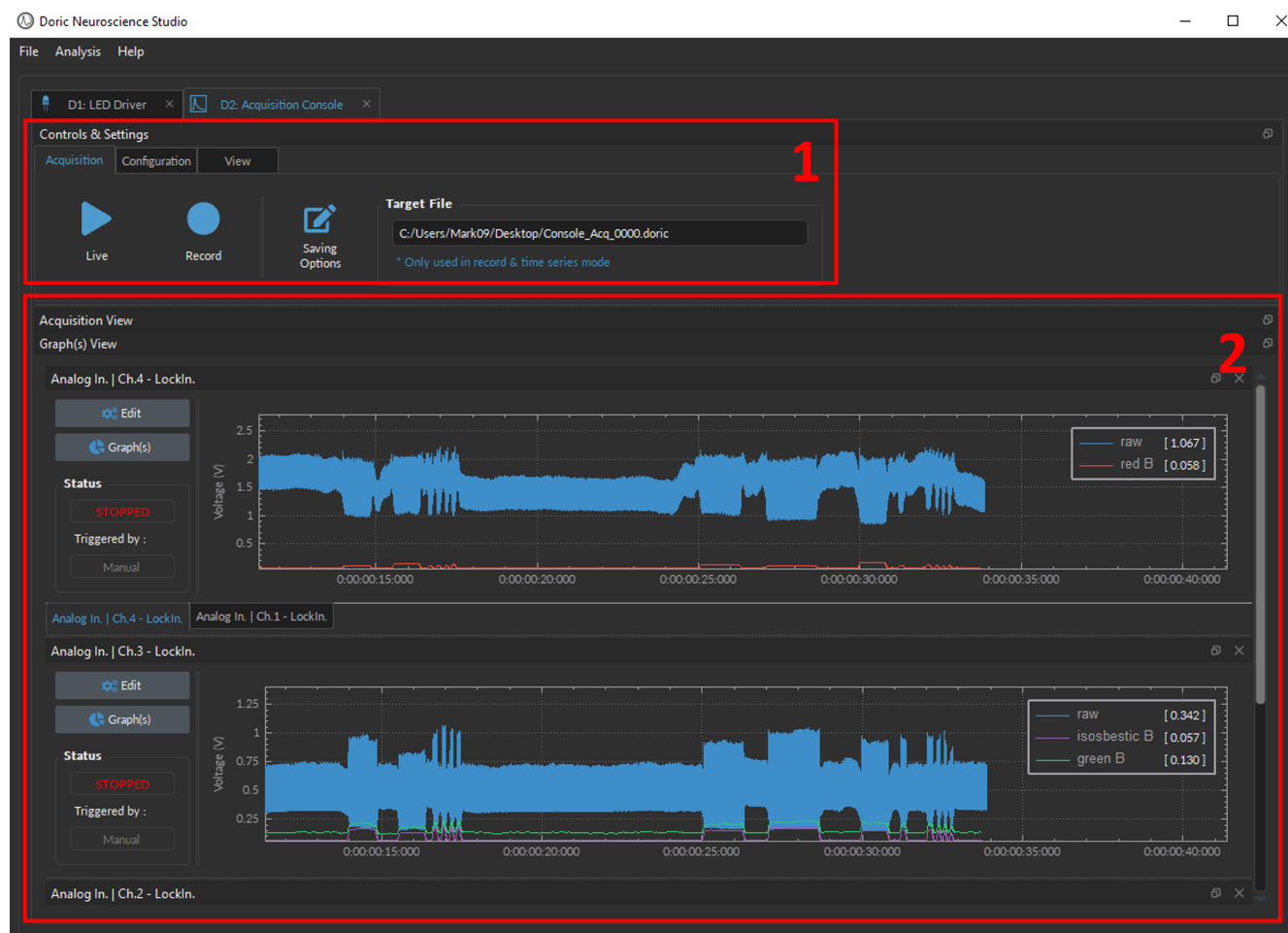
Figure 5.4: *DNS user interface*

5.3 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** (Section 5.3.1), **Configuration** (Section 5.3.2), and **View** (Section 5.3.3).

5.3.1 Acquisition Tab

The **Acquisition** tab is used to start a live/recording session and set the saving parameters. The **Live** and **Record** buttons will not function if channels have yet to be set-up. See section 5.4.1 to configure channels for recording.
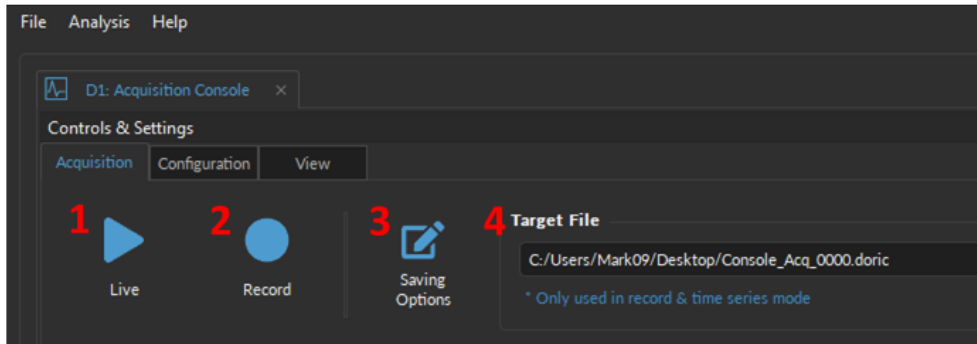
Figure 5.5: *Acquisition Tab*

1. The **Live** button (Fig. 5.5, 1) activates all prepared channels. This mode does not save data, keeping only the most recent 700 000 data points in memory. This mode is not recommended for long or critical measurement sequences. **Live** mode is useful to quickly test the recording software and to ensure that the parameters were properly set.

2. The **Record** button (Fig. 5.5, 2) activates all prepared channels while periodically saving recorded data to the disk. This mode is recommended for long measurement sequences.

3. The **Saving Options** (Fig. 5.5, 3) button opens the **Saving Parameters** window (Fig. 5.6). See section 5.3.1.1 for greater details.

4. The **Target File** (Fig. 5.5, 4) displays the path and file name where the data will be stored once the **Record** button is selected. Select the **Saving Options** button to change the path and file name.
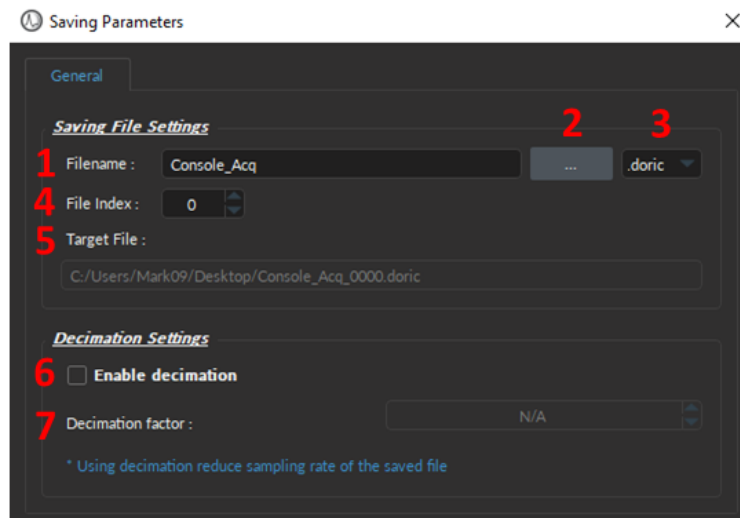
Figure 5.6: *Saving Menu Window*

5.3.1.1 Saving Parameters

The **Saving Parameter** window is used to define how and where the file is saved. This window is opened by selecting the **Saving Options** button in the Acquisition Tab (Fig. 5.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 5.6, 1).

2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 5.6, 2).

3. The **File format** (Fig. 5.6, 3) is **.doric**, an HDF5-based format that supports metadata (signal, video, images, tables, parameters, etc.). Version 6 of *Doric Neuroscience Studio* is no longer compatible with other file formats (.csv, .excel, or .tiff). We provide Matlab, Python, and Octave codes to read **.doric** files HERE. While not recommended, it is possible to export a *.doric* file into .csv format through the **Doric File Reader** module.

4. The **File Index** (Fig. 5.6, 4) box is used to define the current indexation number used for multiple files saved during the same measurement session. The suffix is incremented automatically when recording multiple files.

5. The **Target File** (Fig. 5.6, 5) displays the absolute path and filename where the data will be saved.

6. The **Enable decimation** checkbox (Fig. 5.6, 6) provides a way to reduce the file sizes. This method conserves one point over a number of data points equal to the **Decimation Factor**.

7. The **Decimation factor** text-box (Fig. 5.6, 7) is used to define the number of points saved.[1]

5.3.2 Configuration Tab

The **Configuration** tab is used to set the channels and the global settings (such as sampling rate and Master trigger options), as well as save and load the preset channel configurations.
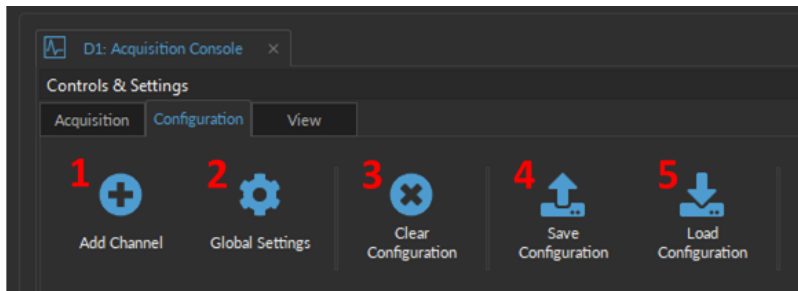
Figure 5.7: *Configuration Tab*

1. The **Add Channel** button (Fig. 5.7, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 5.4. Table **??** describes different types of channels available, their use cases and their individual sections.

2. The **Global Settings** (Fig. 5.7, 2) opens the **Global Options** window in Fig. 5.8, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 5.3.2.1 for more details.

3. The **Clear configuration** button (Fig. 5.7, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.

4. The **Save configuration** button (Fig. 5.7, 4) allows a console configuration to be saved in the **.doric** format. This file preserves the current channel configuration/parameters, the Acquisition View window organization, and any custom trace colors and names.

5. The **Load configuration** button (Fig. 5.7, 5) imports a pre-configured **.doric** file into the module.

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[1]For a data set of 10 points, saved with a **Decimation Factor** of 2, the first point will be saved, the third ... This produces a file of 5 points of data.

5.3.2.1 Global Settings

Through the **Global Settings**, user can set the acquisition **Sampling Rate** and specify the **Master Trigger Options** that will start recordings.

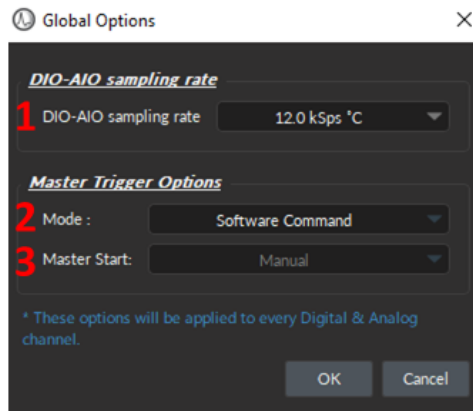Figure 5.8: *Global Options Window*

1. The **DIO-AIO sampling rate** (Fig. 5.8-1) is 12 kSps°C by default. This value was selected because it is the highest value that still produced reliable data given the hardware limitations of the devices. See section 5.3.1.1 to enable the *Decimation* and effectively reduce the saving sampling rate and restrict the data file size.

2. The **Mode** (Fig. 5.8, 2) of the **Master Trigger Options** sets the origin (internal, external or time-series) of the trigger that will start the recording session and synchronize all the external and internal devices. Four options are available for different use cases:

   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 5.5, 2). The **Master Start** is, by definition, always **Manual**.

   - *Triggered* - The recording session starts when a trigger signal is received (from the **Master Start**, either manual or from an external digital source), and continues even if the trigger signal stops. Thus, the **Triggered** mode only controls the START of the recording session (and NOT the endpoint).

   - *Gated* - The recording session starts when a high TTL signal (>4 V) is detected (from the **Master Start**, either manual or from an external digital source), and will stop when a low TTL signal (<0.4 V) is detected. Thus, the **Gated** mode controls both the START and the END signals of the recording session.

   - *Timeseries* - This mode allows users to record pre-defined series over longer periods of time (that can span several days) (Fig 13.9a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 5.9b) pops up. See section 5.3.2.2 for more details.

3. The **Master Start** (Fig. 5.8, 3) defines the source that will automatically start the recording. This source can either be:

   - *Manuel* - the user ultimately starts the recording session by clicking **Record** within *Doric Neuroscience Studio*;

   - *Digital I/O Channel (1-4)* - The specified channel will automatically begin the recording session when it receives a digital trigger pulse from an external device. ***However, this mode still requires that the **Record** button is selected BEFORE the TTL trigger signal is received.***

5.3.2.2 Time Series

The **Time Series** Window (Fig 5.9b) can be opened by clicking on the **Record** button (Fig. 5.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 5.8, 2). Every **Time series** sequence is automatically saved to the *.doric* file defined in **Saving Options** (Section 5.3.1.1).
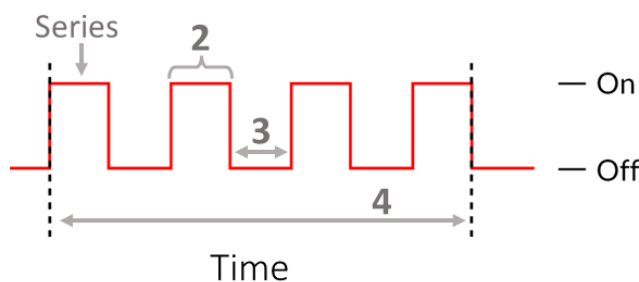
(a) *Time Series Acquisition Timing Diagram*
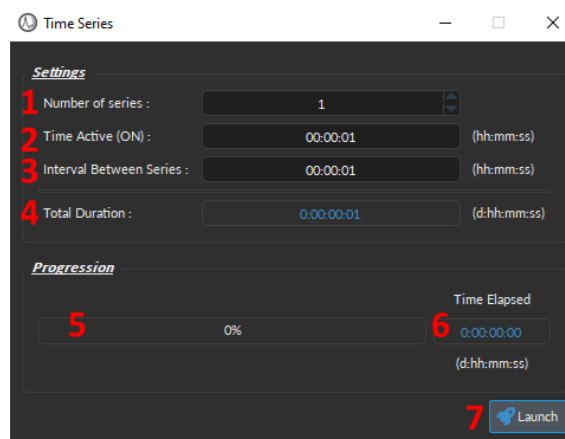
(b) *Time Series Window*

Figure 5.9: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 5.9b) sets the following parameters:

1. The **Number of series** (Fig. 5.9b, 1) defines the amount of times the series is repeated.

2. The **Time Active (ON)** (Fig. 5.9b, 2) defines the duration of the series.

3. The **Interval Between Series** (Fig. 5.9b-3) defines the amount of time between each series, if the **Number of series** is greater than 1.

4. The **Total Duration** (Fig. 5.9b, 4) displays the total amount of time that the timeseries recording will take place.

5. The **Progression bar** (Fig. 5.9b, 5) indicates the progression of the timeseries (in %).

6. The **Time Elapsed** (Fig. 5.9b-6) counter indicates the amount of time that has already passed in d:hh:mm:ss.

7. The **Launch** (Fig. 5.9b, 7) 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.

5.3.3 View Tab

The **View Tab** (Fig. 5.10) is used to modify the presentation of graphs in the **Acquisition view**.
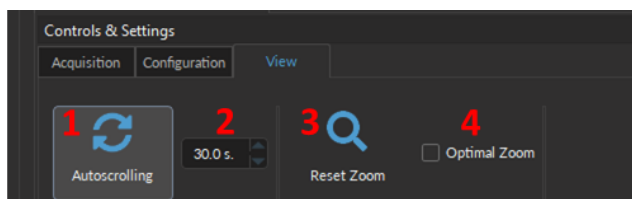
Figure 5.10: *View Tab*

The **View** parameters are as follows:

1. The **Autoscrolling** button (Fig. 5.10, 1), when selected, automatically set the graphs to scroll as new data appears.

2. the **Zoom range** (Fig. 5.10, 2) sets the graph zoom to the value of choice, specified in the text-box.

3. The **Reset Zoom** button (Fig. 5.10, 3) readjusts the graph zoom to the value specified in the zoom range text-box.

4. The **Optimal Zoom** check-box (Fig. 5.10, 4) automatically adjusts the graph range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

5.4   Channel Configurations

5.4.1   Add Channel:

To create a new channel, regardless of the input and/or output type, select the **Add Channel** button, which can be found under the **Configuration** tab (Fig. 5.11). This will open the **Channel(s) Configuration** window (Fig. **??**).

Figure 5.11: *Add Channel button opens the Channel Configuration window*

To generate a new **Channel** using the **Channel(s) configuration** window (Fig. 5.12):
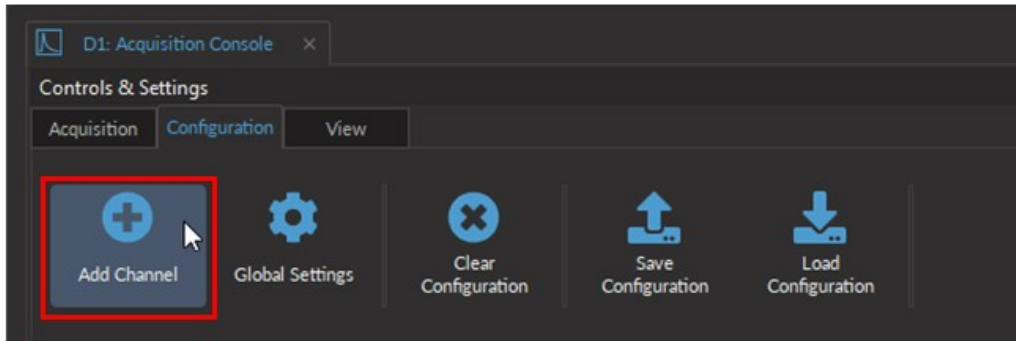
1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 5.12). Table 5.1 describes the use case of each type.

2. Clicking on the icon will display the **Channel Type**-specific options on the right side of the window. Each **Channel Type** has a number of parameters which can be configured to fit the needs of the experiment(s). Details of the parameters and their options will be covered in the following sections. See Table 5.1 for hyperlinks to the relevant sections.

3. Select the **Add** button (Fig. 5.12) to generate the defined channel or to update an already configured channel, but does not automatically close the *Channel Configuration* window. This allows the user to conveniently set up all required channels one after the other.

4. Select the **Close** button to shut the window once all channels are configured.
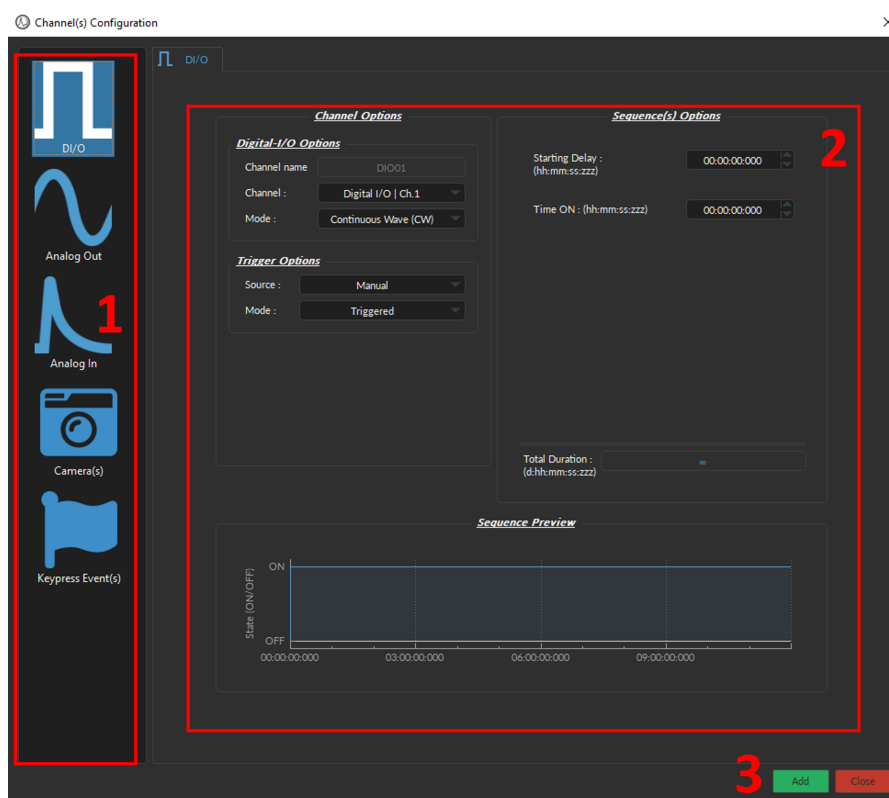
Figure 5.12: *Channel(s) configuration window, Digital I/O input*

5.4.2 Channels Types

Different types of input and output can be configured for the particular of the experiment by creating a new Channel in the Configuration tab or editing an existing one (Fig 5.11). Table 5.1 details the types of inputs and output the console and the software can handle and gives a quick access to their sections.

Table 5.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	Digital I/O	For input and output of TTL signals	5.4.3
	Analog Output	For the output of analog signals, such as sine, stair or customized	5.4.4
	Analog Input	To collect the fluorescent signal (such as GCamp, RCamp, Isosbestic or FRET)	5.4.5
	Camera(s)	To collect images for behaviour experiments	5.4.6
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	5.4.7

5.4.3 Digital I/O Channels

Each **Digital I/O** channel can be configured as an output or an input to create TTL (On/Off) pulse sequences. **Digital Outputs** can provide triggers to external devices (such as light sources) required for the experiment, while remaining synchronized with to recording system. In addition, **Digital Inputs** can record a copy of the trigger of an external driven device used during the experiment (such as the timing of a displayed stimuli or a measured behavior).

The *Channel(s) Configuration* window for the **Digital I/O** Channel is divided into three sections (Fig. **??**): (1) the **Channel Options** (Section 5.4.3.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 5.4.3.2).
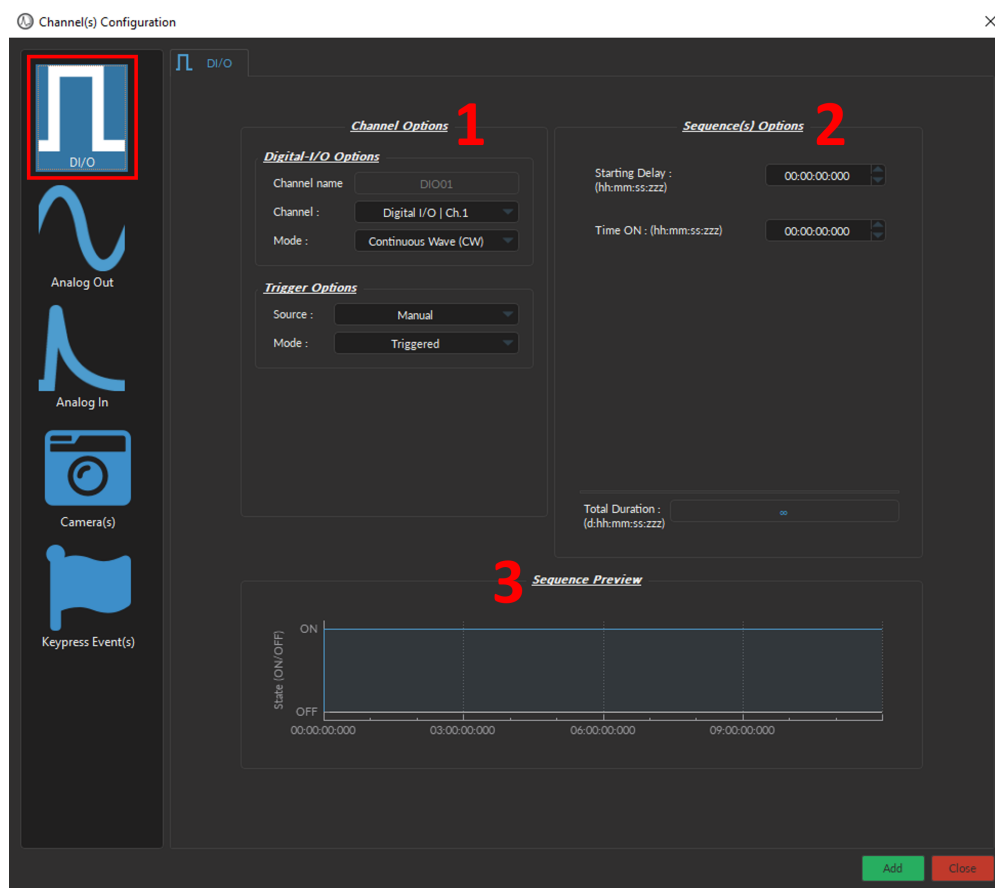

Figure 5.13: *Channel(s) configuration window, Digital I/O - CW mode*

5.4.3.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.
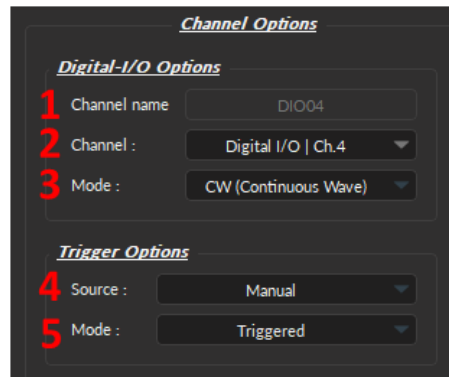
Figure 5.14: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel name** (Fig 5.14, 1) allows user to specify a label for each channel.

2. The **Channel** (Fig 5.14, 2) identifies the channels available to create a Digital I/O. The channel can be changed by selecting a new one from the drop-down list. Each numbered channel on the physical console corresponds to the same number of the digital channel within the software.

3. The **Mode** (Fig 5.14, 3) identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). Three modes are available:

   - The **Continuous wave (CW)** Mode (Fig. 5.15a);
   - The **Square (TTL)** Mode (Fig. 5.15b);
   - The **Input** mode receives a signal that are either 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the console (See Section 5.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.

(a) *Continuous*   (b) *Square*
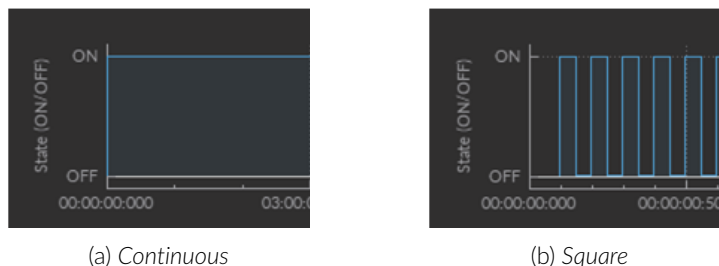
Figure 5.15: *Channel Options - Output Modes*

Trigger Options:

1. The **Source** trigger option (Fig 5.14, 4) allows the choice of a **Manual Trigger** (activated by a user) or an **Input** trigger, coming from a **Digital I/O** channel set in input mode.

2. The **Mode** (Fig 5.14, 5) defines how the trigger activates a sequence. This includes input sequences, which can be triggered/gated by an outside source.

   - In **Triggered** mode (Fig. 5.16a), 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 (Fig. 5.16b), the sequence will start once the voltage reach a high TTL signal (4 V or more) on the input modulation BNC. When the TTL signal reaches a low TTL signal (0.4 V or less), the sequence stops and waits for another high TTL signal to continue. This mode can cut pulses, once the high signal returns. ***ONLY AVAILABLE FOR SQUARE CHANNEL MODE***
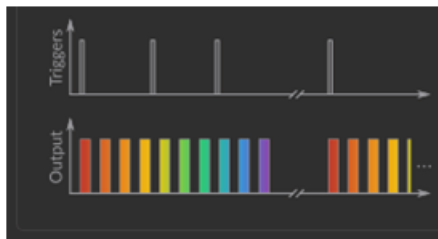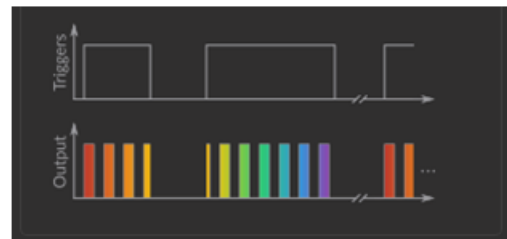
(a) *Trigger*   (b) *Gate*

Figure 5.16: *Trigger Options Modes*

5.4.3.2 Sequence Options & Preview

The **Sequence options** section (Fig. 5.17a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 5.17b) displays the corresponding shape and timing of the sequence. 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**.

The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 5.14), as following:

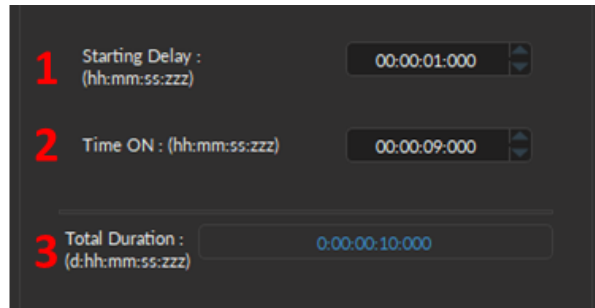- The **CW (Continuous Wave)** channel mode (Fig. 5.13) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

  1. The **Starting Delay** (Fig 5.17, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

  2. The **Time ON** (Fig 5.17, 2) 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** (Fig 5.17, 3) 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**.
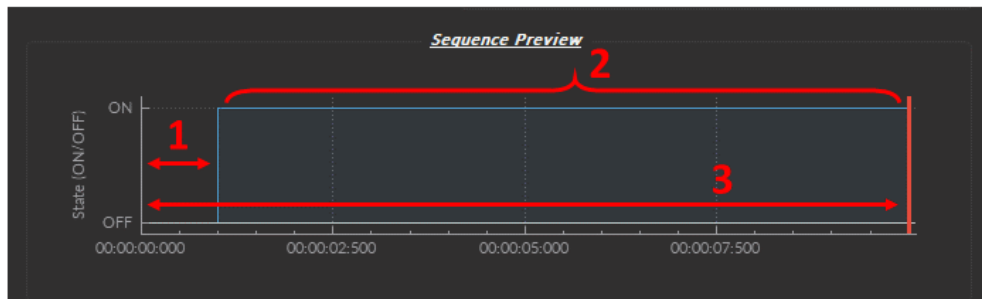
(a) *Sequence Options*

(b) *Sequence Preview*

Figure 5.17: *Channel(s) configuration window, Digital I/O - CW Mode*

- The **Square** channel mode (Fig. 5.14) allows the creation of a square TTL pulse sequence. This includes all sequence options as the **CW** mode (Fig. 5.17, 1-3), with the following additions:

  1. The **Starting Delay** (Fig 5.17, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

  2. The **Frequency** (Fig. 5.18a, 2) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period** (Fig. 5.18a, 2). 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).

3. The **Time ON** (Fig. 5.18, 3) 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.

4. The **Pulse(s) per sequence** (Fig. 5.18, 4) sets the number of pulses within a single sequence. If it is set to 0, the number of pulses will be infinite.

5. The **Number of sequence(s)** (Fig. 5.18, 5) sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** (Fig. 5.18, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).

7. The **Total Duration** (Fig 5.17, 3) 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**.

(a) *Sequence Options*

(b) *Sequence Preview*

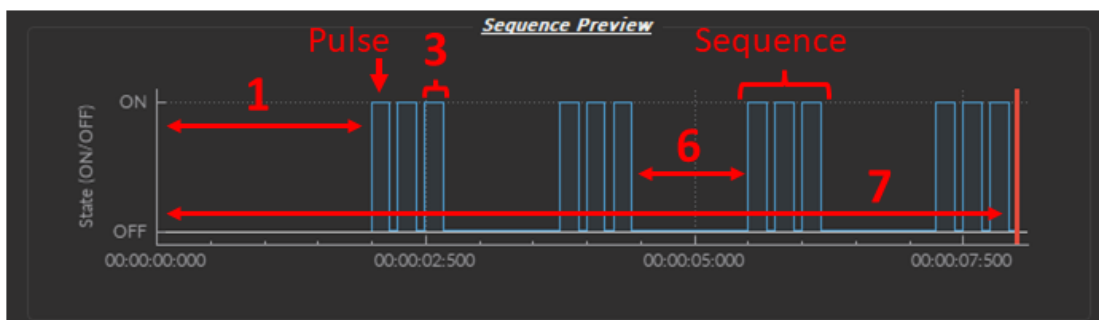Figure 5.18: *Channel(s) configuration window, Digital I/O - Square Mode*

5.4.4 Analog Output
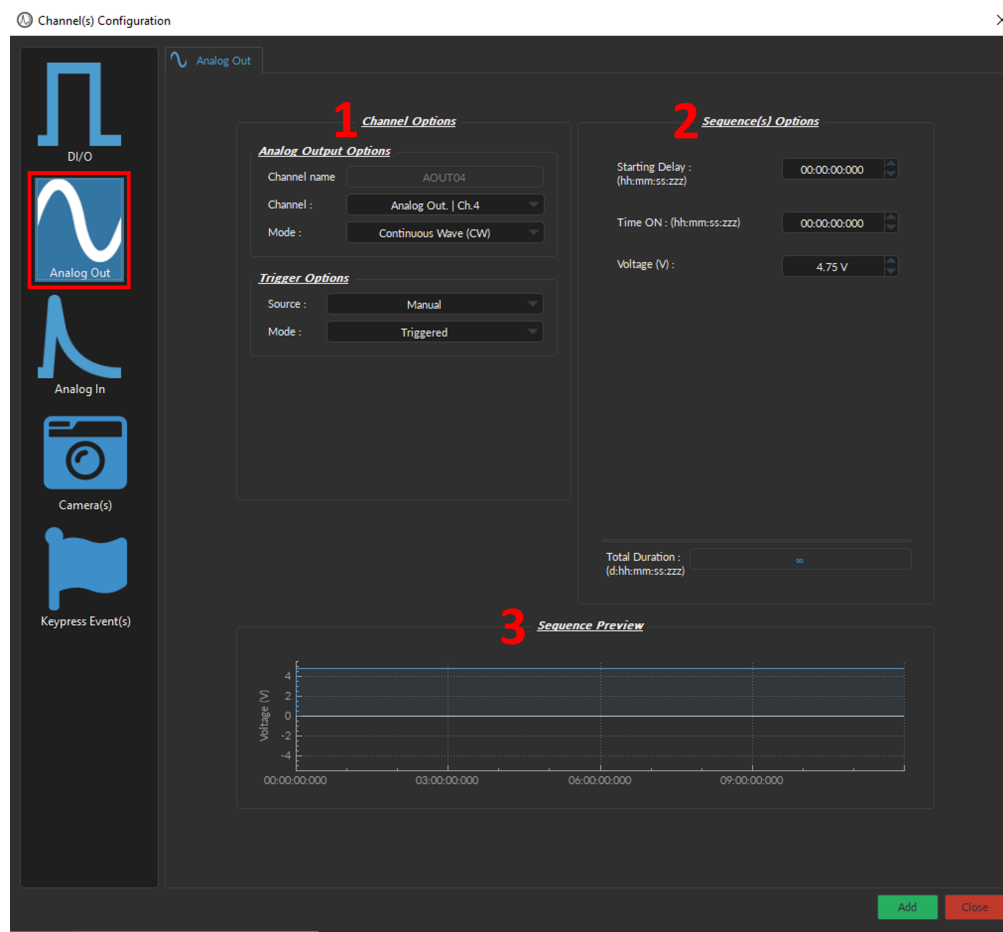
Figure 5.19: *Channel(s) configuration window, Analog Output CW*

The **Analog Output** channel type creates analog pulse sequences. Each numbered channel corresponds to the same analog channel number on the console. Pulse sequences have different parameters depending on the channel **Mode**, which can be **Continuous**, **Square**, **Sine**, **Stair** and **Custom** (Fig. 5.20).

(a) *Continuous* (b) *Square* (c) *Sine* (d) *Stair* (e) *Custom*
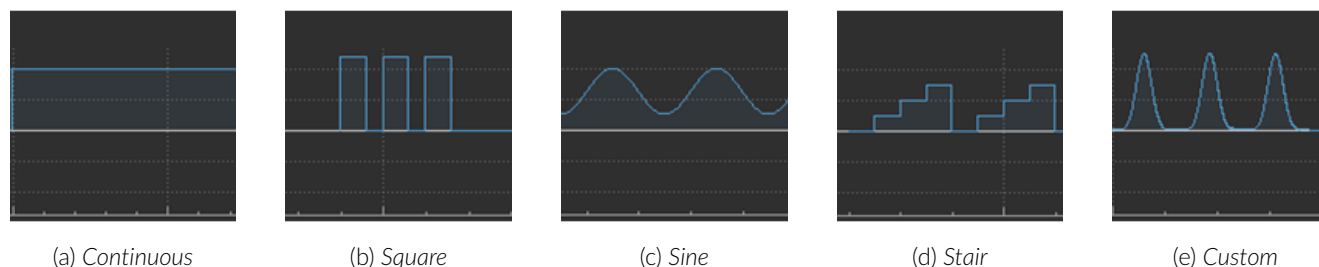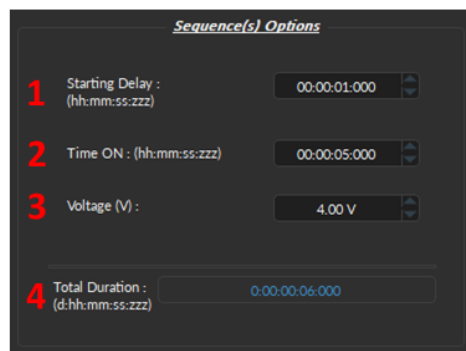
Figure 5.20: *Analog Output Modes*

5.4.4.1 Continuous Wave (CW) Mode

The **CW (Continuous wave)** channel mode (Fig. 5.21) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box (Fig. 5.21a).

1. The **Starting Delay** (Fig. 5.21) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Time ON** (Fig. 5.21) 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. 5.21) defines the voltage of the continuous signal, in volts. The signal cannot go beyond ±4.75 V.

4. The **Total Duration** (Fig. 5.21) 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**.
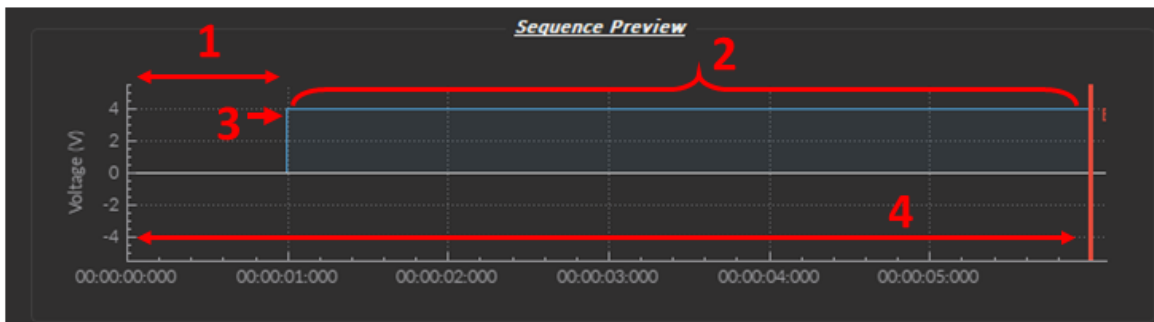
(a) *Sequence Options*

(b) *Sequence Preview*

Figure 5.21: *Channel(s) configuration window, Analog Output CW*

5.4.4.2 Square Mode

The **Square** channel mode (Fig. 5.22) creates a sequence of pulses with the minimum of the pulses at **V-** and the maximum of each pulse at **V+**.

1. The **Starting Delay** (Fig. 5.22, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Frequency** (Fig. 5.22, 2) 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).

3. The **Time ON** (Fig. 5.22, 3) 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.

4. The **Maximum Voltage (V+)** (Fig. 5.22, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.

5. The **Minimum Voltage (V-)** (Fig. 5.22, 5) defines the minimum voltage of each pulse, in volts. The signal cannot go below -4.75 V.

6. The **Pulse(s) per sequence** (Fig. 5.22, 6) set the number of pulses per sequence. If it is set to 0, the number of pulses will be infinite.

7. The **Number of sequence(s)** (Fig. 5.22, 7) sets the number of times that the sequence will be repeated.

8. The **Delay between sequences** (Fig. 5.22, 8) sets the delay between each sequence.

9. The **Total Duration** (Fig. 5.22, 9) 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**.

(a) *Sequence Options*
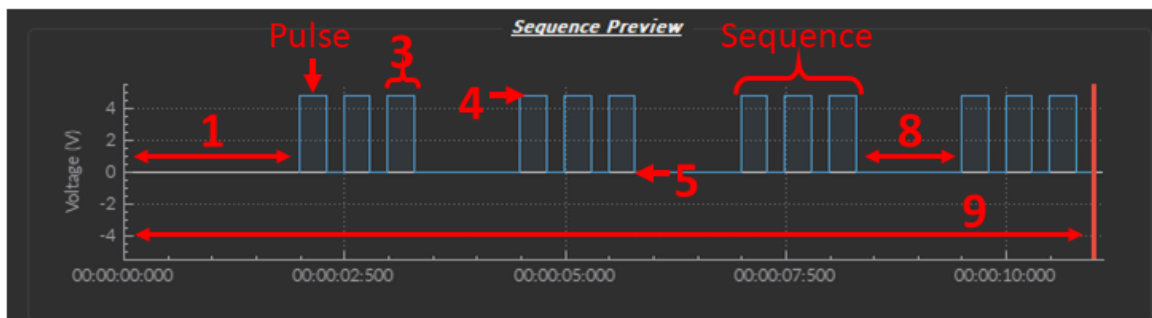
(b) *Sequence Preview*

Figure 5.22: *Channel(s) configuration window, Analog Output Square*

5.4.4.3 Sine Mode

The **Sine** mode (Fig. 5.23) creates a sinusoidal pulse sequence with peaks at **V+** and **V-**.

> **Note:** The **Starting Delay** is not available for this mode (Fig. 5.23a).

1. The **Frequency** (Fig. 5.23, 2) 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 **Phase** option (Fig. 5.23, 2) replaced **Time ON** (Fig. 5.22, 3). This allows the choice of the sine wave phase, in degrees.

3. The **Maximum Voltage (V+)** (Fig. 5.23, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.

4. The **Minimum Voltage (V-)** (Fig. 5.23, 5) defines the minimum voltage of each pulse, in volts. The signal cannot go below -4.75 V.

5. The **Period per Sequence** (Fig. 5.23, 5) is similar to the **Pulse per Sequence** parameter in Square mode (Section 5.4.4.2, Square), but where the period (a single sine wave from peak to peak, Fig. 5.23b, 1) replaces the pulse.

6. The **Number of sequence(s)** (Fig. 5.23, 7) sets the number of times that the sequence will be repeated.

7. The **Delay between sequences** (Fig. 5.23, 8) sets the delay between each sequence.

8. The **Total Duration** (Fig. 5.23, 9) 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**.

(a) *Sequence Options*
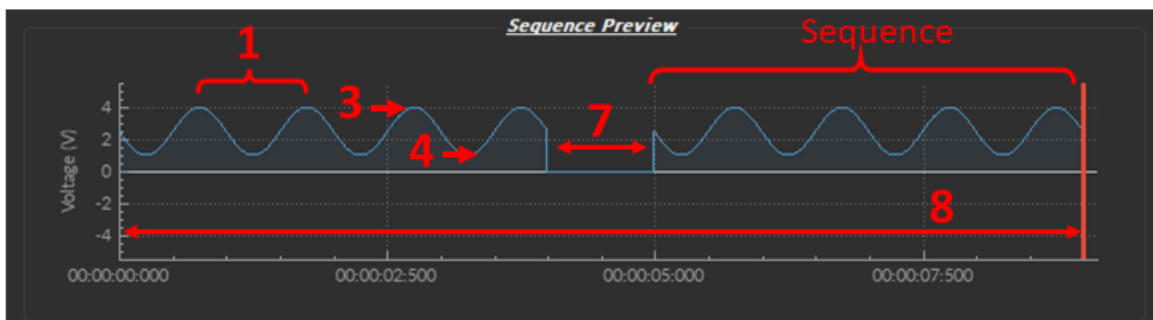
(b) *Sequence Preview*

Figure 5.23: *Channel(s) configuration window, Analog Output Sine*

5.4.4.4   Stairs Mode

The **Stairs** mode (Fig. 5.24) creates a stepwise pulse sequence with peaks at several different Voltage levels **V+**.

1. The **Starting Delay** (Fig. 5.24, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Frequency** (Fig. 5.24b, 2) option replaces the **Time ON** . This parameter applies to a whole pulse, which include all the voltage steps (up to a max of four).

3. The **Number of Steps** sets the amount of voltage levels of a single pulse, up to a maximum of four (Fig. 5.24b, 3). Increasing the number of steps automatically adds an additional parameter to specify the voltage of the added step below.

4. The **Step Voltage** sets the value of stair level X between **-4.75V** and **4.75V** (Fig. 5.24, 4).

5. The **Pulse(s) per sequence** (Fig. 5.24, 5) 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. 5.24, 6) sets the number of times that the sequence will be repeated.

7. The **Delay between sequences** (Fig. 5.24, 7) sets the delay between each sequence.

8. The **Total Duration** (Fig. 5.24, 8) 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**.

(a) *Sequence Options*
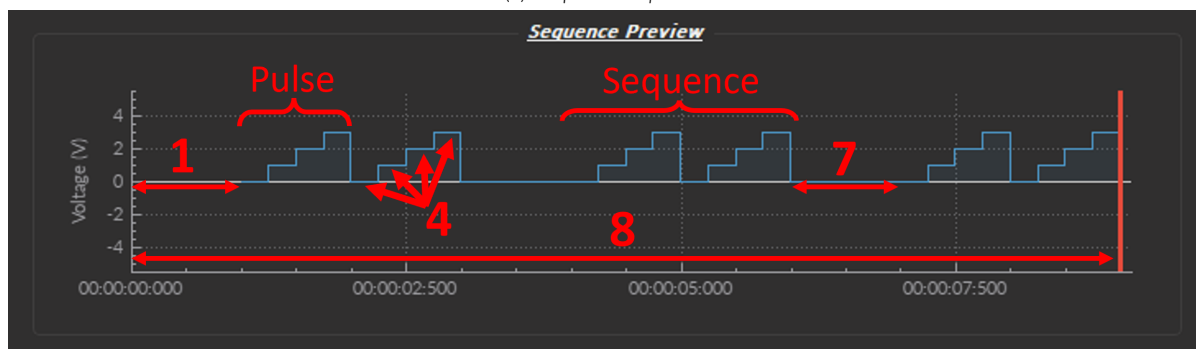
(b) *Sequence Preview*

Figure 5.24: *Channel(s) configuration window, Analog Output Stairs*

5.4.4.5 Custom Mode

The **Custom** mode (Fig. 5.25) provides users with the ability to design a pulse with non-standard shape to fit experimental needs.

1. The **Select File** button (Fig. 5.25a, 1) is used to input a custom .csv file containing the data for the pulse sequence. This must be a .csv format and requires 2500 values in column vector format (i.e. with *line break* between values), as in Fig. 5.25b. The values can be any value between **-4.75V** and **+4.75V**.

2. The **Starting Delay** (Fig. 5.25, 2) defines the time between the activation of the pulse sequence and the beginning of the signal.

3. The **Period** option (Fig. 5.25, 3) replaces the **Time ON** option (Fig. 5.22, 3). This option will stretch or shrink the 2500 value sequence to fit the specified amount of time.

4. The **Period per Sequence** (Fig. 5.25, 4) is similar to the **Pulse per Sequence** found in **Square** modes (Fig. 5.4.4.2, 6), where the pulse is replaced by the period sequence (Fig. 5.25c, Sequence).

5. The **Number of sequence(s)** (Fig. 5.25a, 5) sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** (Fig. 5.25, 6) sets the delay between each sequence.

7. The **Total Duration** (Fig. 5.25, 7) 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**.

(a) *Sequence Options*

(b) *Example .csv file*

(c) *Sequence Preview*

Figure 5.25: *Channel(s) configuration window, Analog Output Custom*

5.4.5   Analog Input

The **Analog Input** channel type acquires signal from the **Analog Input** BNC connector ports. Each numbered channel corresponds to the same analog channel number on the console.

The *Channel(s) Configuration* window for the **Analog Input** is divided into two sections (Fig. 5.26): (1) the **Channel Options** (Section 5.4.5.1) and (2) the **Mode-specific Options** (*Linear*, Section 5.4.5.2; *Interleaved*, Section 5.4.5.3; *Lock-In*, Section 5.4.5.4).
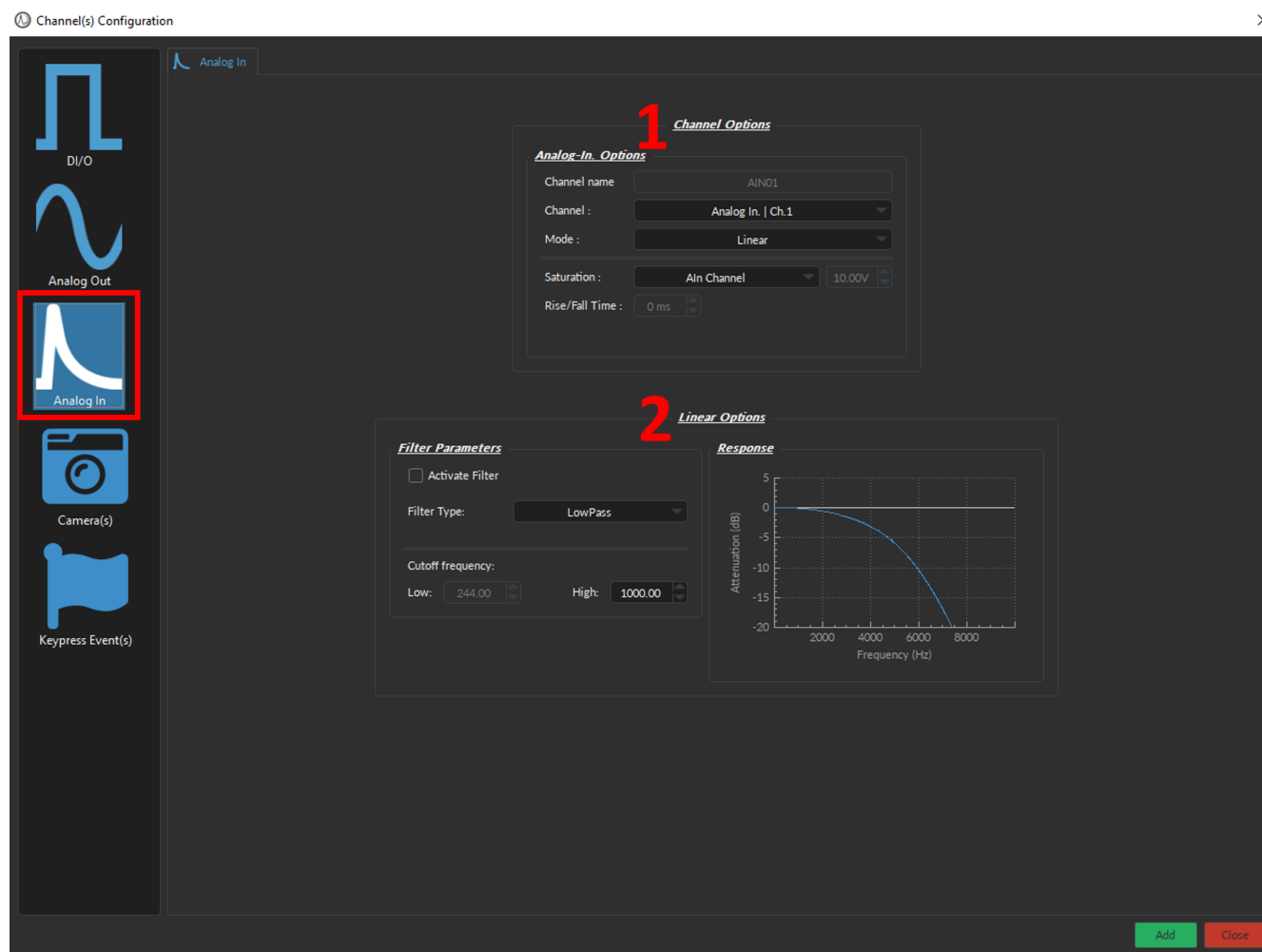
Figure 5.26: *Channel(s) configuration window, Analog Input*

5.4.5.1   Channel Options

The **Channel Options** (Fig. 5.27) defines the channel, source and mode of the digital signal, as following:
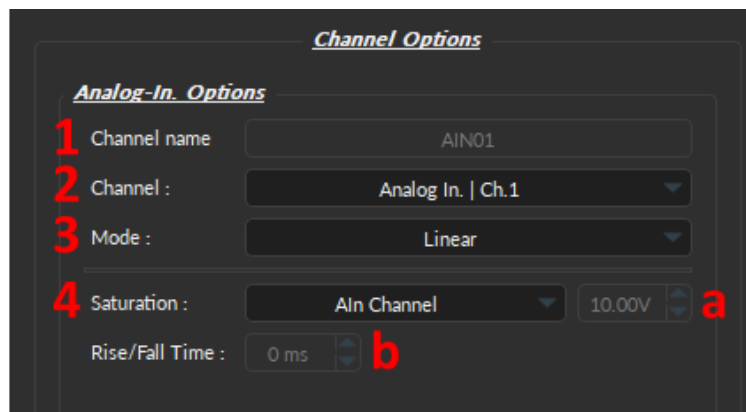
Figure 5.27: *Channel(s) configuration window, Analog Input - Channel Options*

1. The **Channel name** (Fig 5.27, 1) allows user to specify a label for each channel.

2. The **Channel** (Fig 5.27, 2) identifies which of the 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. Each numbered channel on the physical console corresponds to the same number of the digital channel within the software.

3. Three **Mode** are available to record the input signal, each of which have their own defined parameters in the Option box below **Channel Options** (Fig. 5.26, 3):

   - *Linear* - Section 5.4.5.2;
   - *Interleaved* - Section 5.4.5.3;
   - *Lock-In* - Section 5.4.5.4.

4. The **Saturation** (Fig. 5.27, 4) automatically sets the following parameters and depends on the detector acquiring the data (Detectors: *Doric detector*, *Newport Detector*, *Hamamatsu C10709*, and *AIn Channel*):

   a) The **Maximum Voltage** (Fig. 5.27, 4a)
   b) The **Rise/Fall Time** (Fig. 5.27, 4b)

   To manually set either parameter, select **Custom** in the drop-down menu.

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

5.4.5.2 Linear

The **Linear** channel mode (Fig. 5.28) allows the direct measurement of signal received by a channel. The linear mode-specific parameters are as follows:
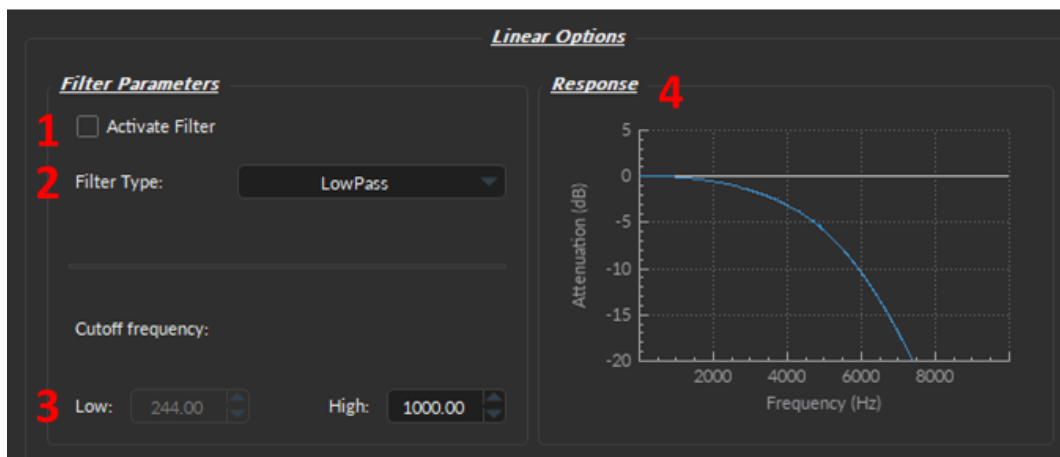
Figure 5.28: *Channel(s) configuration window, Analog Input Linear*

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 Type** drop-down list allows the choice of a filter type from **High-Pass**, **Low-Pass**, **Band-Pass** and **Band-Stop**.

3. 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. Note: the true cut-off value is, by definition, always 3 dB below (Low Cutoff) or above (High Cut-off) the specified value.

4. The **Response** box displays Frequency (Hz) vs Attenuation (dB) trace of the filter according to both the filter type and the cut-off values.

5.4.5.3 Interleaved

The **Interleaved** channel mode allows two 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. 5.29).
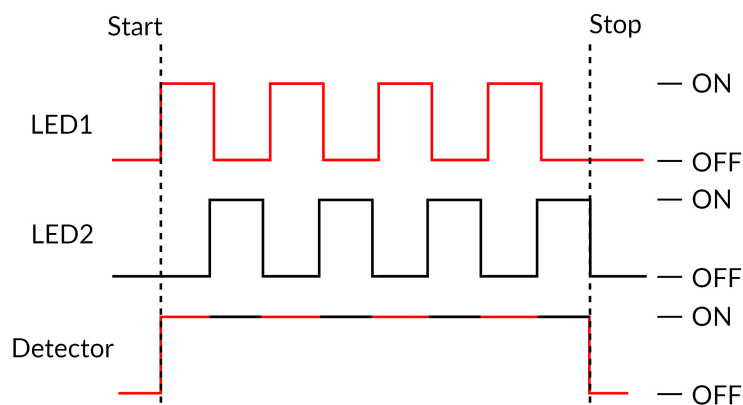
Figure 5.29: *Interleaved Acquisition Timing Diagram*

The interleave preset is using 50% duty cycle for each LED, without delay between them (Fig. 5.29). Thus, depending on the Rise/Fall time of the detector in use (Fig. 5.27, 4b, Detector Rise/Fall Time), there will be more or less crosstalk between the interleaved channels (Fig. 5.30).

> **WARNING:**
> **Crosstalk** occurs between **two interleaved** Digital I/O channels. If possible, use **Lock-In mode** instead, or **switch to a detector** will smaller Rise/Fall Time.

Specifically, when one of the digital channels is ON, it will pick up when the other is turn ON or OFF (Fig. 5.30). Figure 5.30 shows how the Digital Output channel of LED 1 has a small increase in voltage when the LED 2 is turned ON. And, conversely, there is a small dip in voltage in the LED 2 channel when LED 1 is turned OFF (Fig. 5.30).
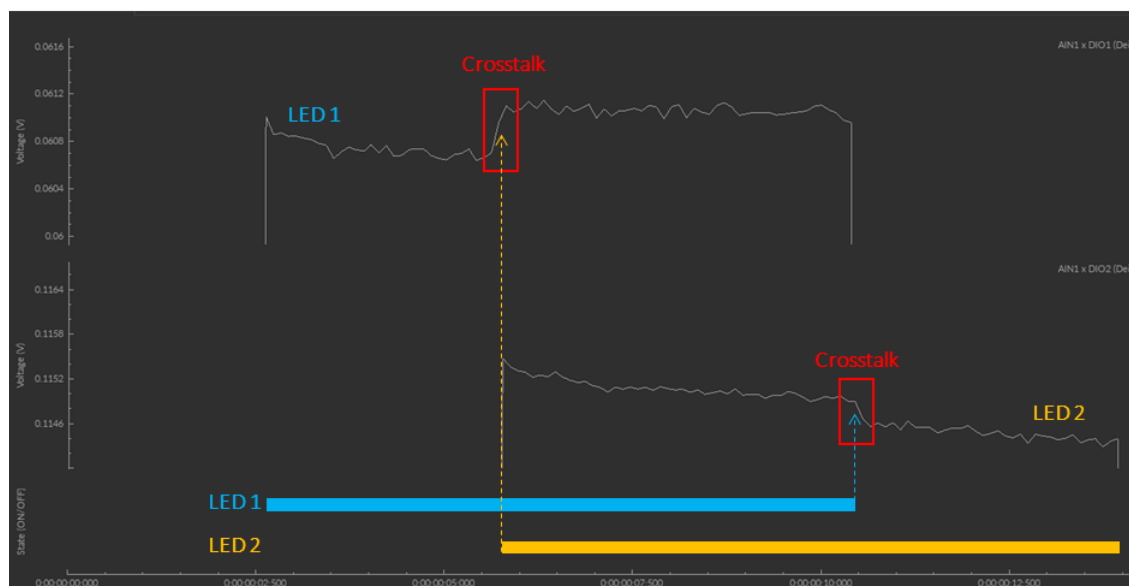
Figure 5.30: *Interleaved Cross-talk*

Strategies to Mitigate Crosstalk:

1. If the sampling rate of the triggered device(s) is high enough (>120Hz), use the **Lock-In mode** (Section 5.4.5.4) instead of the **Interleaved mode**;

2. Switching to a detector with a smaller Rise/Fall Time will reduce the crosstalk. For instance, the *Doric* and *Newport Detectors* have a Rise/Fall Time of 15 ms, while Hamamatsu C10709 has one of 1 ms.

Regardless of the Detector in use, **care should be taken not to misinterpret crosstalk as real signal during data analysis.**
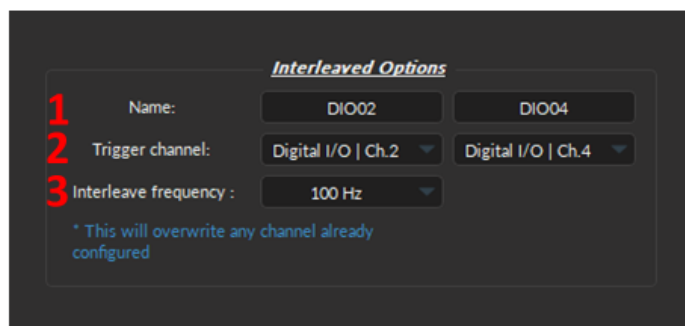
Figure 5.31: *Channel(s) configuration window, Analog Input Interleaved*

To use the **interleaved mode**, specify the parameters in the **Interleaved Options** section of the *Channel Configuration* window (Fig. 5.31):

1. The **Name** (Fig. 5.31, 1) lets users customize the label of the channel to increase clarity of the acquisition system.

2. The **Trigger channel** (Fig. 5.31, 2) drop-down list allows the choice of interleaved outputs (can be either digital or analog). However, once the first channel is selected, the user will only be allowed to select the same type of output (analog or digital) for the second channel.

3. The **Interleave frequency** (Fig. 5.31, 3) drop-down list allows the choice of a pre-configured frequency (either 10, 20, 50 or 100Hz) for the interleaved channels. The two selected trigger channels will be configured to function at the chosen frequency.

> **WARNING:**
> Specifying the interleave frequency will **overwrite** any channel already configured.

5.4.5.4 Lock-In

The **Lock-In** mode can detect fluorescence signals embedded in strong noise (e.g. Isosbestic and a fluorophore) or separate multiple signals from a single input during fiber photometry.

For step-by-step video tutorials on how to set up the **Lock-In** configuration for Basic Fiber Photometry systems, click on the following LINK, under the **Support Tab** (at the bottom of the page).

Each *LED light source* emits a sinusoidal illumination at a given frequency (Fig. 5.32a & 5.32b). The detector collects the fluorescent data at a frequency corresponding to the summation of the LED frequencies (Fig. 5.32c).

(a) *LED 1 frequency*

(b) *LED 2 frequency*
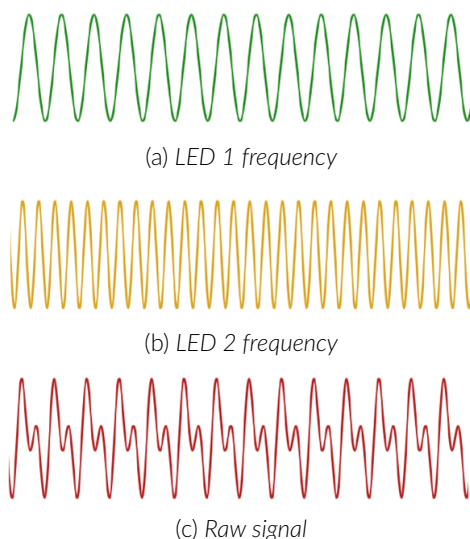
(c) *Raw signal*

Figure 5.32: *Lock-In Acquisition Timing Diagram*

The amplitude changes of the raw signal are due to the collected fluorescence and are dependent of the frequency (Fig. 5.33a). By targeting the known LED frequencies in the raw signal using filters, it is possible to demodulate the fluorescence based on the emission wavelength (Fig. 5.33). The result is separated from the ambient noise that occurred at different frequencies (Fig. 5.33b). The same principle can be applied to demodulate two fluorescent signals.

(a) *Amplitude changes (signal) are frequency dependent*   (b) *Separated signals*
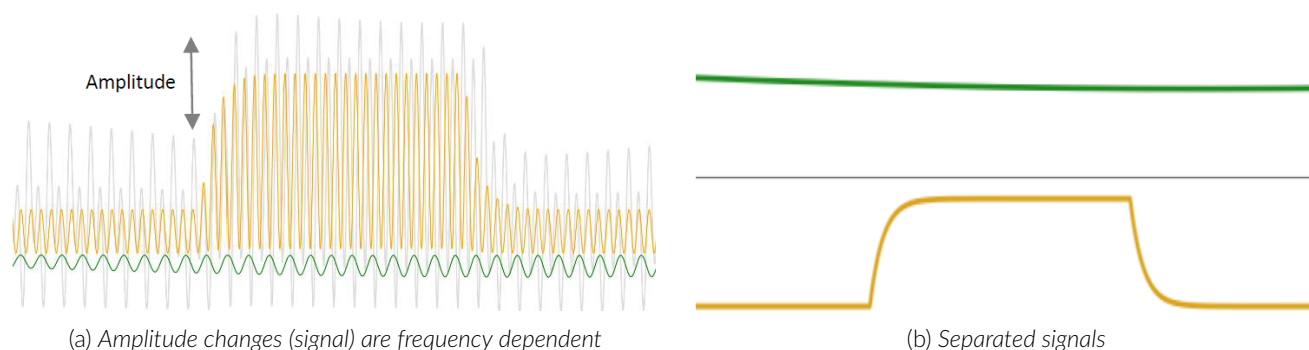
Figure 5.33: *Demodulation separates noise from signal or two signals from each other*

WARNING:
To properly set-up the Lock-In mode, users must have a complete understanding of the wiring of inputs and outputs of they photometry system.

The **Lock-In Mode** parameters are as follows (Fig. 5.34):

1. The **Enable** (Fig. 5.34, 1) row lets users select which output channel to include in the Lock-In settings by clicking the respective check boxes. Each column corresponds to an Analog Out channel of the console (in order, such that left most column = AOUT1). Users should enabled the output(s) channels that will be driving the input specified in Fig. 5.27, 2.

2. **Trace Name** (Fig. 5.34, 2) is the identity of the Input and Output Channel(s) enabled for this Lock-In configuration.

   - The **AIN #** corresponds the console Analog In port number that receives the raw (non-demodulated) signal from the detector. To change the **AIN #**, select a different **Channel** number from the **Analog In Options** box (Fig. 5.27).

   - The **AOUT #** number corresponds the the Analog Out port on the console that sends eletrical information (including the reference frequency) to the *LED Driver*. While you cannot change the **AOUT #** since it is native to each column of the **Carrier Frequency Options** (Fig. 5.34), changing which port is enabled using the checkbox (Fig. 5.34) or physically moving the cable to a different port on the console allows user to specify the connection of the output.

3. **Reference Frequency** (Fig. 5.34, 4) is the oscillating trigger signal that drives the LED (or device(s) of choice). We recommend using the default values since they are optimised for fiber photometry. But, if modified, frequencies will be re-adjusted in steps of 5.96 Hz. In addition, the reference frequency should not be a multiple a of known noise frequency (e.g. 50 and 60 Hz), or a multiple of another reference frequency.

4. **LED maximum current** (Fig. 5.34, 5) is the largest current that the LED can handle. This value should be set either in low power mode (recommend) or based on the intrinsic maximum current of the LED in use (500 mA or 1000 mA, depending of the type of LED).

   - **Low Power Mode (200 mA)** - allows reduced power for the same voltage. This allows low-power signals to be more stable in time. The **maximal current** is reduced to one tenth of light source normal maximal current. 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).
   *Recommended for Fiber Photometry using *Doric FMC or RFMC* systems*

   - **500 mA** - the **LED maximum current** for the following LEDs: 365 nm, 385 nm & 405 nm.

   - **1000 mA** - the **LED maximum current** for most *Doric* LEDs, except the three mentioned above.

   - **Custom** - this setting allows users to manually adjust the Vmax and Vmin of the LED, regardless of LED maximum current. Care should be taken to remain below the maximum voltage, or the excitation signal will be cropped at the true maximum value (see Fig. 5.35).

Figure 5.34: *Channel(s) configuration window, Analog Input Lock-In*
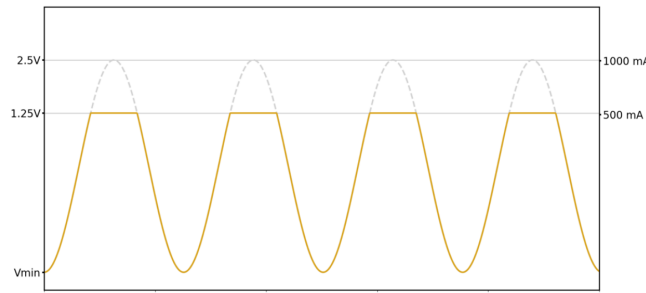
Figure 5.35: *Cropped LED excitation signal*

5. **LED power** (Fig. 5.34, 6) is the percentage of maximum current (converted to voltage) that will be used as **Vmax** and in **External Mode** during the recording, since the LED driver outputs a current proportional to the voltage with a conversion factor of 400 mA/V in standard operation mode, and 40 mA/V in low-power mode.

   - **Note:** The **LED current** should always be set to its maximum on the *LED driver* (and in **External Mode**), while increasing or decreasing **Vmax** should always be done by changing the *FP console* **LED power**.

6. **Vmax Preview** - automatically displays the maximum voltage based on the **LED maximum current** and the **LED power** selected above (Fig. 5.34, 7). **Vmax** can be changed if the **Custom** LED maxiumum current mode is selected. The **Vmax** should never be below 0.3 V, nor above 4.7 V.

   - **Note:** If you are using GCaMP and its isosbestic, we recommend that the isosbestic demodulated trace be about half the power of the GCaMP demodulated trace to reduce the risk of photobleaching (as in Fig. 5.34, 6).

7. **Vmin Preview** (Fig. 5.34, 8) - the default value is set to 0.2 V, but can be changed if the **Custom** LED maxiumum current mode is selected. The **Vmin** should never be below 0.1 V.

8. **Lock-in LPF Frequency** (Fig. 5.34, 9) define the **Cutoff Frequency** of the low-pass filter that extracts the signal and is set to 12 ksps by default. This value was selected because in photometry experiments, the greatest source of noise to the filter is around the carrier frequency above 200 Hz. Thus, with the current filtering algorithm, a cutoff frequency of 12 Hz (corresponding to a decimation factor of 200x) gives the best filtering results.

   - **Note 1:** The saved file **effective sampling rate** is set to 5x the lowpass filter frequency, using a decimation factor (which can be disabled in Saving Options, see Fig. 5.36).

   - **Note 2:** The **Cutoff Frequency** (the frequency at which a -3 dB attenuation will occur) should be chosen as a value close to that of the phenomena observed. A lower cutoff frequency may not result in smaller noise figures.
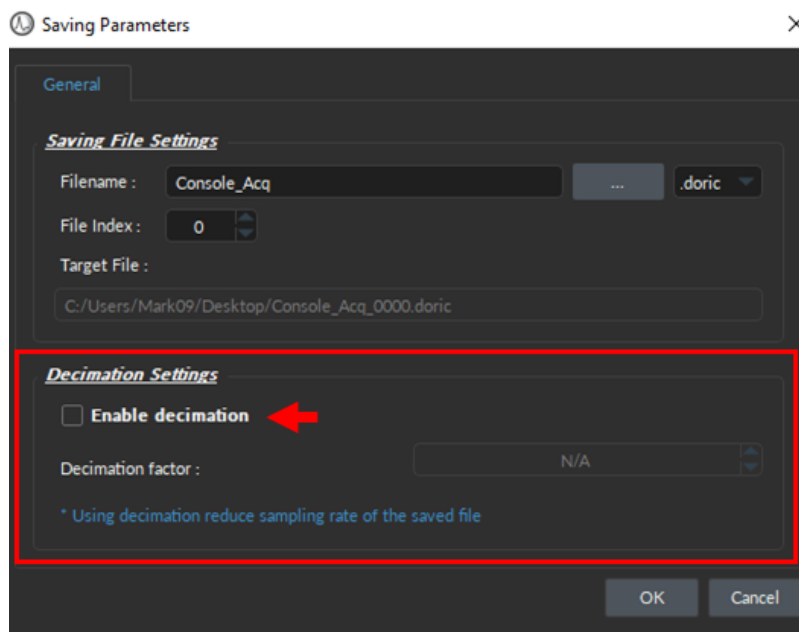
Figure 5.36: *Enable/Disable the decimation factor that reduces Sampling Rate of the saved files*

5.4.6 Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for its measurement.
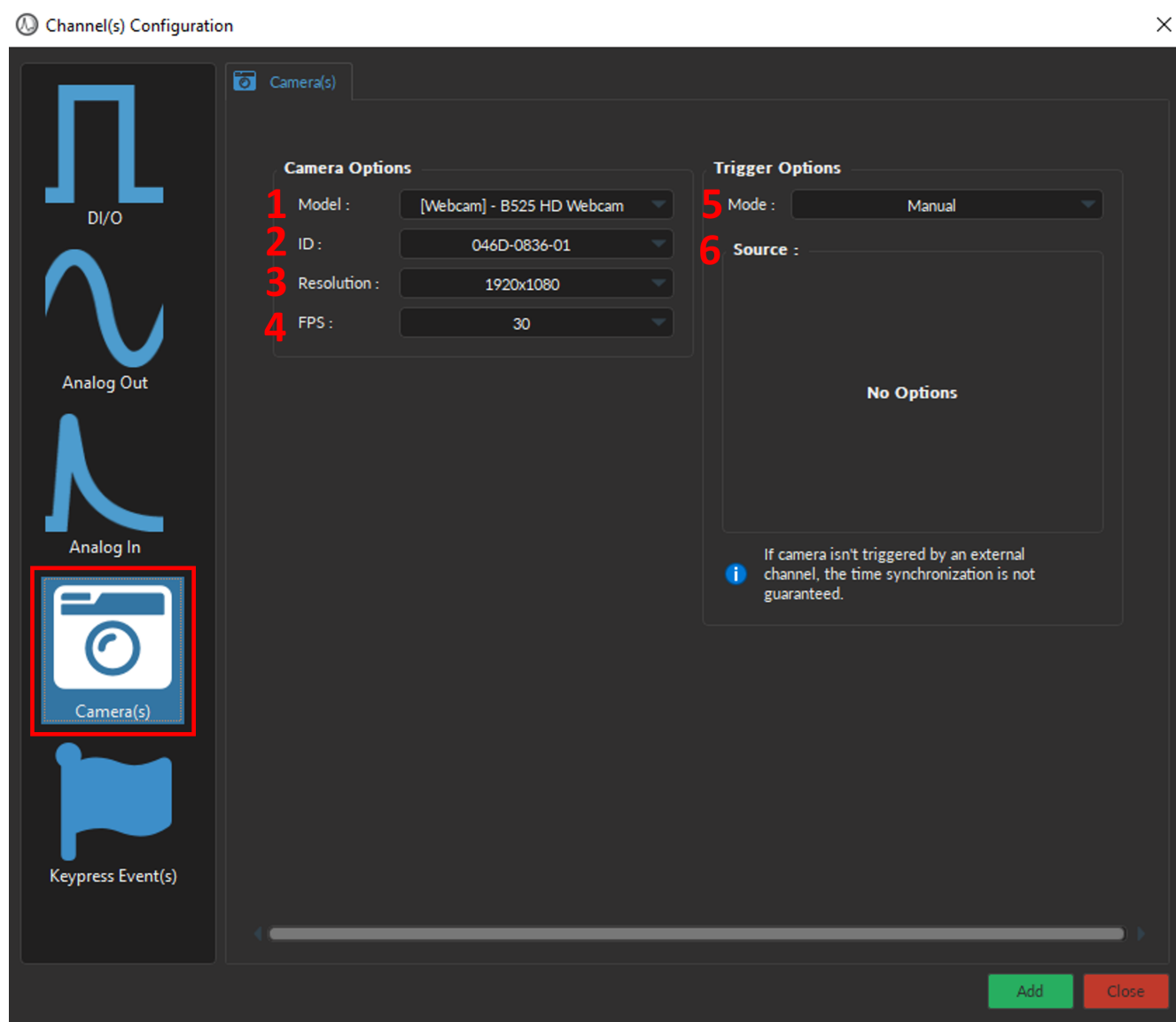
Figure 5.37: *Channel(s) configuration window, Camera*

WARNING:
A camera cannot be used for <u>BOTH</u> **Acquisition Console** and **Camera** modules.
When creating a Camera Channel, if *"No available camera detected..."*, <u>disconnect</u>
the camera in the **Device Selection** window to close the extra module.

Camera Options:

1. The **Model** (Fig. 5.37, 1) allows you to select the camera of choice based on the type of camera.

2. **ID** drop-down list (Fig. 5.37, 2) is used to select a camera based on its unique ID. The ID is particularly useful if multiple cameras of the same model are required for the experiment.

3. The **Image Size** (Fig. 5.37, 3) is used to set the resolution of the image. The large the number of pixels used for width x height, the better the resolution. Currently, image size can ranges between 160x120 to 1920x1080 pixels.

4. The **FPS** (Fig. 5.37, 4) is used to specify the frame rate of the camera (i.e. the number of images displayed per second). FPS can be any value between from 5-30 for web cameras and up to 60 FPS for the *Doric Behavior Camera*.

Trigger Options:

5. The **Mode** (Fig. 5.37, 5) sets the type of trigger that will control the camera. Depending on the type of camera, at most three modes are available:

⚠️ **WARNING:**
If the camera isn't triggered by an external channel,
the **time synchronization is NOT guaranteed**. ⚠️

- **Manual** - Selecting the *Live* or *Record* buttons located in the Acquisition Tab will the trigger the start of the camera recording. ***The time difference between the actual start time and when the first frame is received depends on the camera itself.*** Around a 1 second delay is pretty common for web cameras.

  The time delay (in ms) between the photometry and video data is recorded in the *DifferenceMasterStart-ToFirstImage* attribute, located in *.doric* file under the **Web Camera ID** folder (Fig. 5.38). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

Figure 5.38: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **External** - Will drive the camera using external TTL signal through the trigger cable (Frequency: 30 Hz (or camera FPS); Time ON: 5 ms). This signal can come from any external device connected to the opposite end of the trigger cable. If using *Doric Neuroscience Studio* to synchronize the recording, use *External (Preconfigured)* mode below instead. ***ONLY offered for the *Doric Behavior Camera*.***

- **External (Preconfigured)** - This is the recommended mode to synchronize the camera with the rest of the Acquisition system. This mode automatically creates an additional Digital I/O channel configured to drive the camera at the proper frequency and Time ON. ***ONLY offered for the *Doric Behavior Camera*.***

6. The **Source** (Fig. 5.37, 6 & Fig. 5.39) is only used for the **External (Preconfigured)** mode, and displays the **Digital I/O** channel with the preconfigured parameters that will be created at the same time as the **Camera Channel** (Fig. 5.39). For detailed description of each Digital I/O parameter see the corresponding section in the Fiber Photometry System Manuel. Briefly, key parameters include:

a) The **Channel** (Fig. 5.39, a) corresponds to the physical Digital I/O channel number on the Console that is connected to the trigger cable of the *Doric Behavior Camera*.

b) The **Mode** (Fig. 5.39, b) is by default set to the *Square (TTL)* which provides the external trigger signal to the camera. This parameter cannot be changed.

c) The **Frequency** (Fig. 5.39, c) corresponds to the **FPS** set in the **Camera Options**. Changing the **FPS** will automatically change the **Frequency** in the **Sequence(s) Options**.

d) The **Duty Cycle** (Fig. 5.39, d) is by default 50%. The frame will be taken at the start of each square pulse.

Figure 5.39: *Channel(s) configuration window, Camera - External (Preconfigured)*

5.4.7 KeyPress Event(s)

Keypress Event(s) are ideal when manually labelling or annotating events during experiments. Specifically, selecting any keyboard key during a recording will save the output synchronized to other measurements. Keypress events can be used to:

- Flag disruptions during the experiment, such as lights on, door opened, construction noise, etc.

- Record experimentally relevant events/stimuli, such as airpuff, licks or any other behavior.

WARNING:
Keyboard event(s) timing are **accurate within 1 second** due to variations in Windows priority management and buffering of the signals.

5.4.7.1 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the botton of the window (Fig. 5.40, left). To remove a KeyPress, use - button (Fig. 5.40, right).

- **NOTE:** Selecting the + button (without clicking the *Add* button or the *Close* of the *Channel Configuration* window) will **automatically** add the Keypress Event channel at the **bottom** of the Acquisition View window, below any pre-existing channels (Fig.5.40).
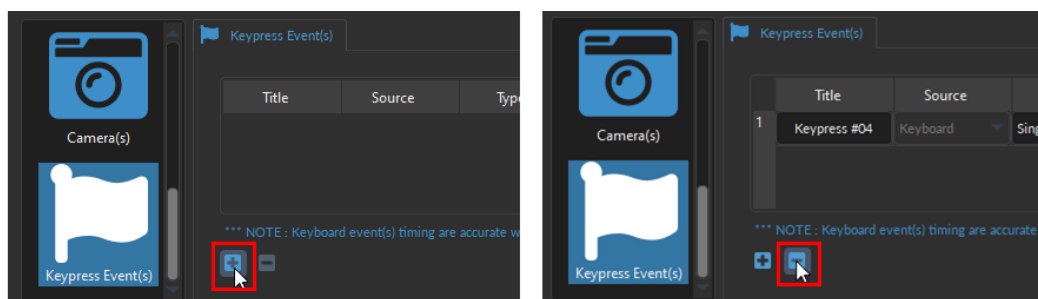
Figure 5.40: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 5.41) in the **Acquisition View**.
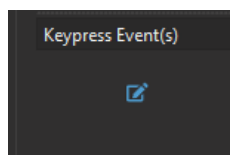
Figure 5.41: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 5.45:

1. The **Title** allows you to give a name for the Keypress event.

2. The **Source** is by default *Keyboard*.

3. Three **Types** of Keypress Event(s) can be specified with the drop-down list:

   - **Single** - Records single event at the touch of a key (Fig. 5.42a).
   - **Toggled** - Records the start and end of an event using the same key. First press denotes the start of the event while a second press denotes the end of it (Fig. 5.42b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 5.42c). Every keypress is a new event, with the start of the event occuring when the key was depressed.

(a) *Single*

(b) *Toggled*
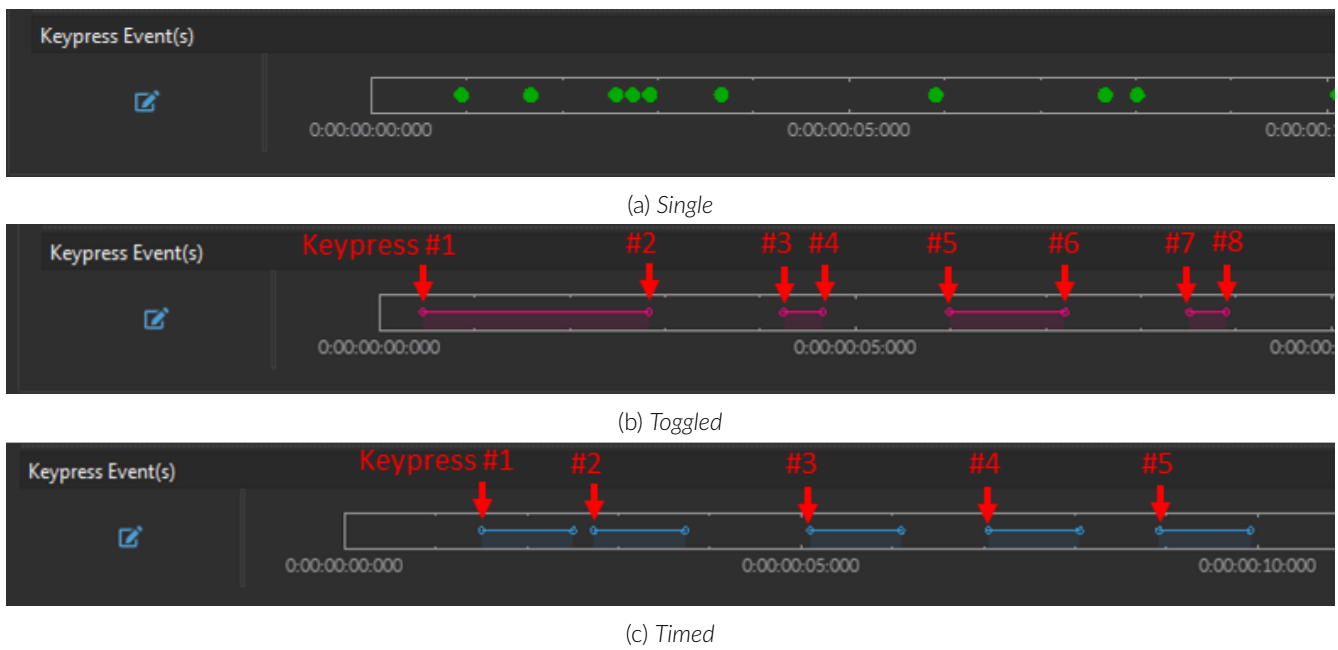
(c) *Timed*

Figure 5.42: *Three types of Keypress Event(s)*

4. The **Duration** is only used for the **Timed** Keypress type to specify the predetermined amount of time a Keypress Event will span. The duration is set in hh:mm:ss:zzz.

5. Select the **Color** field to open the **Select Color** window. Basic colors are provided, in addition to custom colors can be created and stored.

6. The **Shortcut Key(s)** can be any keyboard key, including space bar, enter, backspace, any letters, number and special characters (*, !, ? etc.). To specify the key, click inside the *Shortcut Key(s)* cell, then press the keyboard key of choice. If a key was properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 5.45, column 6).

7. The **Information** column provides space to make notes or write a short description of the Keypress Event.
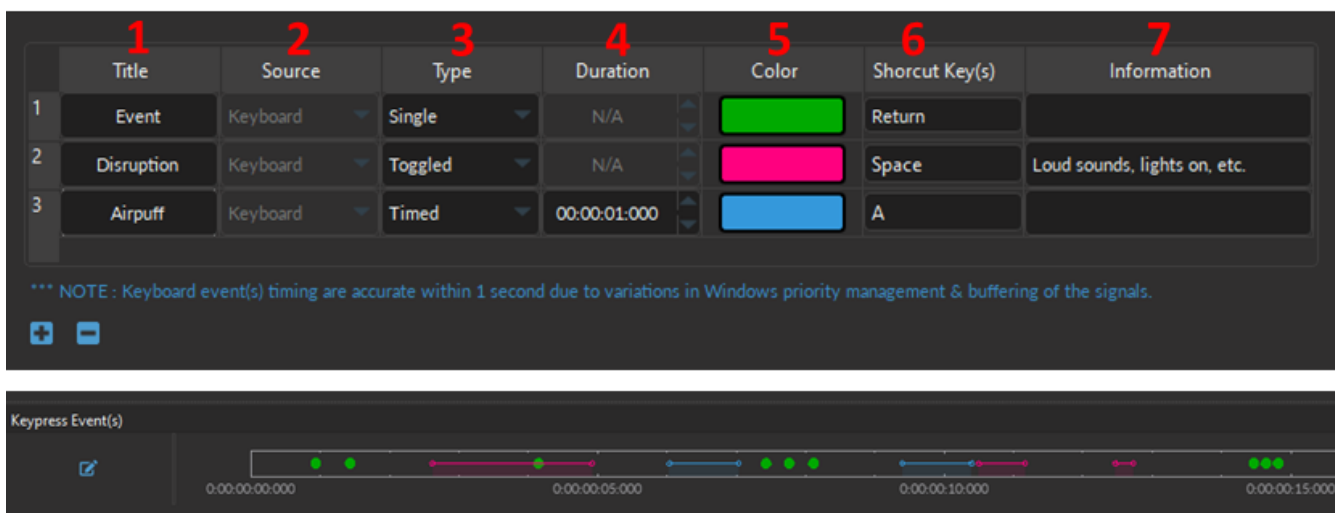
Figure 5.43: *Channel(s) configuration window, KeyPress Event(s)*
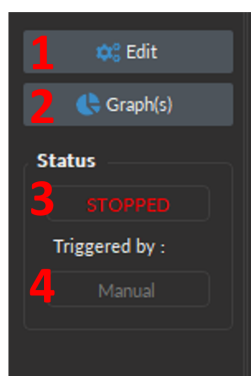
Figure 5.45: *Common parameters*

5.5   Acquisition View

The **Acquisition View** displays all the information concerning active channels: **Control box** (Fig.5.44) and the **Graphs** (Fig.5.45).

If neither **Control Box** nor **Graphs** are displayed in the **Acquisition View**, this means channels have yet to be config-ured. User can either use the **Load Configuration** button (see Section 5.3.2) to load a .*doric* file with previously saved channel parameters, or user can manually add channels using the **Add Channel** button (see Section 5.3.2).

Figure 5.44: *Channels*

5.5.1   Channel Control Box

Each channel **Control box** shows the following basic elements (Fig.5.45), with additional elements available for specific channel types:

1. The **Channel name** (Fig. 5.45, 1) is located on the upper left of the **Control box**, 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 (Fig. 13.36).

2. The **Edit** button (Fig. 5.45, 2) opens the **Channel Configuration** window, were channel parameters can be modified (See section 5.4.1).

3. The **Graph(s)** (Fig. 5.45, 3) button opens the **Graph Options** window (Fig. 13.36) corresponding to the channel whose graph will be modified. This window allows users to configure visualization and naming parameters of each channel graph (Fig. 13.36). If a channel has multiple traces, parameters to configure each trace individually will appear automatically on different rows (Fig. 13.36). **Graph(s) Options** parameters (Fig. 13.36) are as follows:
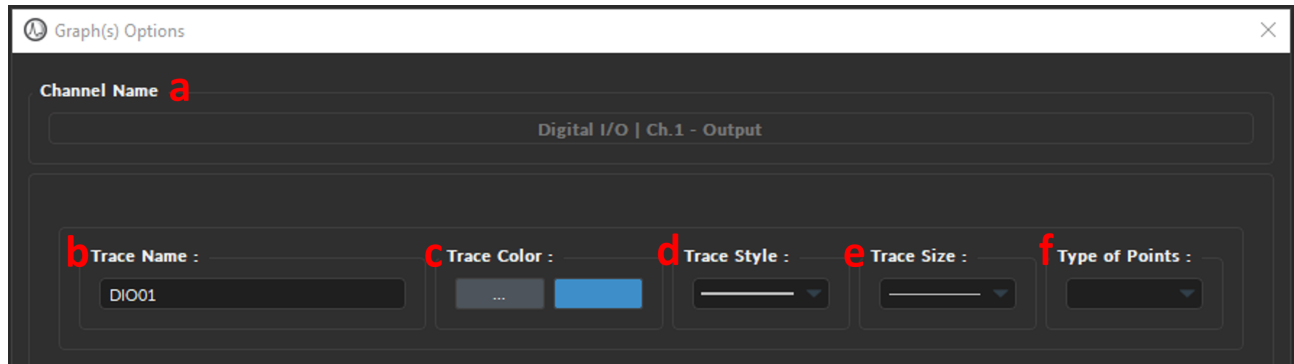
Figure 5.46: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 13.36, 1) is the default name assigned by the software, which includes the type of channel (Digital / Analog In or Out) and the location of said channel on the console (BNC connector 1-4).

b) The **Trace Name** text-box (Fig. 13.36, 2) allows users to specify a name for the trace, instead of the default name generated by the software.

c) The **Trace Color** button (**...**) (Fig. 13.36, 3) opens the **Color Select** window (Fig. 13.37), 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.

d) The **Trace style** drop-down list (Fig. 13.36, 4) 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.

e) The **Trace size** drop-down list (Fig. 13.36, 5) allows the selection of the trace size. Using a bigger **Trace size** than the default may result in slower display and performance degradation.

f) The **Type of points** drop-down list (Fig. 13.36, 6) 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.
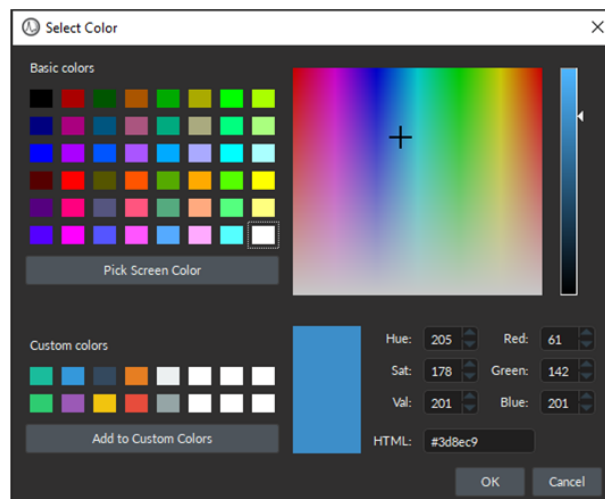
Figure 5.47: *Select Color Window*

4. The **Status** bar (Fig. 5.45, 4) displays acquisition status. **STOPPED** is displayed when the acquisition is inactive, and **STARTED** when acquisition is active.

5. The **Triggered by:** (Fig. 5.45, 5) text-box displays the source of the trigger for that channel, which can either be Manuel (i.e. selecting the **Record**/**Live** button) or a specific channel that provides external trigger signal.

5.5.2 Channel Graph Visualisation

Besides editing the trace of the channel **Graph**, which can be done through the **Edit** button of the **Control box** (section 5.5.1), other features of **Graph** view can be directly manipulated by selecting elements of the **Graph** itself. This section includes changing axis properties, manual zoom, and determining instantaneous values.
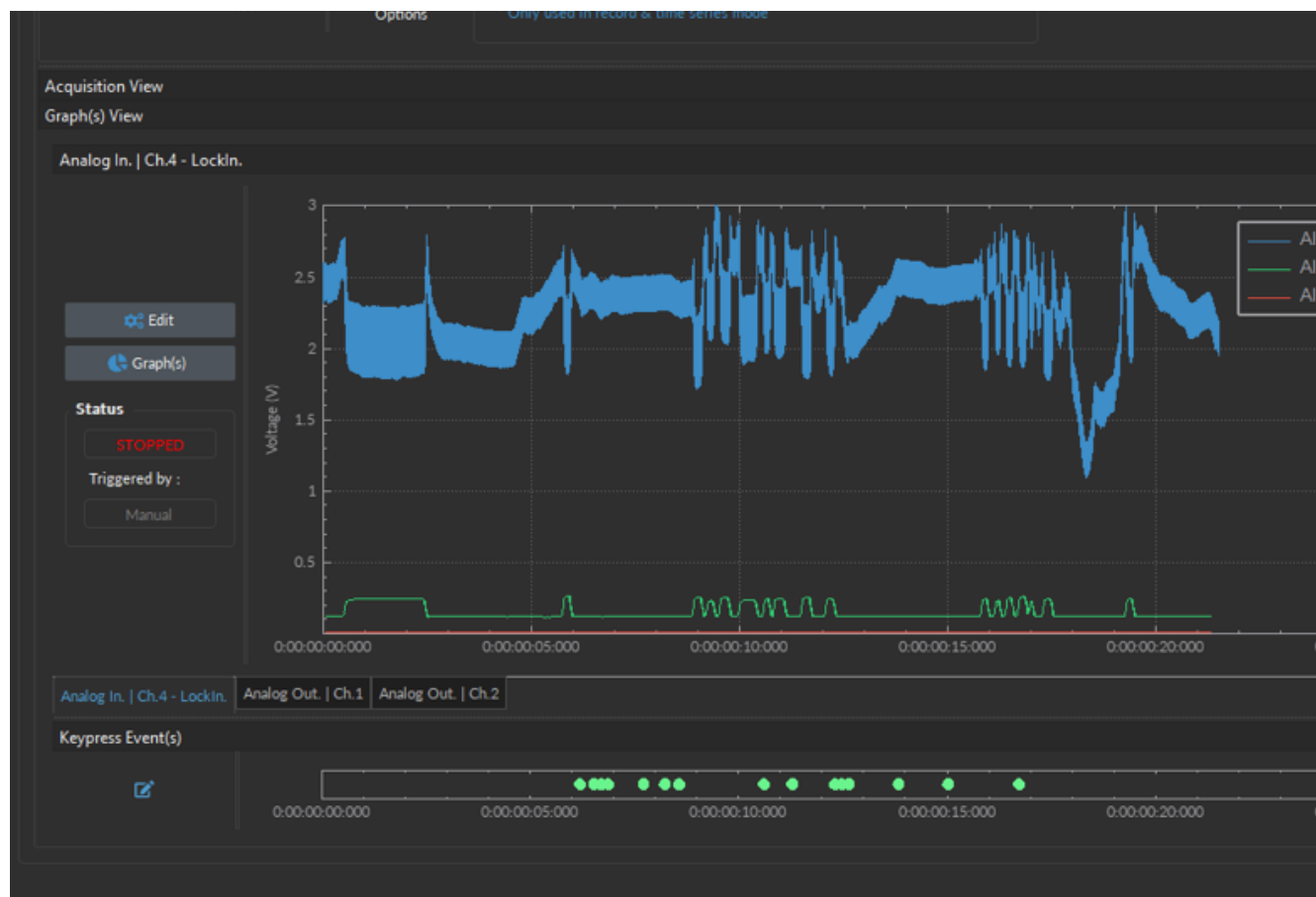
Figure 5.48: *Acquisition View - Graph*

- *Axis Options* - Each **Graph** (Fig. 13.36) has both a **Voltage** or **State** as the vertical axis and **Time** as the horizontal axis. Double-clicking either axis will open an **Axis Options** window where the axis limits can be set, similar to the **Zooming Range** in the **View Tab**. Any changes done on a horizontal axis will change the axis limits for every channel.
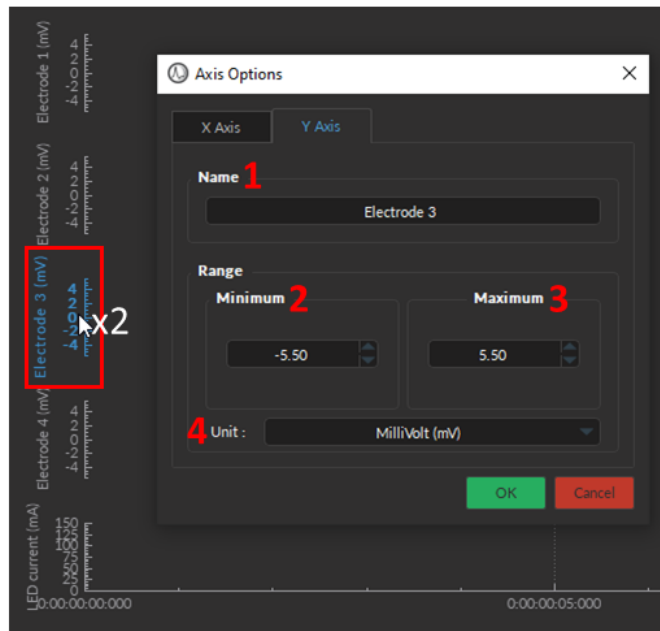
Figure 5.49: *Double click on any axis to open its Axis Options window*

- By clicking and **dragging the graph sideways or upwards**, one can scroll through nearby values on either axis, keeping the zoom range constant. Any changes done on a horizontal axis will change the axis limits for every channel.

- Using the **Mouse Scroll Wheel**, one can change the zoom range of the graph. Any changes done on a horizontal axis will change the axis limits for every channel.

- The **Instant values** box can be activated by double-clicking the **Input graph** box and selecting **Show instant values** (Fig. 13.40). 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**. To remove instantaneous value, double click on the dot.

Figure 5.50: *Acquisition View - Instant values*

- The **Channel tabs** 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.

- Analog output channels display an **Active state** graph (Fig. 5.51, left panel). This graph displays whether the channel is outputting a signal (On, V≠0) or not (Off, V=0).

- Output channels display a **Preview** graph (Fig.5.51, right panel), showing a preview of the pulse sequence.
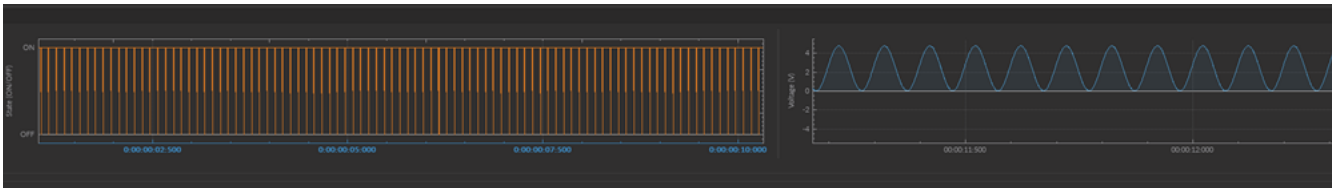
Figure 5.51: *Acquisition View - Output graph*

Behavior / Web Camera

A Behavior Tracking Camera is a great addition to any experiment, providing complementary information that can establish correlations between neuronal activity and animal behavior. *Doric Neuroscience Studio* offers two different camera modules (1) the **Behavior Camera** module and (2) the **Web Camera** module. Both these modules can be integrated in the **Acquisition Console** module as a **Camera Channel**, providing a simultaneous view of the fiber photometry and behavior recordings (Fig. 6.14). See section 6.2 for more details.
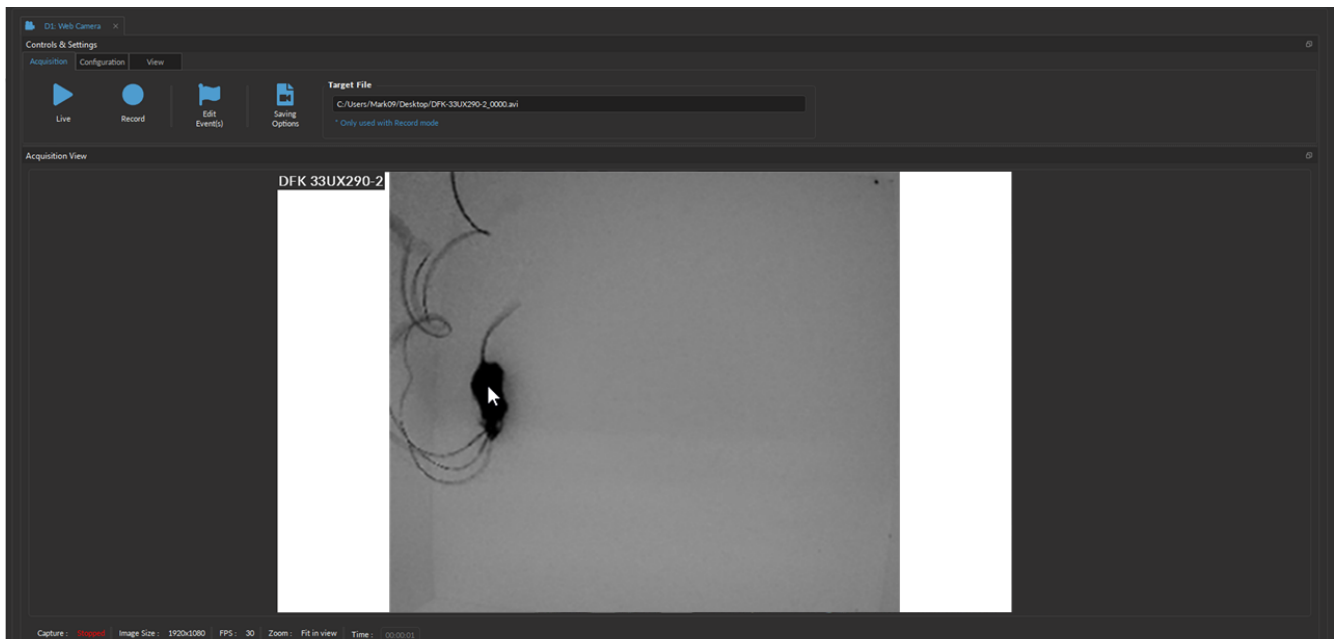
Figure 6.1: *Web Camera Module*

While most of the features and the user interface layout are identical between both modules, there are a few key differences (Table 6.1).

1. The **Behavior Camera** module is specially created for *Doric Behavior Camera* and provides a framework for streaming high-speed video and synchronizing it with other data acquisition devices required for the experiment.

2. The **Web Camera** module supports a large number of USB3 Cameras (including webcams) but cannot be synchronized with the neural recording using Ex. TTL trigger.

   To check whether a camera is compatible with this module, open *Device Manager* on the computer where the camera is connected (Fig. 6.2). If the camera name is under the *Camera* device tab (Fig. 6.2), then in most cases it will be compatible with the **Web Camera** module.

Table 6.1: *Comparison between camera modules*

Features	Web Cameras	Behavior Camera
Camera	USB3 cameras	*Doric Behavior Camera*
Acquisition Console Integration	x	x
Synchronization	Manual [1]	Manual or Ex. TTL
Expanded Capture Options		x
Compatible with Behavior Analyzer	x	x
Compatible with DANSE software	x	x
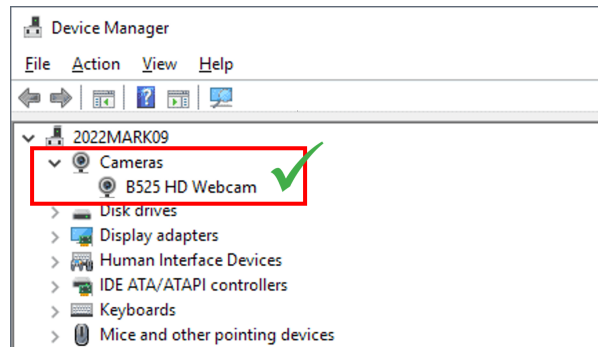
Figure 6.2: *Compatible Web Cameras located under Device Manager Camera tab*

6.1 Control & Settings

The **Control & Settings** of both the *Web Camera* and the *Behavior Camera* modules are split into three tabs that allow the configuration and control of the camera:

1. The **Acquisition** tab - Section 6.1.1

2. The **Configuration** tab - Section 6.1.2

3. The **View** tab - Section 6.1.3

6.1.1 Acquisition Tab

The **Acquisition** tab (Fig. 6.3) contains the controls to start, stop and save behavior footage, and includes the following elements:
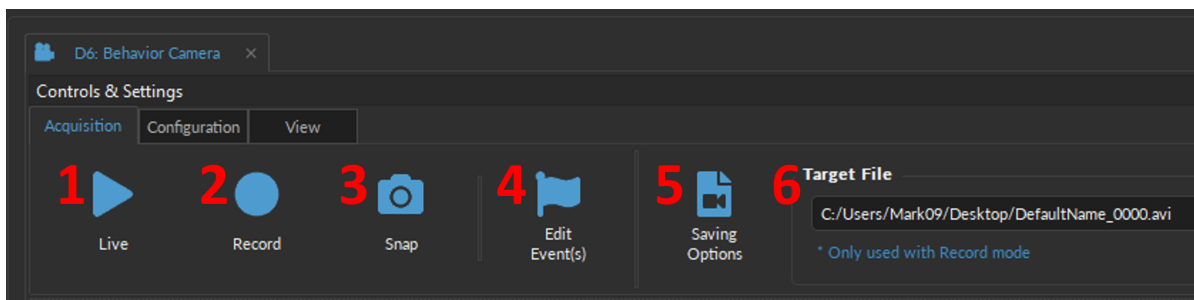
Figure 6.3: *Control & Settings, Acquisition tab*

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[1]It is possible to synchronize a non-Doric camera with TTL pulse using a Digital Output Channel, but not while using Web Camera Module. Thus, users will also have to use 3rd-party/camera manufacturer software to record the footage.

1. The **Live** button (Fig. 6.3, 1) acquires images and displays them. These images are for display only and cannot be saved.

2. The **Record** button (Fig. 6.3, 2) acquires a continuous image stream and saves it to a user-defined file as one .AVI file.

3. The **Snap** button (Fig. 6.3, 3; NOT available for the *Web Camera* module) will take a picture and automatically open a window where users can save the image in a variety of available file formats (including .bmp, .jpeg, .tiff, among many more).

4. The **Edit Event(s)** button (Fig. 6.3, 4) opens the **Keypress Event(s)** window, which allows users to flag behavior events or experimental disruption at the press of a keyboard key. See Section 6.1.5.

5. The **Saving Options** button (Fig. 6.3, 5) opens the **Saving Options** window (Fig. 6.4), which is split into two tabs. The **General** tab (Fig. 6.4a) is used to set file saving parameters, while the **Encoding** (Fig. 6.4b) is used to adjust the camera acquisition parameters.

(a) *General tab* (b) *Encoding tab*
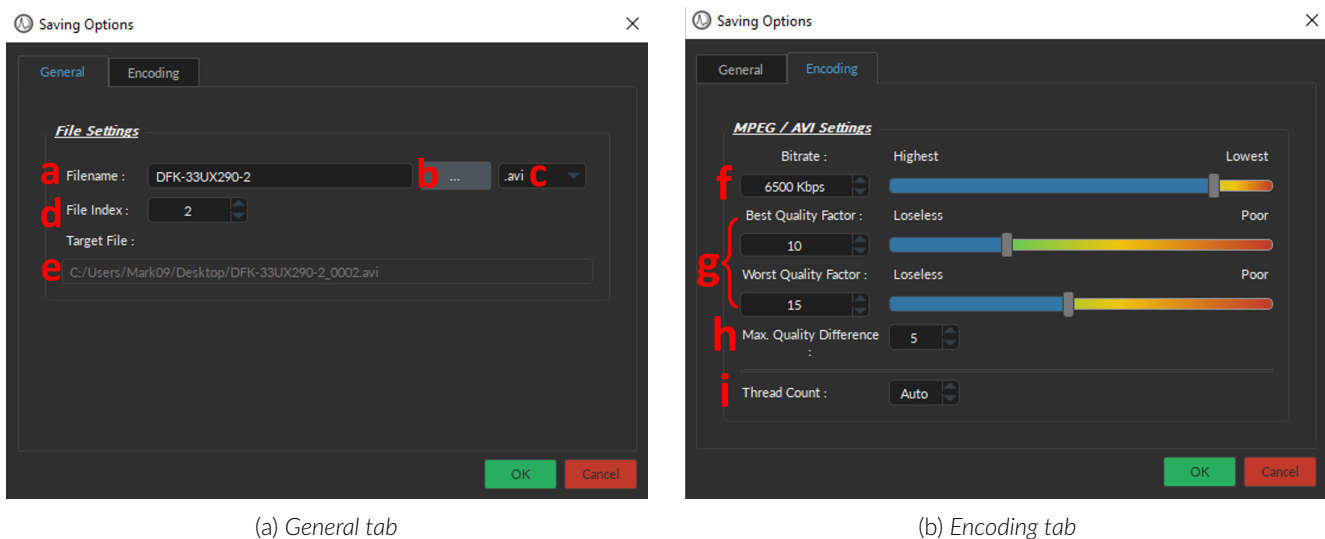
Figure 6.4: *Saving Options window*

Specifically, using the **General** tab user can specify the following (Fig. 6.4a):

a) The **Filename** box (Fig. 6.4a, a) is used to define the name of the recorded video file.

b) The **[...]** button (Fig. 6.4a, b) is used to define the target directory where the video will be saved.

c) For the **File format**, all videos are saved in the *.avi* format. (Fig. 6.4a, c).

d) The **File Index** box (Fig. 6.4a, d) is used to automatically add a four-digit number immediately after the **Filename** where the file will be saved. The suffix is incremented automatically when recording multiple files.

e) The **Target File** displays the final path + filename + extension. This file name will ultimately be displayed as the **Target File** in the **Acquisition** tab (Fig. 6.3, 5).

The *Encoding* tab is used to choose video encoding quality (Fig. 6.4b). Most elements can be changed using the appropriate **Text Box** or **Slider**.

f) The **Bitrate** (Fig. 6.4b, f) sets the number of bits recorded per second. Larger resolution images require a higher **Bitrate**.

g) The **Best Quality Factor** and **Worst Quality Factor** (Fig. 6.4b, g) 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** indicates the highest-compression frames accepted.

h) The **Max Quality Difference** box (Fig. 6.4b, h) indicates the maximal compression difference between two subsequent video frames.

i) The **Thread Count** (Fig. 6.4b, i) 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 it is more demanding on the CPU.

6.1.2   Configuration Tab

The **Configuration** tab is useful to save and load camera settings and includes the following options (Fig. 6.5):

(a) Web Camera
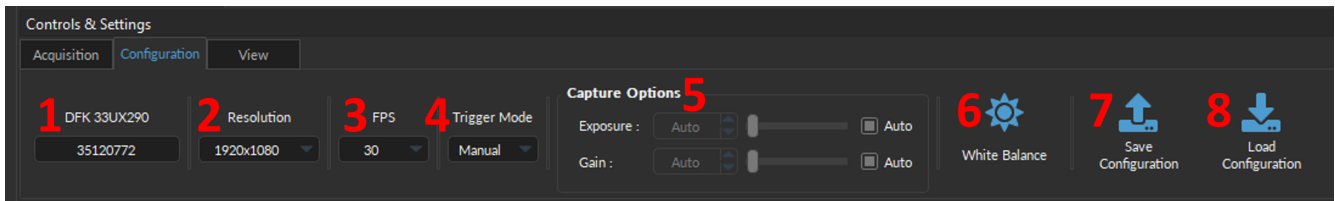
(b) Behavior Camera

Figure 6.5: *Camera Module Comparison, Configuration Tab*

1. The **Name** (Fig. 6.5, 1) displays the serial number of the camera currently in use.

2. The **Resolution** or **Image Size** (Fig. 6.5, 2) displays the width x height of the camera image in pixels. Larger width and height will have better resolution, but will also make for larger files. The available resolution ranges between 368x256 to 1920x1080.

3. The **FPS** (Fig. 6.5, 3) stands for *frames per seconds* and represents the frequency at which frames are displayed. Higher FPS makes for smoother motion in the video, but will also make for a larger video file. The available FPS ranges between 1-60 FPS.

4. The **Trigger Mode** (Fig. 6.5b, 4 - NOT available for *Web Camera* module) is used to set how the camera will be controlled and synchronized with the rest of the recording system.

   - *Manual* - user controls the camera by selecting the **Record** or **Live** buttons.
   - *External* - the camera will wait for an external TTL pulse when clicking the **Live** or **Record** buttons. Note that for this mode, the camera must be also connected to the Digital I/O port of the console with a triggering cable. The Digital I/O should be configured using the *Square (TTL)* mode with a *Frequency* of 30Hz (or matching FPS used with camera mode) and *Time ON* of 5ms.

5. The **Capture Options** (Fig. 6.5b, 5 - NOT available for *Web Camera* module) controls the brightness of the image in two different ways.

   - *Exposure* (in ms) - the duration when the camera sensors are exposed to light. The larger the exposure, the brighter the image.
   - *Gain* (in dB) - is an amplification factor applied to all pixel values. Increasing the gain will increase the brightness of the signal and noise evenly.

   The **Auto** checkbox will automatically set either setting based on the current live detection. We recommend using the Auto settings.

6. The **White Balance** (Fig. 6.5b, 6 - NOT available for *Web Camera* module) adjusts the colors of the image to match the color of the light source in order for white objects to appear white This calculation is run over the previous 5 seconds.

7. The **Save Configuration** button (Fig. 6.5, 7) stores the current settings (from Acquisition, Configuration, and View tabs) in a *.doric* file for future use.

8. The **Load Configuration** button (Fig. 6.5, 8) allows users to open a previously saved *.doric* configuration file and will automatically preset all the parameters of the **Web Camera** module.

6.1.3 View Tab

The **View** tab (Fig. 6.6) sets the parameters of video images, such as zoom, orientation, and calibration of the conversion factor between pixel and real distance in mm. Calibration is required for proper behavior analysis in the **Behavior Analyzer** module.
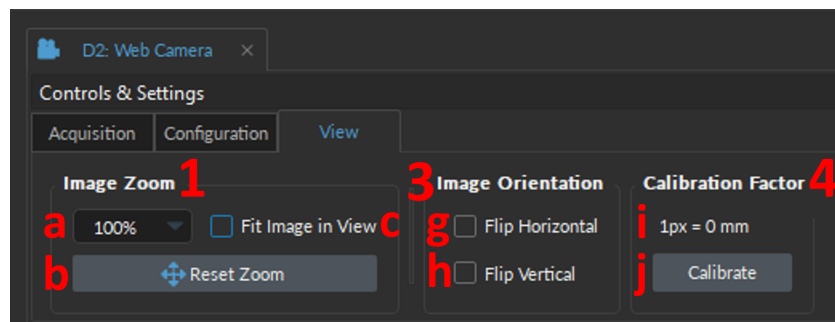
(a) *Web Camera*

(b) *Behavior Camera*

Figure 6.6: *Camera Module Comparison, View Tab*

1. The **Image Zoom** (Fig. 6.6, 1) sets the image magnification factor. This factor only affects the live display of the feed. The entire image (at 100%) will be saved in the .doric file, no matter the zoom settings selected.

    a) The **Zoom %** drop-down list (Fig. 6.6, a) specifies the zoom factor for the image display, which ranges between 10%-500%.

    b) The **Reset Zoom** (Fig. 6.6, b) button returns the zoom factor to 100%.

    c) The **Fit Image** checkbox (Fig. 6.6, c) automatically adjusts the image to fit the entire Acquisition View.

2. The **Image Offset** (Fig. 6.6b, 2) parameters are available when the **Resolution** of the image is smaller than the maximum available (1920 x 1080), essentially cropping the saved image feed. Note that the available offset depends on the difference between the maximum and current **Resolutions** and is independent from the **Image Zoom**.

    d) The **Auto-center** checkbox (Fig. 6.6b, d) centers the camera and is the default setting. Unchecking the box unlocks the X & Y slider setting to manually set the offset.

e) The **X Offset** slider (Fig. 6.6b, e) allows users to move the camera image horizontally by the selected number of pixels.

f) The **Y Offset** slider (Fig. 6.6b, f) allows users to move the camera image's vertical axis by the selected number of pixels.

3. The **Image Orientation** (Fig. 6.6, 3) contains parameters that control the direction of the image displayed in the **Acquisition View**:

g) The **Flip Horizontal** checkbox (Fig. 6.6, g) displays a mirrored image where the left side becomes the right, and vice versa.

h) The **Flip Vertical** checkbox (Fig. 6.6, h) displays a mirrored image where the top becomes the bottom and vice versa.

4. The **Calibration Factor** box (Fig. 6.6, 4) contains:

i) The **Current Calibration Factor** is the conversion ratio between the value of 1 pixel and the unit of choice (mm, cm, or in). If the image has yet to be calibrated, it will be 0mm by default.

j) The **Calibrate** button opens the hidden **Calibration Settings** box (Fig. 6.7, 5) which are required to calculate a new **Calibration Factor**. The image calibration can only be done once the **Live** / **Record** mode was started and stopped. Note that once the **Calibrate** button is selected, it turns into the **Apply** button (Fig. 6.7, j).
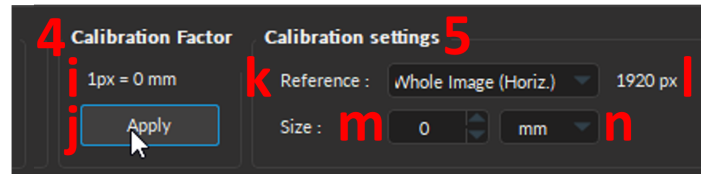
Figure 6.7: *Camera Module - Calibration*

REMINDER:
Image calibration is required **BEFORE** data collection when using the
Animal Tracking and the **Motion Score** functions.

5. The **Calibration Settings** contain the parameters required to calculate the **Calibration Factor**. Once updated, the new **Calibration Factor** will be displayed above the **Apply** button (Fig. 6.7, i).

k) The **Reference** drop-down list (Fig. 6.7, k) offers three options of elements of the image to use as a reference when calculating **Calibration Factor**.

The following options are available as references:
- The *Whole Image (Horiz.)* - uses the width of the images as the reference.
- The *Whole Image (Vert.)* - uses the height of the images as the reference.
- A *User Defined (Line)* - uses a user-drawn line within the image as a reference (Fig. 6.8). This line can online be horizontal or vertical. For optimal results use an object/dimension that fills most of the image.

l) The **Current Reference Dimensions** (in pixels) is displayed to the right of the drop-down list (Fig. 6.7, l - 1920px).

m) The **Size & Units** text-boxes (Fig. 6.7, m & n) specify the real dimensions of the reference and its unit (mm, cm or inches). Select the **Apply** button (Fig. 6.7, j) to recalculate the **Calibration Factor** using the new **Size & Units**.
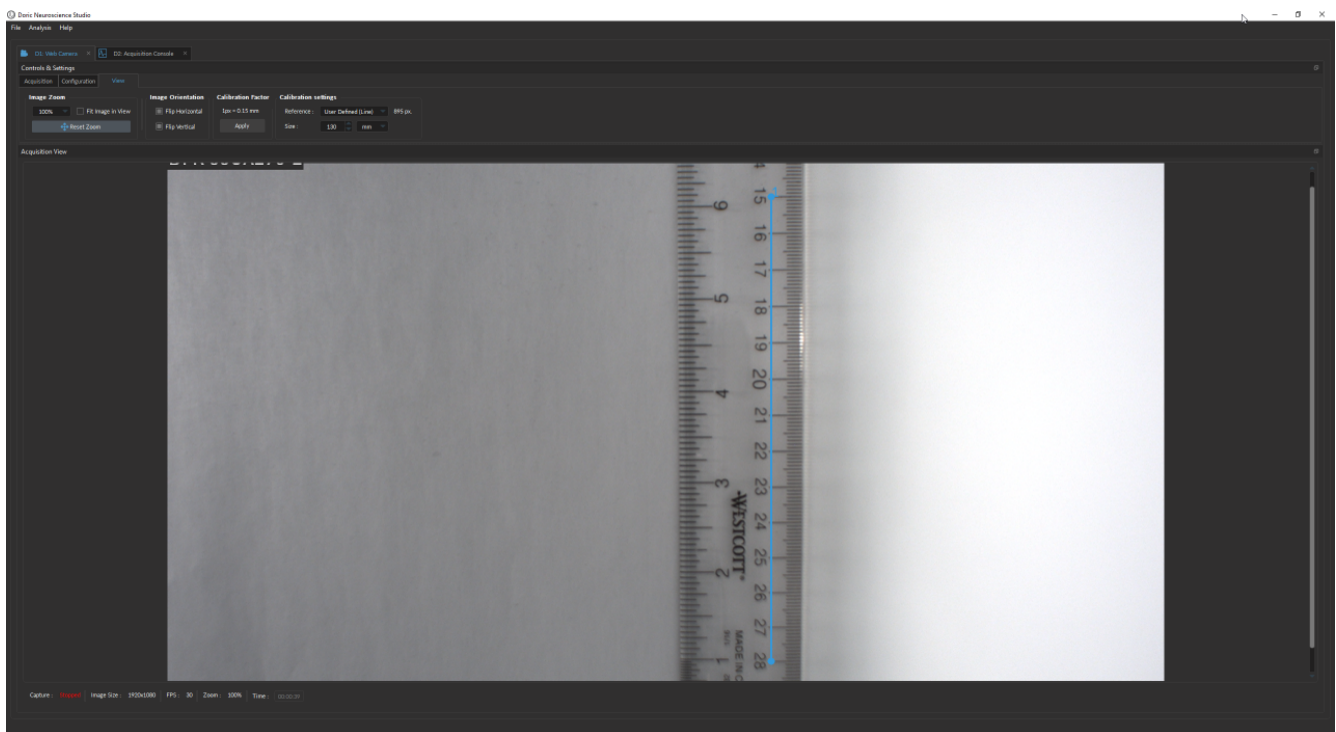
Figure 6.8: *Camera Module - Calibration User-defined line*

6.1.4 Live feed monitoring bar

The constant live feed allows user to quickly track the status of the camera feed.
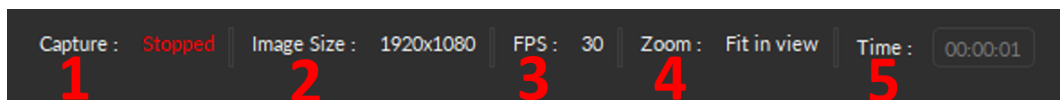
Figure 6.9: *Camera Module - Live Feed Monitoring bar*

1. The **Capture** status displays whether the camera is *Stopped*, *Active* or if using the **External Trigger Mode**, *Waiting for image*.

2. The **Resolution** or **Image Size** displays the value selected in Section 6.1.2 - no. 2.

3. The **FSP** displays the value selected in Section 6.1.2 - no. 3.

4. The **Zoom** displays the value selected in Section 6.1.3 - no. 1a, or 1c.

5. The **Time** displays the time since the camera was turned on.

6.1.5 KeyPress Event(s)

Keypress Event(s) are ideal when manually labelling or annotating events during experiments. Specifically, selecting any keyboard key during a recording will save the output synchronized to other measurements. Keypress events can be used to:

- Flag disruptions during the experiment, such as lights on, door opened, construction noise, etc.

- Record experimentally relevant events/stimuli, such as airpuff, licks or any other behavior.

> **WARNING:**
> Keyboard event(s) timing are **accurate within 1 second** due to variations in Windows priority management and buffering of the signals.

6.1.5.1 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the bottom of the window (Fig. 6.10, left). To remove a KeyPress, use the - button (Fig. 6.10, right).

- **NOTE:** Selecting the + button (without clicking the *Add* or the *Close* buttons of the *Channel Configuration* window) will **automatically** add the Keypress Event channel at the **bottom** of the Acquisition View window, below the video feed (Fig.6.10).
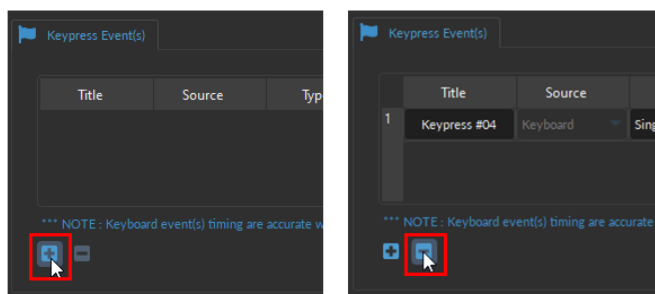
Figure 6.10: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 6.11) in the **Acquisition View**.
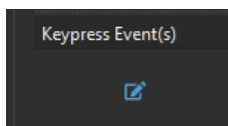
Figure 6.11: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 6.13:

1. The **Title** allows you to give a name for the Keypress event.

2. The **Source** is by default *Keyboard*.

3. Three **Types** of Keypress Event(s) can be specified with the drop-down list:

   - **Single** - Records single event at the touch of a key (Fig. 6.12a).
   - **Toggled** - Records the start and end of an event using the same key. First press denotes the start of the event while a second press denotes the end of it (Fig. 6.12b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 6.12c). Every keypress is a new event, with the start of the event occurring when the key was depressed.

(a) *Single*

(b) *Toggled*
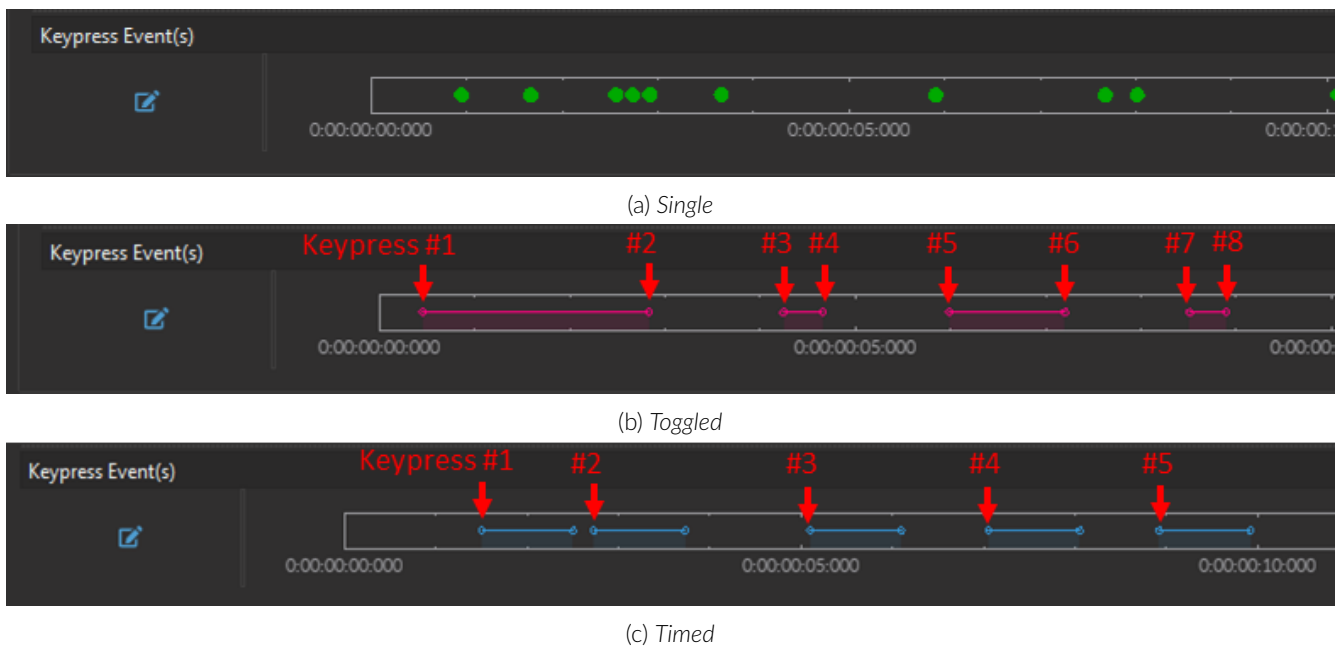
(c) *Timed*

Figure 6.12: *Three types of Keypress Event(s)*

4. The **Duration** is only used for the **Timed** Keypress type to specify the predetermined amount of time a Keypress Event will span. The duration is set in hh:mm:ss:zzz.

5. Select the **Color** field to open the **Select Color** window. Basic colors are provided, in addition to custom colors can be created and stored.

6. The **Shortcut Key(s)** can be any keyboard key, including space bar, enter, backspace, any letters, numbers, and special characters (*, !, ? etc.). To specify the key, click inside the *Shortcut Key(s)* cell, then press the keyboard key of choice. If a key was properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 6.13, column 6).

7. The **Information** column provides space to make notes or write a short description of the Keypress Event.
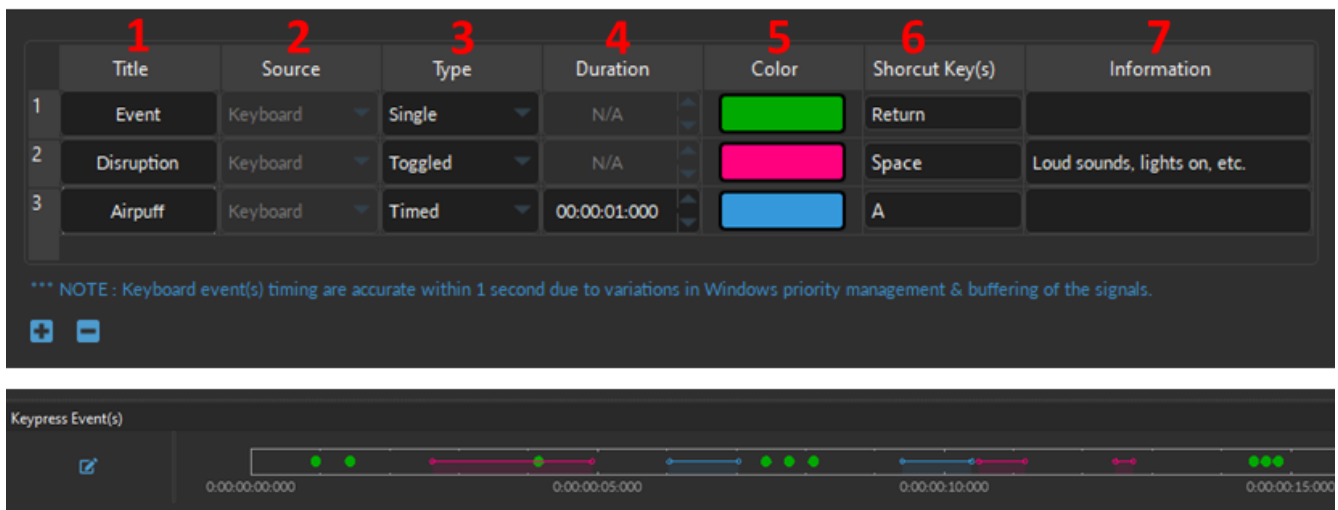
Figure 6.13: *Channel(s) configuration window, KeyPress Event(s)*

6.2 Integrating Camera into Acquisition Console module

To streamline data acquisition of fiber photometry experiments that combine behavior measurements, *Doric Lenses* offers a simple way to integrate either **Web Camera** or **Behavior Camera** modules into the **Acquisition Console** module.

There are several advantages of using this integration:

1. **Simultaneous view** of photometry signal and the video feed (Fig. 6.14, 1 & 2 respectively). Note that while this module can support multiple camera integration, it can only view one video feed at a time.

2. Video and photometry data are **saved within a single *.doric* file**, even when multiple Cameras Channels are used.

3. **Web Cameras** can be *Manually* synchronized with the photometry recording. (See Section 6.2.1, no.5 for synchronization limitations of this mode.)

4. A **Behavior Camera** that is run in the *External (Preconfigured)* mode will automatically create a Dig I/O channel to trigger the camera (Section 6.2.1, no. 5) with proper configurations, simplifying set-up.
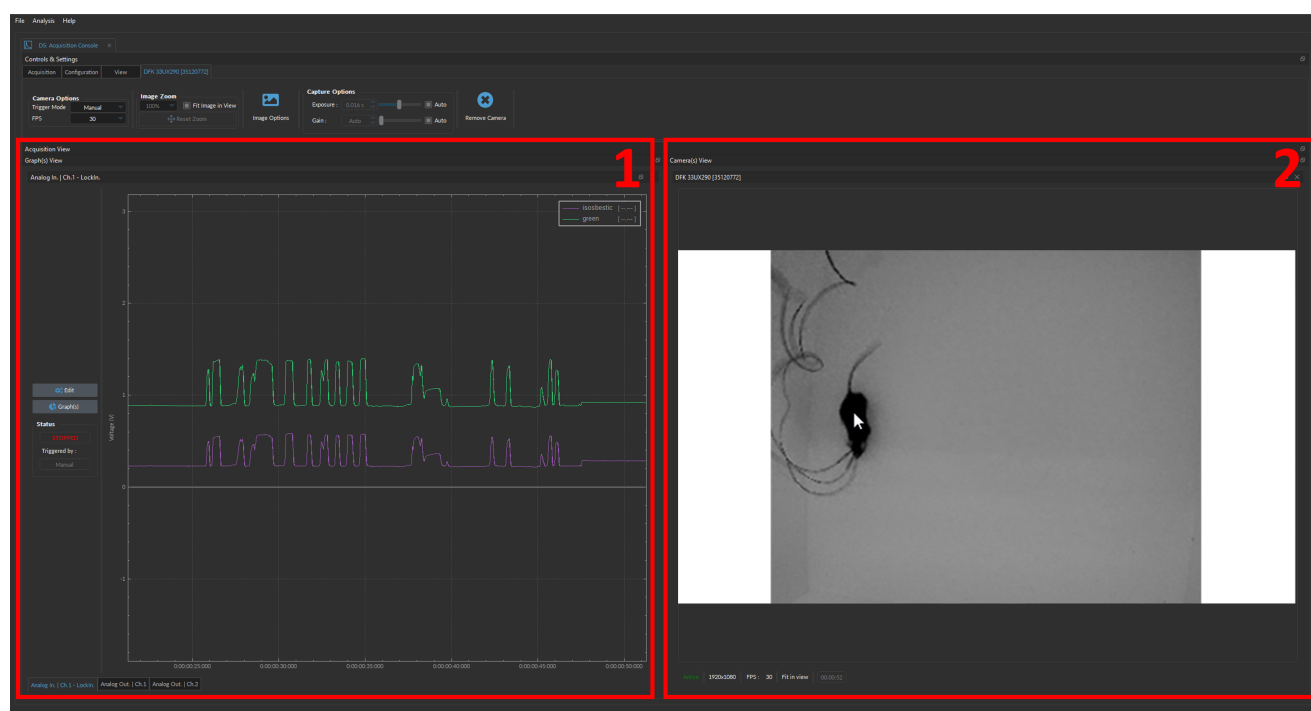
Figure 6.14: *Integrating Camera into Acquisition Console module*

6.2.1 Adding Camera Channel

To create a **Camera Channel** in the **Acquisition Console** module, select the *Add Channel* button, which can be found under the *Configuration* tab (Fig. 6.15). This will open the *Channel(s) Configuration* window (Fig. 6.16).
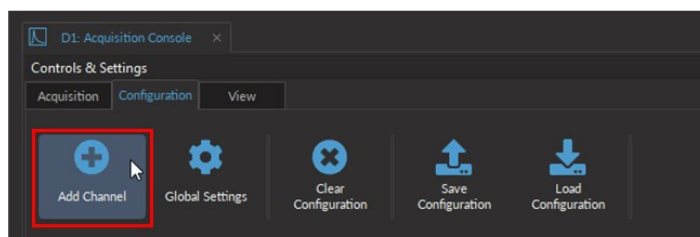
Figure 6.15: *Add Channel button opens the Channel Configuration window*
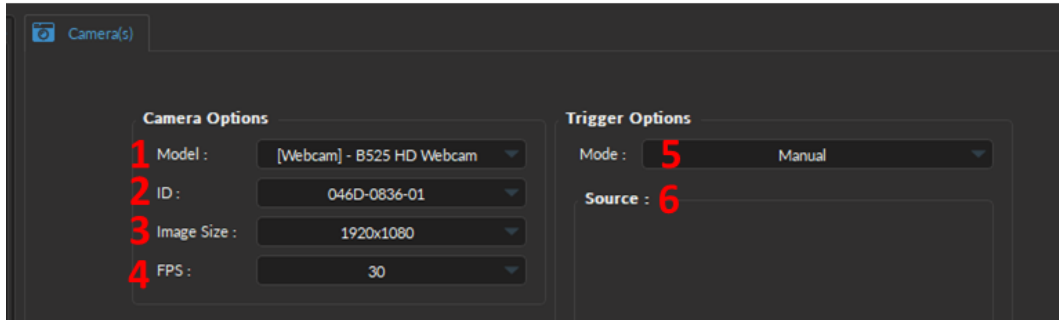
Figure 6.16: *Channel(s) configuration window, Camera Channel*

Camera Options:

1. The **Model** (Fig. 6.16, 1) allows you to select the camera of choice based on the type of camera.

2. **ID** drop-down list (Fig. 6.16, 2) selects a camera based on its unique ID. The ID is particularly useful if multiple cameras of the same model are required for the experiment.

3. The **Image Size** (Fig. 6.16, 3) sets the resolution of the image. The large the number of pixels used for width x height, the better the resolution. Currently, image size can range between 160x120 to 1920x1080 pixels.

4. The **FPS** (Fig. 6.16, 4) specifies the frame rate of the camera (i.e. the number of images displayed per second). FPS can be any value between 5 and 30 for web cameras and up to 60 FPS for the *Doric Behavior Camera*.

Trigger Options:

5. The **Mode** (Fig. 6.16, 5) sets the type of trigger that will control the camera. Depending on the type of camera, at most three modes are available:

WARNING:
If the camera isn't triggered by an external channel,
the **time synchronization is NOT guaranteed**.

- **Manual** - Selecting the *Live* or *Record* buttons located in the *Acquisition Tab* will the trigger the start of the camera recording. ***The time difference between the actual start time and when the first frame is received depends on the camera itself.*** Around a 1 second delay is pretty common for web cameras.

  The time delay (in ms) between the photometry and video data is recorded in the *DifferenceMasterStart-ToFirstImage* attribute, located in *.doric* file under the **Web Camera ID** folder (Fig. 6.17). This attribute can be used to retroactively align the video and fiber photometry data during analysis.
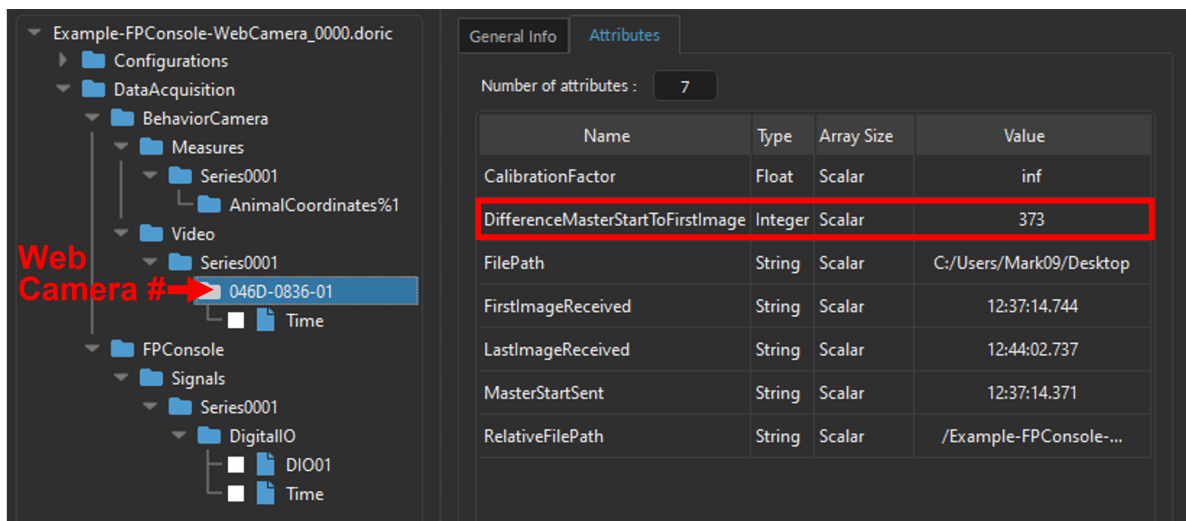
Figure 6.17: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **External** - Will drive the camera using external TTL signal through the trigger cable (Frequency: 30Hz (or camera FPS); Time ON: 5ms). This signal can come from any external device connected to the opposite end of the trigger cable. If using *Doric Neuroscience Studio* to synchronize the recording, use *External (Preconfigured)* mode below instead. ***ONLY offered for the *Doric Behavior Camera*.***

- **External (Preconfigured)** - This is the recommended mode to synchronize the camera with the rest of the Acquisition system. This mode automatically creates an additional Digital I/O channel configured to drive the camera at the proper frequency and Time ON. ***ONLY offered for the *Doric Behavior Camera*.***

6. The **Source** (Fig. 6.16, 6 & Fig. 6.18) is only used for the **External (Preconfigured)** mode and displays the **Digital I/O** channel with the preconfigured parameters that will be created at the same time as the **Camera Channel** (Fig. 6.18). For a detailed description of each Digital I/O parameter see the corresponding section in the Fiber Photometry System Manuel (Section 5.4.3). Briefly, key parameters include:

    a) The **Channel** (Fig. 6.18, a) corresponds to the physical Digital I/O channel number on the Console that is connected to the trigger cable of the *Doric Behavior Camera*.

    b) The **Mode** (Fig. 6.18, b) is by default set to the *Square (TTL)* which provides the external trigger signal to the camera. This parameter cannot be changed.

    c) The **Frequency** (Fig. 6.18, c) corresponds to the **FPS** set in the **Camera Options**. Changing the **FPS** will automatically change the **Frequency** in the **Sequence(s) Options**.

    d) The **Duty Cycle** (Fig. 6.18, d) is by default 50%. The frame will be taken at the start of each square pulse.
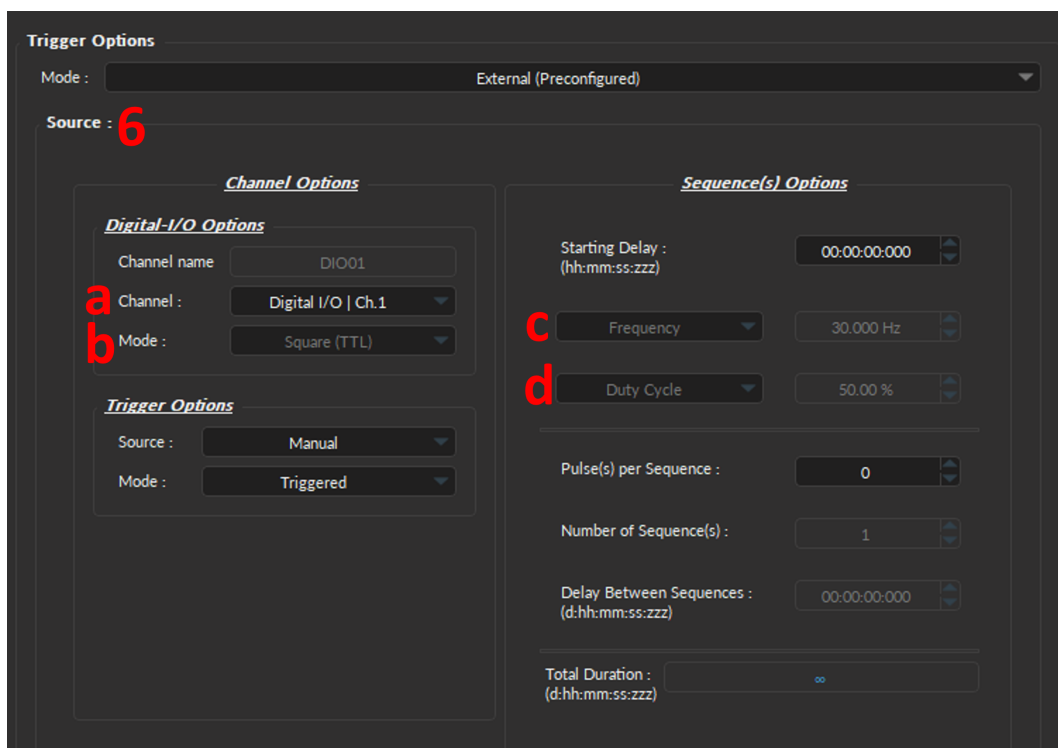
Figure 6.18: *Camera Channel - External (Preconfigured) mode*

6.2.2 Camera Control & Settings within Acquisition Console

Most of the Camera **Control & Settings** parameters (from Section 6.1) of the individual **Web Camera** and **Behavior Camera** modules are also integrated within the **Control & Settings** tabs of the **Acquisition Console** module.
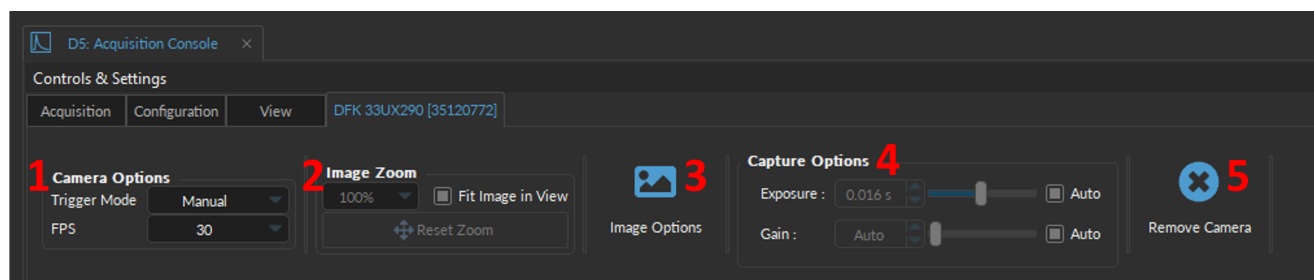
(a) *Camera Tab*
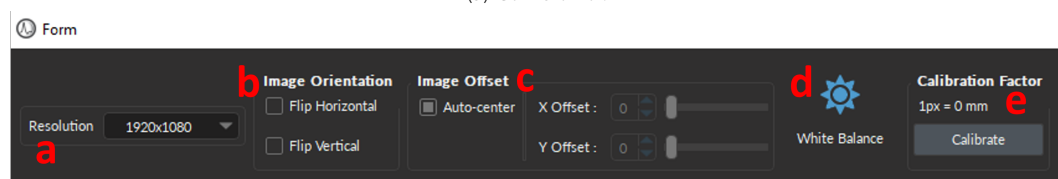
(b) *Image Options window*

Figure 6.19: *Acquisition Console, Control & Settings*

While controls common between camera and fiber photometry modules remain the same (such as Acquisition Tab's Live, Record, etc. are located in the same tab), camera-specific parameters can be found in the CAMERA-NAME tab (Fig. 6.19a). If multiple **Camera Channels** were created, each camera would have its own tab, with a unique camera name.

For a detailed description of these parameters, see the equivalent parameter description from the **Web Camera** and **Behavior Camera** modules in the following sections:

1. The **Camera Options** (Fig. 6.19a, 1) - Section 6.1.2, no. 3 & 4.

2. The **Image Zoom** (Fig. 6.19a, 2) - Section 6.1.3, no. 1.

3. The **Image Options** (Fig. 6.19a, 3) - Opens the **Image Options** window (Fig. 6.19b), which contains the following:

   a) The **Resolution** (Fig. 6.19b, a) - Section 6.1.2, no. 2.
   b) The **Image Orientation** (Fig. 6.19b, b) - Section 6.1.3, no. 3.
   c) The **Image Offset** (Fig. 6.19b, c) - Section 6.1.3, no. 2.
   d) The **White Balance** (Fig. 6.19b, d) - Section 6.1.2, no. 6
   e) The **Calibration Factor** (Fig. 6.19b, e) - Section 6.1.3, no. 4.

4. The **Capture Options** (Fig. 6.19a, 4) - Section 6.1.2, no. 5.

5. The **Remove Camera** button (Fig. 6.19a, 5) closes the camera view & tab integrated within the **Acquisition Console** module. If multiple cameras are integrated within this module, this button will only close the camera of that current tab.

Microscope

The **Microscope Driver** module of the Doric Neuroscience Studio provides an interface to control our *Fluorescence Microscope Driver*. This module enables image acquisition and its export in 16 bit .tif or in .doric (hdf5-based) files.

7.1 Device Selection Window

Once *Doric Neuroscience Studio* is opened, the *Device Selection* window should automatically pop up, if the device is turned ON and properly connected to the computer with USB port (as in Fig. 7.1).

To add a device to the studio, select the device of choice in the *Available device(s)* sections (bottom half of window), then click. If the device in question does not show up, double-check that it is indeed turned ON and the two ends of the USB cord are properly connected within the USB port. Then click *Refresh*. When properly connected to the system, the device will appear in the *Connected/Opened device(s)* section of the Window (see the green check-mark in Fig. 7.1).
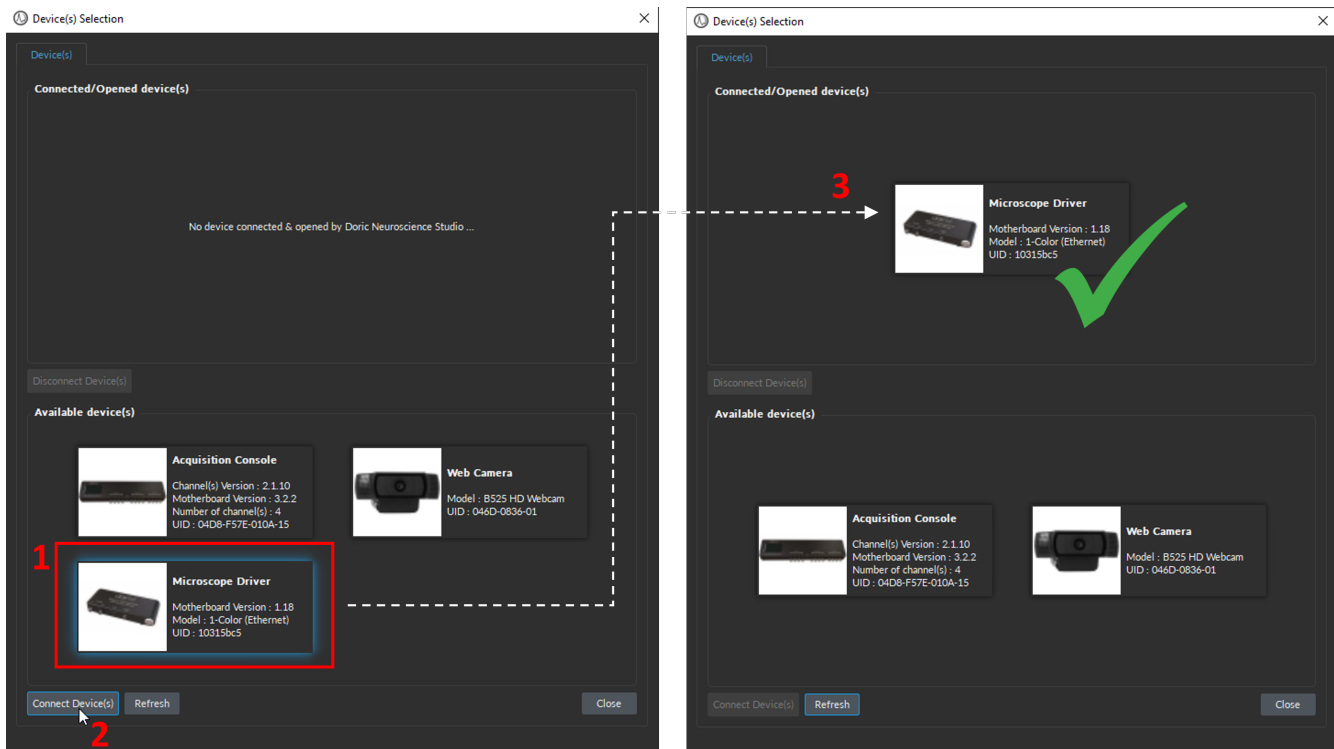
Figure 7.1: *Double click on the device of choice to connect it to DNS*

NOTE: If you have switched to DNS v6, older devices will require a firmware update to be recognized by the new version of the software. This update can be easily done using *Doric Maintenance Tools (DMT)* application and must be done one by one for each device. Further instructions can be found **HERE**.

Manually opening the *Device(s) Selection* window:
To manually open the *Device(s) Selection* window, select the *File*, then *Device Selection* (as per Fig. 7.2) or use the hot key: *Ctrl+N*.
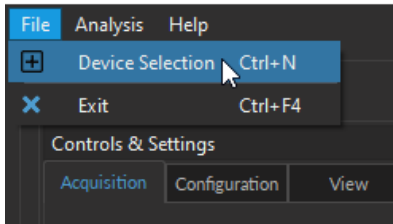
Figure 7.2: *Open Device Selection Window*

Mask Required Warning If the following warning message pops up (as in Fig. 7.3), see Section 7.5. Note that only *2-color fluorescence microscope* and the *eFocus fluorescence microscope* required manual Mask loading.
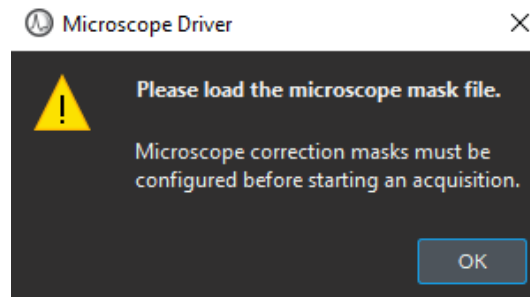
Figure 7.3: *Install Mask warning message*

7.2 Overview

The **Microscope Driver** interface (Fig 7.4) of *Doric Neuroscience Studio* software is split into two sections: **(1) Control and settings tabs** (Section 7.3) are used to manage different elements of the software (Acquisition, Configuration, and View); and (2) the **Acquisition view** (Section 7.4) displays visualization of the **Microscope View** (Section 7.4.1) and the **ROIs View** (Section 7.4.2).
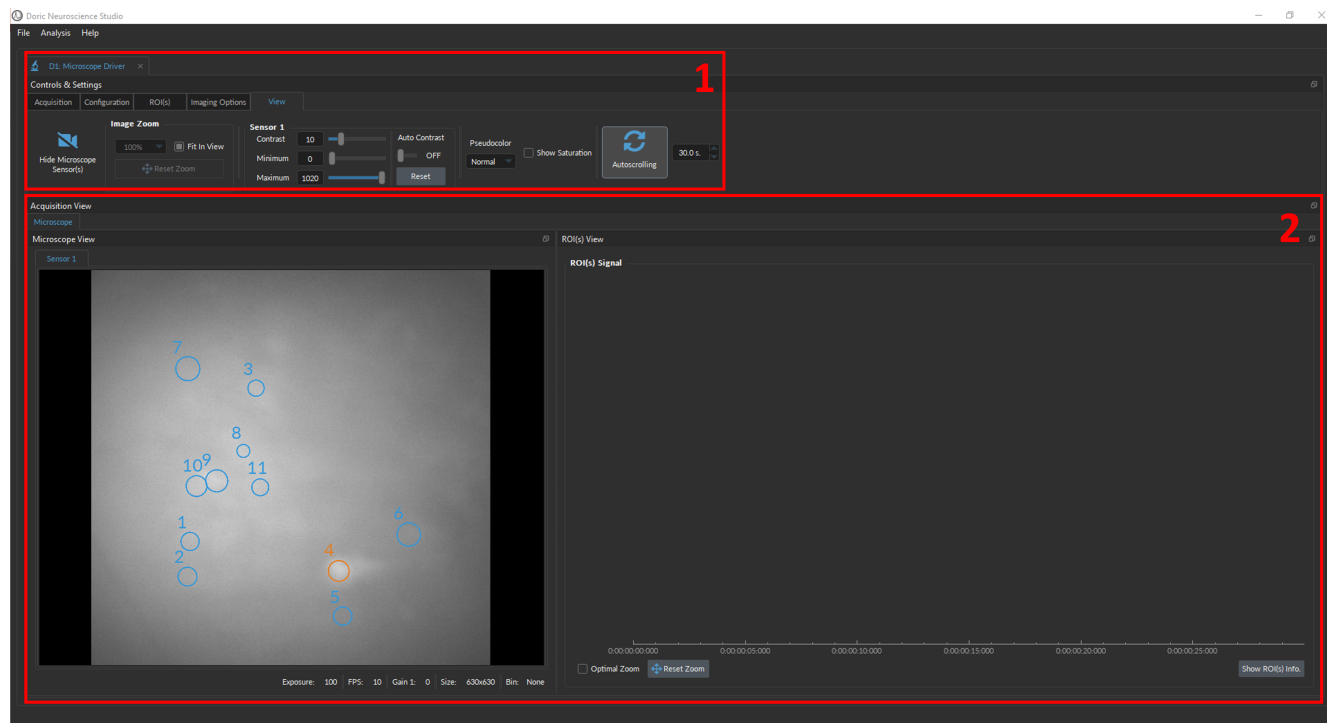
Figure 7.4: *DNS user interface*

7.3 Control and Settings tabs

The **Control and settings** are used to manage the different parts of the software and are split into five tab and are treated in the following sections:

1. *Acquisition* - Section 7.3.1

2. *Configuration* - Section 7.3.2

3. *ROIs* - Section 7.3.3

4. *Imaging Options* - Section 7.3.4

5. *View* - Section 7.3.5

7.3.1 Acquisition Tab

The **Acquisition** tab is used to start a live/recording session and set the saving parameters.

1. The **Live** button (Fig. 7.5, 1) activates all prepared channels. This mode does not save data, keeping only the most recent 700 000 data points in memory. This mode is not recommended for long or critical measurement sequences. **Live** mode is useful to quickly test the recording software and to ensure that the parameters were properly set and that the recording is working as intended.

2. The **Record** button (Fig. 7.5, 2) activates all prepared channels while periodically saving recorded data to the disk. This mode is recommended for long measurement sequences.
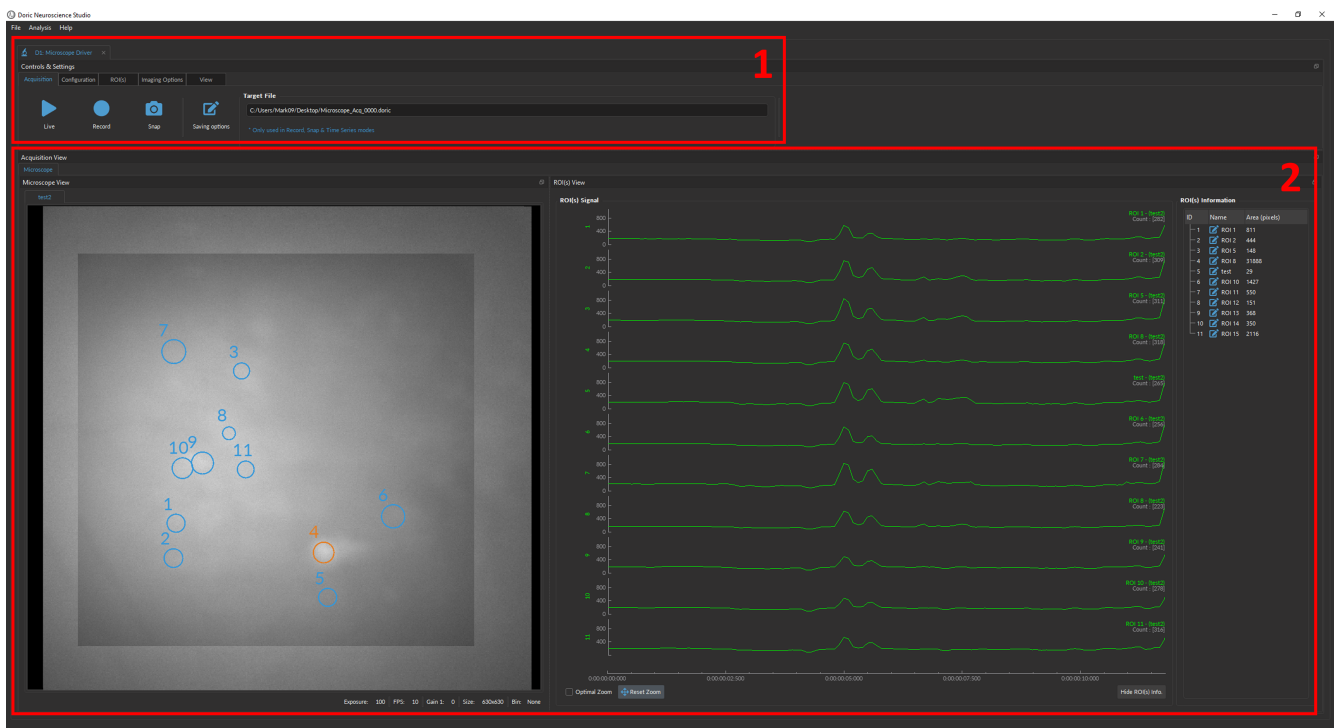
Figure 7.5: *Acquisition Tab*

3. The **Snap** button (Fig. 7.5, 3) will take a picture and automatically open a window where users can save the image in a variety of available file formats (including .bmp, .jpeg, .tiff, among many more).

4. The **Saving Options** (Fig. 7.5, 4) button opens the **Saving Parameters** window (Fig. 7.6). See section 7.3.1.1 for greater details.

5. The **Target File** (Fig. 7.5, 5) displays the path and file name where the data will be stored once the **Record** button is selected. Select the **Saving Options** button to change the path and file name.

7.3.1.1 Saving Parameters

The **Saving Parameter** window is used to define how and where the file is saved. This window is opened by selecting the **Saving Options** button in the Acquisition Tab (Fig. 7.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 7.6, 1).

2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 7.6, 2).

3. The **File format** (Fig. 7.6, 3) is **.doric**, an HDF5-based format that supports metadata (signal, video, images, tables, parameters, etc.). Version 6 of *Doric Neuroscience Studio* is no longer compatible with other file formats (.csv, .excel, or .tiff). We provide Matlab, Python, and Octave codes to read **.doric** files HERE. While not recommended, it is possible to export a *.doric* file into .csv format through the **Doric File Reader** module.

4. The **File Index** (Fig. 7.6, 4) box is used to define the current indexation number used for multiple files saved during the same measurement session. The suffix is incremented automatically when recording multiple files.

5. The **Target File** (Fig. 7.6, 5) displays the absolute path and filename where the data will be saved.

6. The **Saving Choices** check-box (Fig. 7.6, 6) when enabled will save the ROIs fluorescent fluctuations within the *.doric* file in addition to the raw ImageStack, as per Fig. 7.7. By default the ROI(s) Data will be included.

Figure 7.6: *Saving Options Window*

(a) *Without ROI(s) Data*

(b) *Including ROI(s) Data*

Figure 7.7: *Saving Choices can include or exclude ROI(s) Data within data file*

7.3.2 Configuration Tab

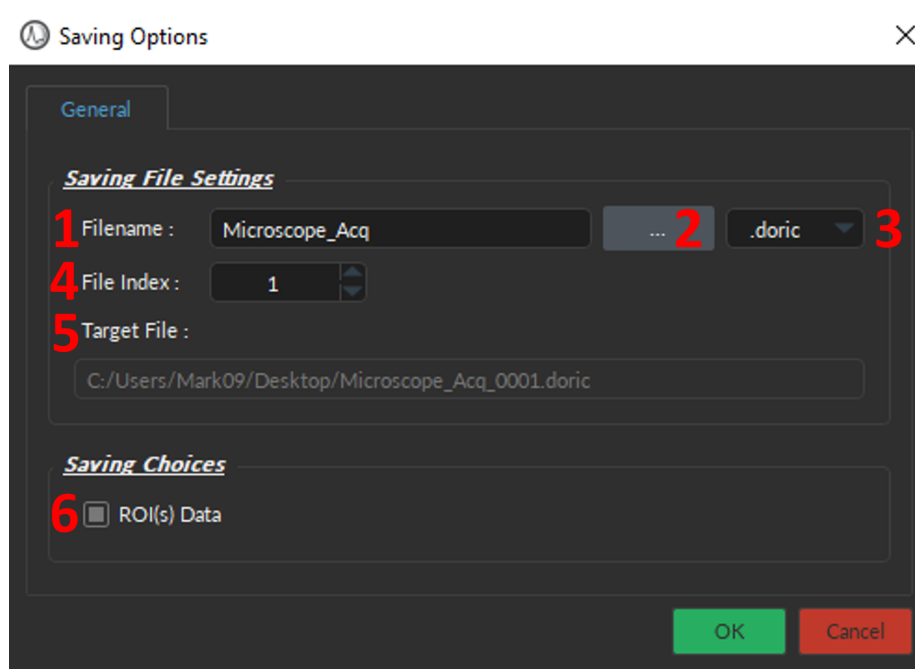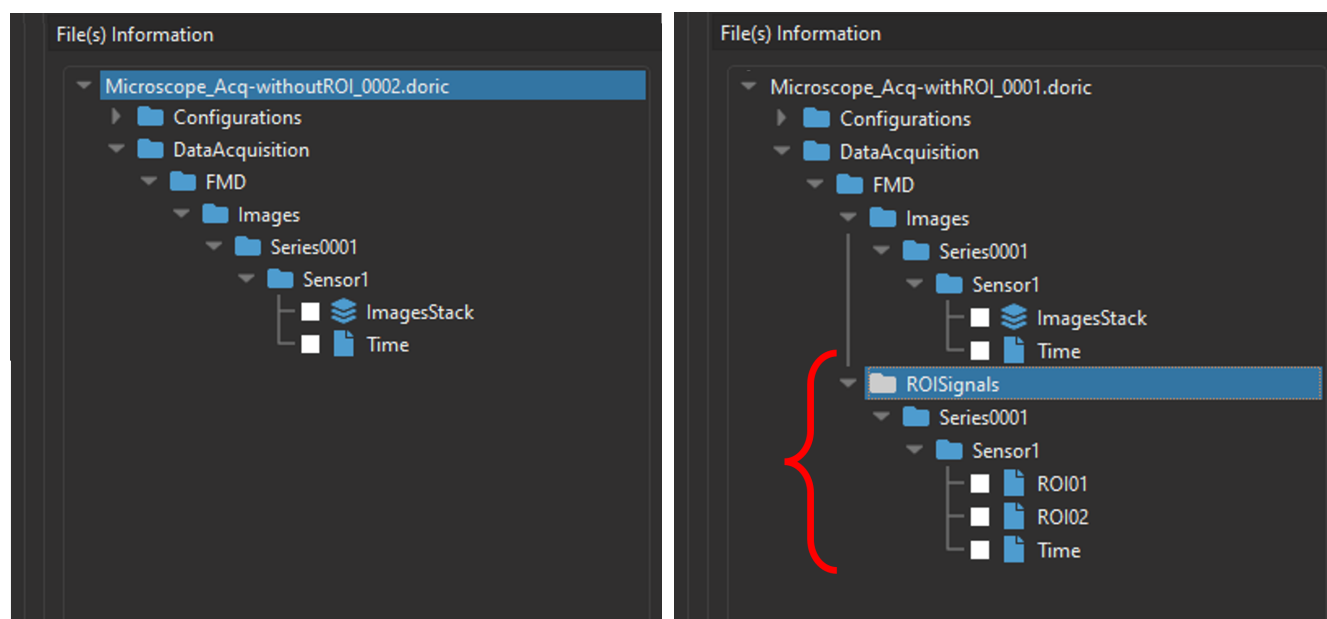The **Configuration** tab is used to set global parameter such as Master trigger options, as well as save and load the preset *Microscope Driver* configurations.
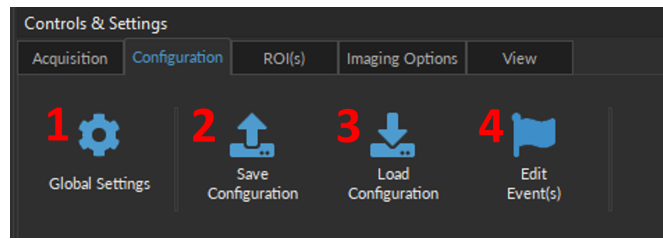
Figure 7.8: *Configuration Tab*

1. The **Global Settings** (Fig. 7.8, 1) opens the **Global Options** window in Fig. 7.9, where user can specify the master start options and Trigger Out options. See Sections 7.3.2.1 for more details.

2. The **Save configuration** button (Fig. 7.8, 2) allows a console configuration to be saved in the *.doric* format. This file preserves the current channel configuration/parameters, the Acquisition View window organization, and any custom trace colors and names. If ROI(s) were created within the **Microscope View**, these will also be saved within the same .doric configuration file.

3. The **Load configuration** button (Fig. 7.8, 3) imports a previoulsy configured *.doric* file into the module.

4. The **Edit Event(s)** button (Fig. 7.8, 4) opens the **Keypress Event(s)** window (Fig. 7.14), which allows users to flag behavior events or experimental disruption at the press of a keyboard key. See Section 7.3.2.3.

7.3.2.1   Global Settings

Through the **Global Settings**, user can specify the **Master Trigger Options** that will start recordings.
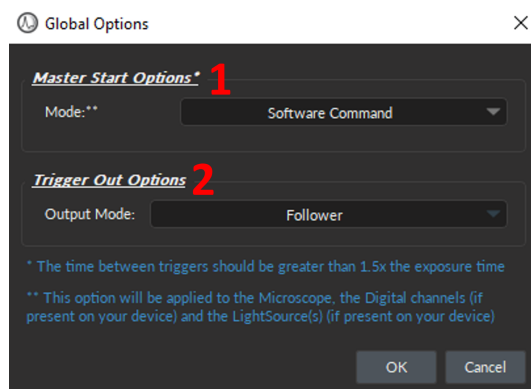
Figure 7.9: *Global Options Window*

1. The **Master Start Options** (Fig. 7.9, 1) of the sets the origin (internal, external or time-series) of the trigger that will start the recording session and synchronize all the external and internal devices. Four options are available for different use cases:

   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 7.5, 2).
   - *Triggered* - A n number of images will be acquired when a TTL signal is received from the IN trigger port of the *Microscope Driver*. This number is specified by the value associated with **Images per Trigger** parameter that only appears for this mode. ***This mode still requires that the Record button is selected BEFORE the TTL trigger signal is received.***
   - *Gated* - The recording session starts when a high TTL signal (>4 V) is received from the IN trigger port of the *Microscope Driver* and will stop when a low TTL signal (<0.4 V) is detected. Thus, the **Gated** mode controls both the START and the END signals of the recording session. ***This mode still requires that the Record button is selected BEFORE the TTL trigger signal is received.***

- *Timeseries* - This mode allows users to record pre-defined series over longer periods of time (that can span several days) (Fig 13.9a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 7.10) pops up. See section 7.3.2.2 for more details.

2. The **Trigger Out Options** will output a TTL train from the OUT Trigger port on the *Microscope Driver*. There are two available output modes:

- *Follower* - Will output a signal that is continuously ON as during the entirety of the recording.

- *With Each Frame* - Will output a TTL signal at every time point when a image is captured.

7.3.2.2   Time Series

The **Time Series** Window (Fig 7.10) can be opened by clicking on the **Record** button (Fig. 7.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 7.9, 2). Every **Time series** sequence is automatically saved to the *.doric* file defined in **Saving Options** (Section 7.3.1.1).

(a) *Time Series Acquisition Timing Diagram*

(b) *Time Series Window*
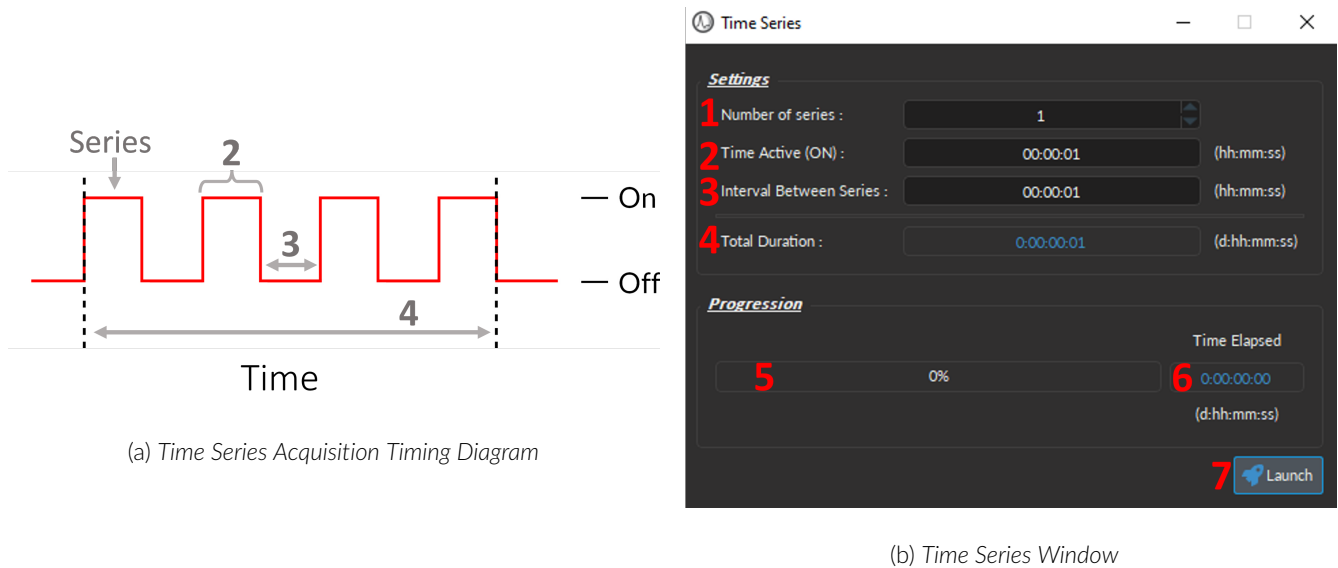
Figure 7.10: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 7.10) sets the following parameters:

1. The **Number of series** (Fig. 7.10, 1) defines the amount of times the series is repeated.

2. The **Time Active (ON)** (Fig. 7.10, 2) defines the duration of the series.

3. The **Interval Between Series** (Fig. 7.10-3) defines the amount of time between each series, if the **Number of series** is greater than 1.

4. The **Total Duration** (Fig. 7.10, 4) displays the total amount of time that the timeseries recording will take place.

5. The **Progression bar** (Fig. 7.10, 5) indicates the progression of the timeseries (in %).

6. The **Time Elapsed** (Fig. 7.10-6) counter indicates the amount of time that has already passed in d:hh:mm:ss.

7. The **Launch** (Fig. 7.10, 7) 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.

7.3.2.3 KeyPress Event(s)

Keypress Event(s) are ideal when manually labelling or annotating events during experiments. Specifically, selecting any keyboard key during a recording will save the output synchronized to other measurements. Keypress events can be used to:

- Flag disruptions during the experiment, such as lights on, door opened, construction noise, etc.

- Record experimentally relevant events/stimuli, such as airpuff, licks or any other behavior.

> **WARNING:**
> Keyboard event(s) timing are **accurate within 1 second** due to variations in Windows priority management and buffering of the signals.

7.3.2.4 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the botton of the window (Fig. 7.11, left). To remove a KeyPress, use - button (Fig. 7.11, right).

- **NOTE:** Selecting the + button (without clicking the *Add* button or the *Close* of the *Channel Configuration* window) will **automatically** add the Keypress Event channel at the **bottom** of the Acquisition View window, below the video feed (Fig.7.11).
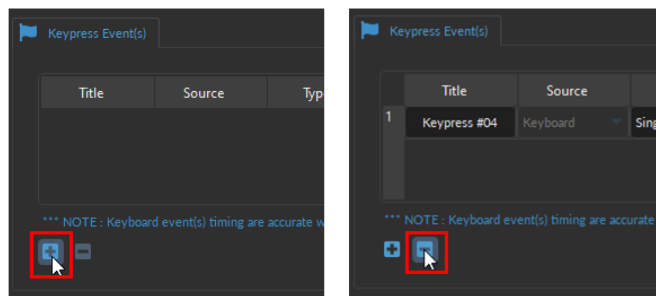
Figure 7.11: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 7.12) in the **Acquisition View**.
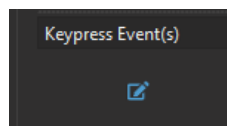
Figure 7.12: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 7.14:

1. The **Title** allows you to give a name for the Keypress event.

2. The **Source** is by default *Keyboard*.

3. Three **Types** of Keypress Event(s) can be specified with the drop-down list:

   - **Single** - Records single event at the touch of a key (Fig. 7.13a).
   - **Toggled** - Records the start and end of an event using the same key. First press denotes the start of the event while a second press denotes the end of it (Fig. 7.13b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 7.13c). Every keypress is a new event, with the start of the event occuring when the key was depressed.

(a) *Single*

(b) *Toggled*
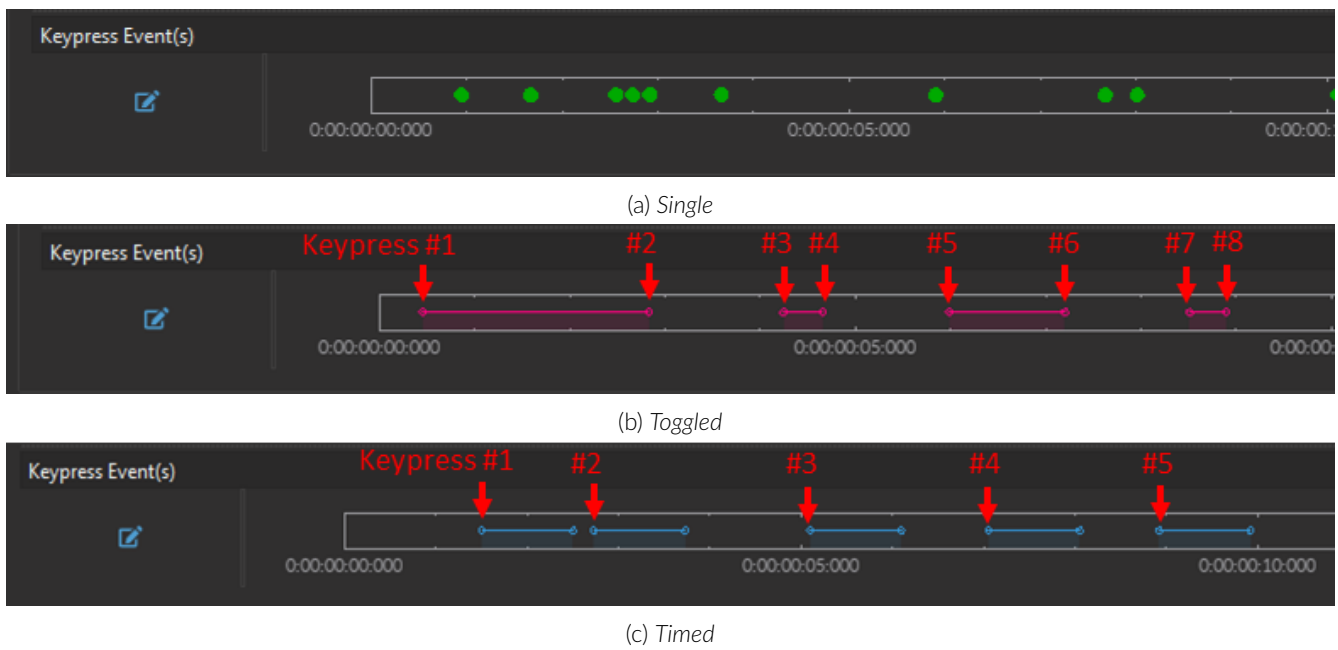
(c) *Timed*

Figure 7.13: *Three types of Keypress Event(s)*

4. The **Duration** is only used for the **Timed** Keypress type to specify the predetermined amount of time a Keypress Event will span. The duration is set in hh:mm:ss:zzz.

5. Select the **Color** field to open the **Select Color** window. Basic colors are provided, in addition to custom colors can be created and stored.

6. The **Shortcut Key(s)** can be any keyboard key, including space bar, enter, backspace, any letters, number and special characters (*, !, ? etc.). To specify the key, click inside the *Shortcut Key(s)* cell, then press the keyboard key of choice. If a key was properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 7.14, column 6).

7. The **Information** column provides space to make notes or write a short description of the Keypress Event.
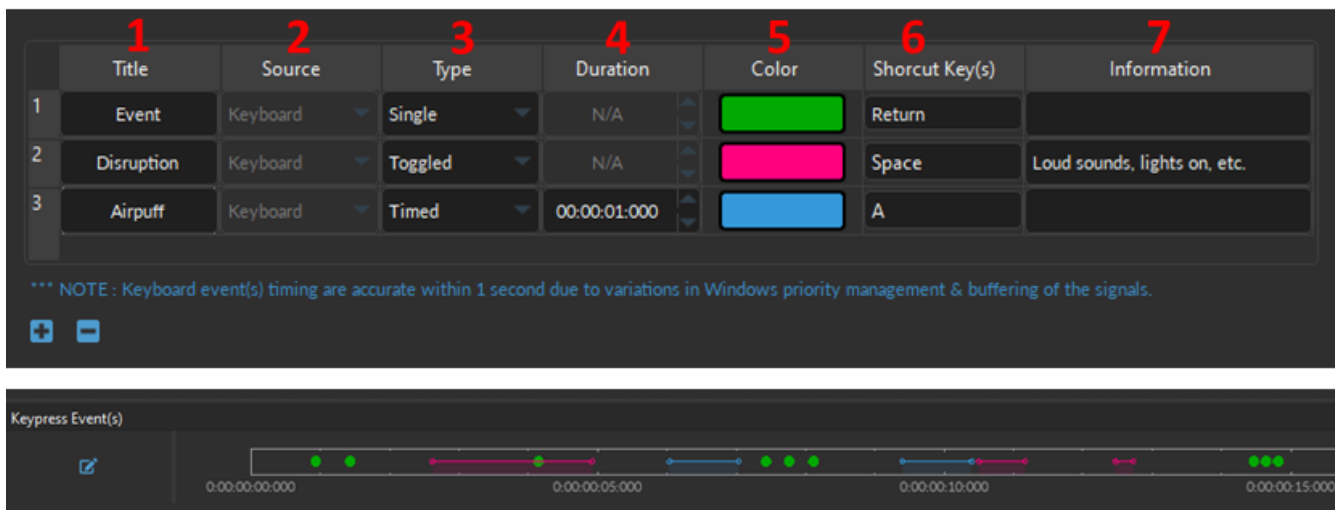
Figure 7.14: *Channel(s) configuration window, KeyPress Event(s)*

7.3.3 ROIs Tab

The **ROI(s)** tab includes all features related to the region of interests (ROI(s)), including:
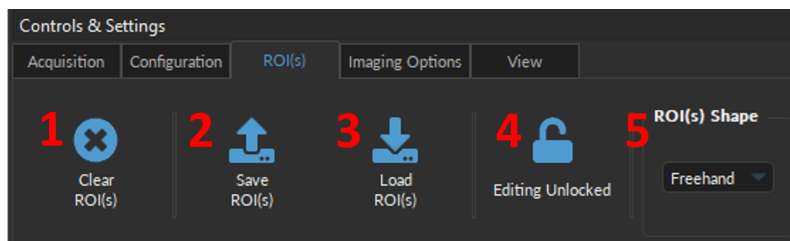
Figure 7.15: *ROIs Tab*

1. The **Clear ROI(s)** button (Fig. 7.15, 1) will delete all drawn regions on interest within the **Microscope View**. Note that unless the ROI(s) were previously saved, these ROI(s) cannot be recuperated.

2. The **Save ROI(s)** button (Fig. 7.15, 2) will save the region of interests drawn in the **Microscope View** in a *.doric* file, so that the identical ROI can be re-imported into the module at a later time. At least one ROI must be drawn for this feature to work.

3. The **Load ROI(s)** button (Fig. 7.15, 3) will import a previously saved .doric file. Note that this ROI(s) configuration can be edited, but must be re-saved in order for changes to be conserved.

4. The **Editing Unlocked** button (Fig. 7.15, 4) when enabled will prevent new ROI(s) from being drawn. However, it does not prevent moving or reshaping a selected ROI.

5. The **ROI(s) Shape** drop-down (Fig. 7.15, 5) sets the geometry of the ROI, which can be added at any point, even when not under the **ROI(s) tab** and in **Live** mode (but not when using **Record** mode). Four **ROI(s) Shapes** are available: *Freehand*, *Circle*, *Rectangle*, and *Square* (Fig. 7.16, 1-4). Multiple different shapes can be used within a single **Sensor View**.

Figure 7.16: *ROI(s) Shape*

Note: In order to use draw ROI(s), a frame must be loaded into the **Microscope View**, which can be done using either **Snap**, **Live** or **Record** buttons (Fig. 7.5, 1-3).

7.3.4 Imaging Options Tab

Figure 7.17: *Imaging Options Tab*

1. The **Exposure (ms)** textbox (Fig. 7.17, 1) specifies the length of time that the microscope sensor collects light from the sample. There are trade-offs between exposure time, image brightness, and phototoxicity.

2. The **Sensor 1** (Fig. 7.17, 2)

   - *Sensor Name* text-box lets users rename the sensor with a more intuitive/experiment-specific label.
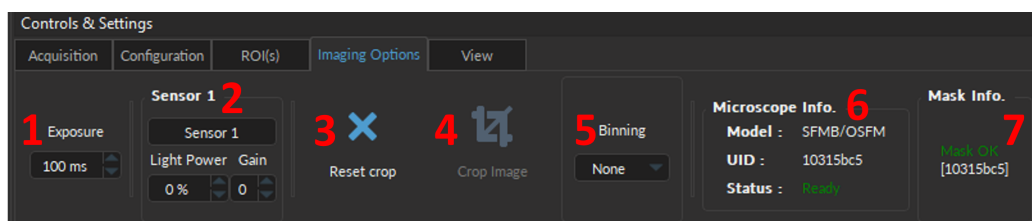   - *Light Power* text-box defines the power emitted by the excitation light source. The light sources will be activated when the image acquisition is started. The maximum optical power (in mW) depends on the light source model.
   - *Gain* text-box corresponds to the relative amplification measure applied to the sensor. Note that increasing the gain will simultaneously increase both the signal and noise. Three options of gain (0-2) are available.
   - *Light Source* drop-down list - ONLY AVAILABLE for *2-color fluorescence microscope* and the *eFocus fluorescence microscope* (Fig. 7.26, Sensor 1 & 2). This allows users to set the excitation source, which can be either **LED** or **LISER**.

3. The **Rest Crop** button (Fig. 7.17, 3) will remove any crop applied to the image and reset the image size to its maximal value. The change will only appear when a new **Capture** sequence is activated.

4. The **Crop Image** button (Fig. 7.17, 4) will chop unwanted section of the **Microscope View** by drawing a square on the video feed. When a new **Capture** sequence is activated, only the cropped region will be captured. Note that the image size will automatically be adjusted to the new resolution.

5. The **Binning** drop-down (Fig. 7.17, 5) averages the pixels values based on the binning value selected: either none, 2x2 or 4x4 pixel squares will be average together. This reduces the number of pixels for smaller save file sizes. Note that **Size** of the frame will be automatically adjusted based on the binning:

   - *None* - unchanged image size
   - *2 x 2* - update size by factor of 0.5 per dimension.
   - *4 x 4* - update size by factor of 0.25 per dimension.

6. The **Microscope Info.** (Fig. 7.17, 6)

   - *Model* - displays type of microscope currently connected to the software.
   - *UID* - displays the connected microscope's unique serial number.
   - *Status* - displays the

7. The **Mask Info.** (Fig. 7.17, 7) displays the which is used to calibrate the microscope image. For 1-colour microscopes (SFMB, eSFMB and eTFMB), the masque are automatically loaded when the **Microscope Driver** module is opened.

7.3.5   View Tab

The **View Tab** (Fig. 7.18) is used to modify the presentation of graphs in the **Acquisition view**.

Figure 7.18: *View Tab*

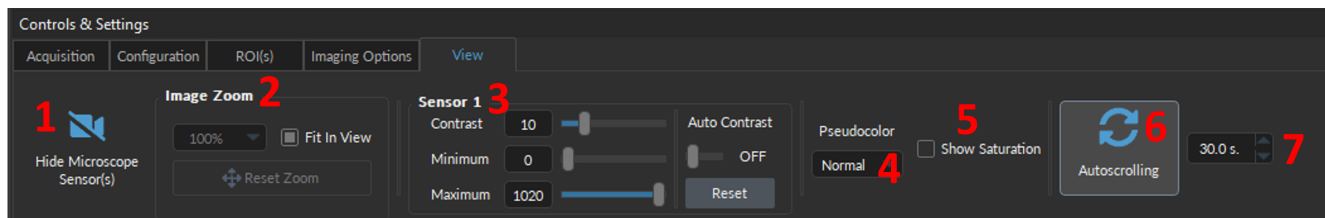The **View** parameters are as follows:

1. The **Hide Microscope Sensor(s)** button (Fig. 7.18, 1) will remove the **Microscope View** from the **Acquisition View**, automatically enlarging the **ROIs / Signal View**. Disabling **Hide Microscope Sensor(s)** (renamed **Show Camera Feed(s)**) will make the **Microscope View** reappear.

2. The **Image Zoom** (Fig. 7.18, 2) inlcudes the following:

- The **Zoom %** specifies the zoom factor for the image display, which ranges between 10%-500%.
- The **Fit In View** checkbox automatically adjusts the image to fit the entire **Microscope View**.
- The **Rest Zoom** button returns the zoom factor to 100%.

3. The **Sensor 1** (Fig. 7.18, 3) 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.

- The **Contrast** slider set the standard deviation of the pixel intensity, and thus is related to the difference between the highest and lowest intensity values of the image. The **Contrast** factor can range from 1 to 50.
- The **Minimum** slider sets the lowest pixel value cutoff. Should the minimum be above 0, all pixels with lower count will display a minimal value.
- The **Maximum** slider sets the largest pixel value cutoff. Should the Max be below 1018, all pixels with a higher count will appear saturated.
- The **Auto Contrast** (Fig. 7.18, 4) will active an automatic contrast adjustment algorithm, and will set the contrast to maximize the difference between the maximum and minimum values based on current values collected. The **Reset** button re-adjusts the contrast functions to their default settings, before the algorithm was enabled.

4. The **Pseudocolor** drop-down (Fig. 7.18, 5) maps the pixels values to a pseudocolor range (of 13 possibles options) to facilitate viewing.

5. The **Show Saturation** checkbox (Fig. 7.18, 6) is only available when using the **Auto Contrast** setting. When enabled, saturation pixels will turn red. This function is only available if no pseudocolor is selected.

6. The **Autoscrolling** button (Fig. 7.18, 7), when selected, automatically set the graphs to scroll as new data appears.

7. The **Zoom range** text-box (Fig. 7.18, 8) sets the graph zoom to the value of choice, specified in the text-box.

7.4   Acquisition View

The **Acquisition View** is split into two sections (Fig. 7.19):

1. The **Microscope View** (Fig. 7.19, 1) - Section 7.4.1.

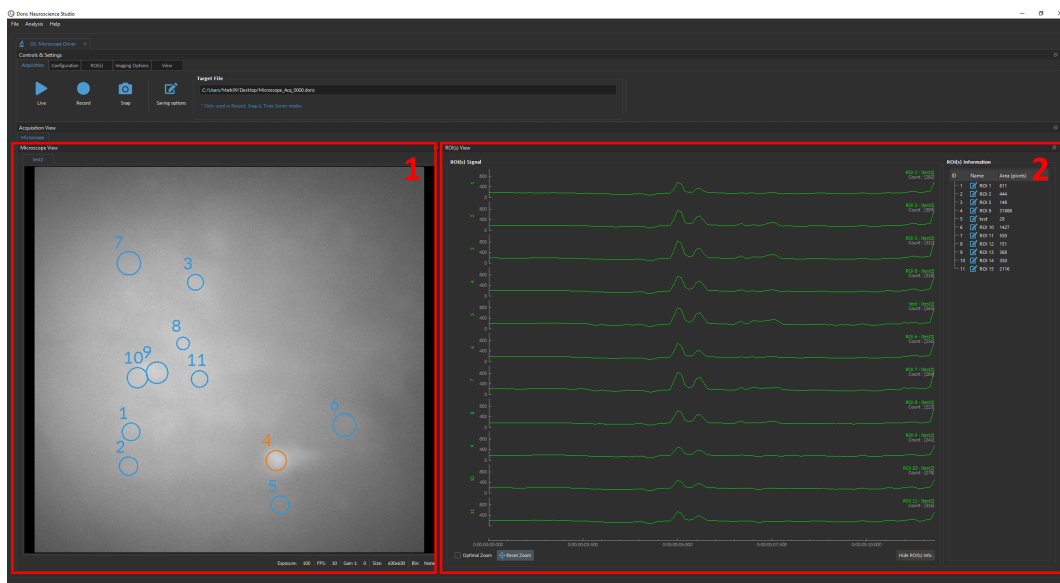2. The **ROI(s) View** (Fig. 7.19, 2) - Section 7.4.2.

Figure 7.19: *Acquisition View*

7.4.1 Microscope View

The **Microscope View** displays the live video feed from the microscope **Sensor(s)**. This view can also be split into two sections:

Figure 7.20: *Microscope View*

1. The **Sensor(s)** tab (Fig. 7.22, 1) - displays each sensor's live video feed, where the ROI(s) can be drawn, edited, or deleted. For multi-sensor microscopes, changing the tab allows you to see the image available to each sensor, and for some microscopes, you can also select the overlay. See the **ROI(s)**, the **Imaging Options**, and/or the **View** tabs to modify microscope parameters (Sections 7.15, 7.17, 7.18, respectively).

   To edit ROI(s) directly manipulate ROI(s) within the **Sensor** video feed:

   - **Draw ROI** - click the area within the **Sensor View** that will be assigned as a ROI and draw and outline around the area. To change the shape type see Section 7.3.3, no. 5.
   - **Select ROI** - click either the edge or within the ROI will select it. Proper selection will turn ROI orange instead of blue and automatically highlight the corresponding ROI in the **ROI(s) Information** tab (Fig. 7.25).
   - **Delete individual ROI** - Select a ROI (as detailed above) and press the **Delete** key on the Keyboard. To delete all ROIs, see Section 7.3.3, no. 1.
   - **Displace ROI** - Select the ROI and hove above the center of the ROI until a *Move* icon (Fig. 7.21a) appears. Click and drag the ROI to its new desired location.
   - **Resize ROI** - Select the ROI and hove above the orange trace of the ROI until a *Resize* icon (Fig. 7.21b) appears. Click and drag the ROI to reduce or enlarge the shape. *Resize* option is not available for the *Freehand* shape.
   - **Select multiple ROIs** - Press *Ctrl* while selecting a second+ ROI, such that each selected ROI turns orange (Fig. 7.21c). This selection allows multi-ROI deletion or displacement.
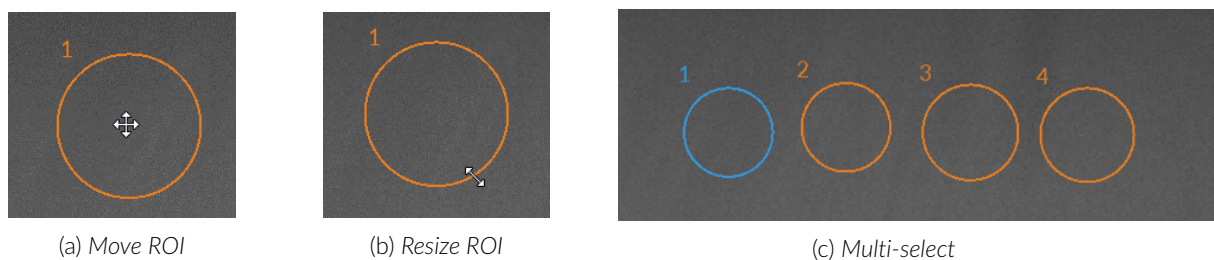
(a) *Move ROI*　　　　　　　(b) *Resize ROI*　　　　　　　(c) *Multi-select*

Figure 7.21: *Edit ROI(s)*

2. The **Microscope Monitoring Bar** (Fig. 7.22, 2) displays the parameters currently being used during the recording:
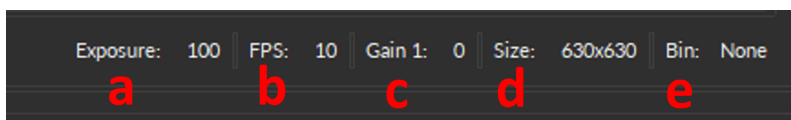
Figure 7.22: *Microscope Monitoring Bar*

a) The **Exposure** (Fig. 7.22, 1) displays the value specified in Section 7.3.4, no. 1.

b) The **FPS** (Fig. 7.22, 2) the number of frames per second of the device. The maximum attainable frame rate in frames per second (fps) cannot surpass 1 divided by the exposure time in seconds. Thus increasing or decreasing the exposure in Section 7.3.4, no. 1 will change the *FPS*.

c) The **Gain** (Fig. 7.22, 3) displays the value specified in Section 7.3.4, no. 2.

d) The **Size** (Fig. 7.22, 4) displays the image resolution. If the image is **Cropped** (Section 7.3.4, no. 4) this value will be automatically updated.

e) The **Bin** (Fig. 7.22, 5) displays the value (none, 2x2 or 4x4) specified in Section 7.3.4, no. 5.

7.4.2　ROI(s) View

The **ROI(s) View** displays the ROI traces calculated by averaging the pixel intensity value within each ROI. The following elements can be found in the **ROI(s) View**:

Figure 7.23: *ROI(s) View*

The following elements can be found in the **ROI(s) View**:

1. The **ROI(s) signal graph** (Fig. 7.23, 1) displays the raw signal trace for each ROI(s).

    a) The **ROI(s) ID** (Fig. 7.24, a) - specifies which ROI the signal graph belongs to. The graphs are displayed in order of ROI created.

    b) The **y-axis** (Fig. 7.24, b) represents the mean signal intensity of the ROI, which is unit-less.

    c) The **x-axis** (Fig. 7.24, c) represents the time in d:hh:mm:ss:zzz.

    d) The **Trace** (Fig. 7.24, d) is the curve of the signal, corresponding to fluctuations in pixel intensity, from which $\Delta F/F_0$ will be calculated.

    e) The **Legend** (Fig. 7.24, e)
       - *ROI label* - displays the ROI **Name** (specified within the **Name** column of **ROI(s) Infromation** tab; Fig., 7.25, b), followed by the **Sensor Name** in parenthesis (which can be specified in Fig. 7.17, 2).
       - *Counts* - displays the value of the last data point of the ROI trace (in average pixel intensity value).
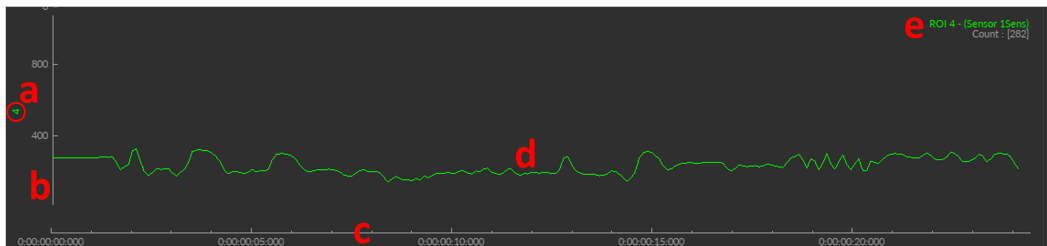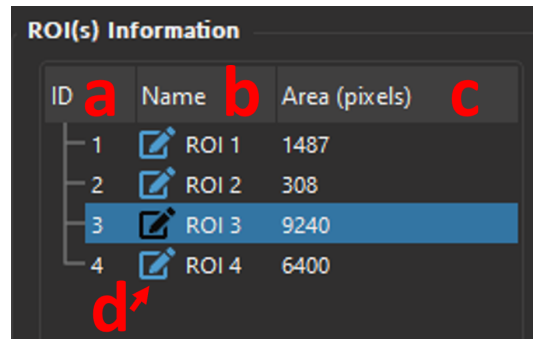

Figure 7.24: *ROI(s) View, graphs*

2. The **Optimal Zoom** checkbox (Fig. 7.23, 2) automatically adjusts the graph range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

3. The **Reset Zoom** button (Fig. 7.23, 3) readjusts the graph zoom to the value specified in the zoom range text-box.

4. The **Show ROI(s) Info.** button (Fig. 7.23, 4) opens or closes the **ROI(s) Information** Tab in Fig. 7.23, 5.

5. **ROI(s) Information** Tab (Fig. 7.23, 5) displays a table with ROI basic data, including:

   a) *ID* (Fig. 7.25, a) displays the number associated with ROI.

   b) *Name* (Fig. 7.25, b) displays the label associated with the ROI. Double-click on the text-box to rename the ROI.

   c) *Area* (Fig. 7.25, c) displays the number of pixels that make-up that fill the perimeter of the ROI.

   d) *Edit* button (Fig. 7.25, d) will highlight in orange the corresponding ROI in the **Microscope View**. To edit or delete the selected ROI, see section 7.4.1, no. 1.

Figure 7.25: *ROI(s) View, ROI(s) information*

7.5   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 *Doric 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 every time *Doric Neuroscience Studio* is installed on a different computer.

2. Open the software and connect the *Microscope Driver*. Then navigate to the **Imaging Options** tab and click **Select Mask File** (Fig. 7.26). This opens a file selection window. Travel to the location of the mask file, select it and click **OK**.
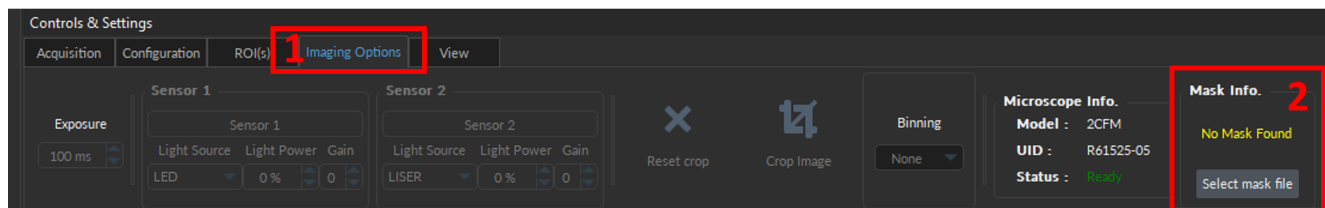
Figure 7.26: *Add Mask in the Imaging Options tab*

3. Once the file is selected, the following message will be displayed in the **Mask Info.** box above the **Select Mask File** button: **Loading Masks** and then **Mask OK** (Fig. 7.27). This is replaced by **Masks Loaded** once loading is complete.
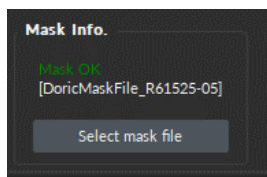
Figure 7.27: *Mask correctly added*

4. With the masks installed, the microscope is ready for use.

Fiberless system

The Doric Neuroscience Studio's Fiberless and Wireless *Electrophysiology Console* module takes care of pairing the headstage to the Console, collecting electrophysiological data and synchronizes of electrophysiology with optogenetics, behaviour and other measurements. Through its intuitive user interface, scientists can configure channels with great flexibility, fitting a wide range of experimental setups.
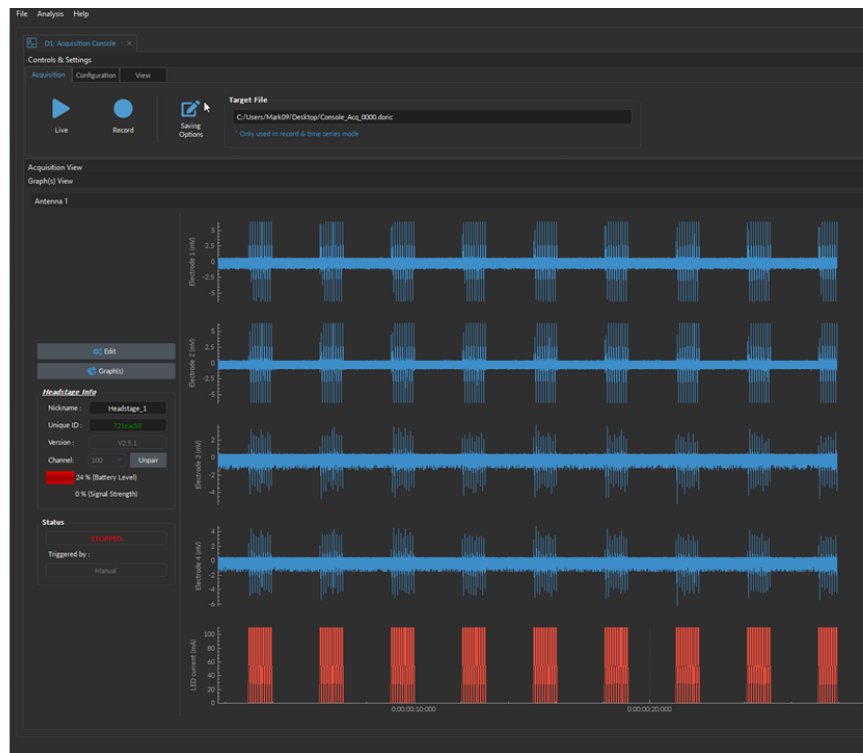
Figure 8.1: *Electrophysiology console user interface*

8.1   Connecting devices to DNS

Once *Doric Neuroscience Studio* is opened, the *Device Selection* window should automatically pop up, if the device is turned ON and properly connected to the computer with USB port (as in Fig. 8.2).

To add a device to the studio, **double click** on the device of choice in the *Available device(s)* sections (bottom half of window). If the device in question does not show up, double check that it is indeed turned ON and the two ends of the USB cord are properly connected within the USB port. Then click *Refresh*. When properly connected to the system, the device will appear in the *Connected/Opened device(s)* section of the Window (see the green check mark in Fig. 8.2).
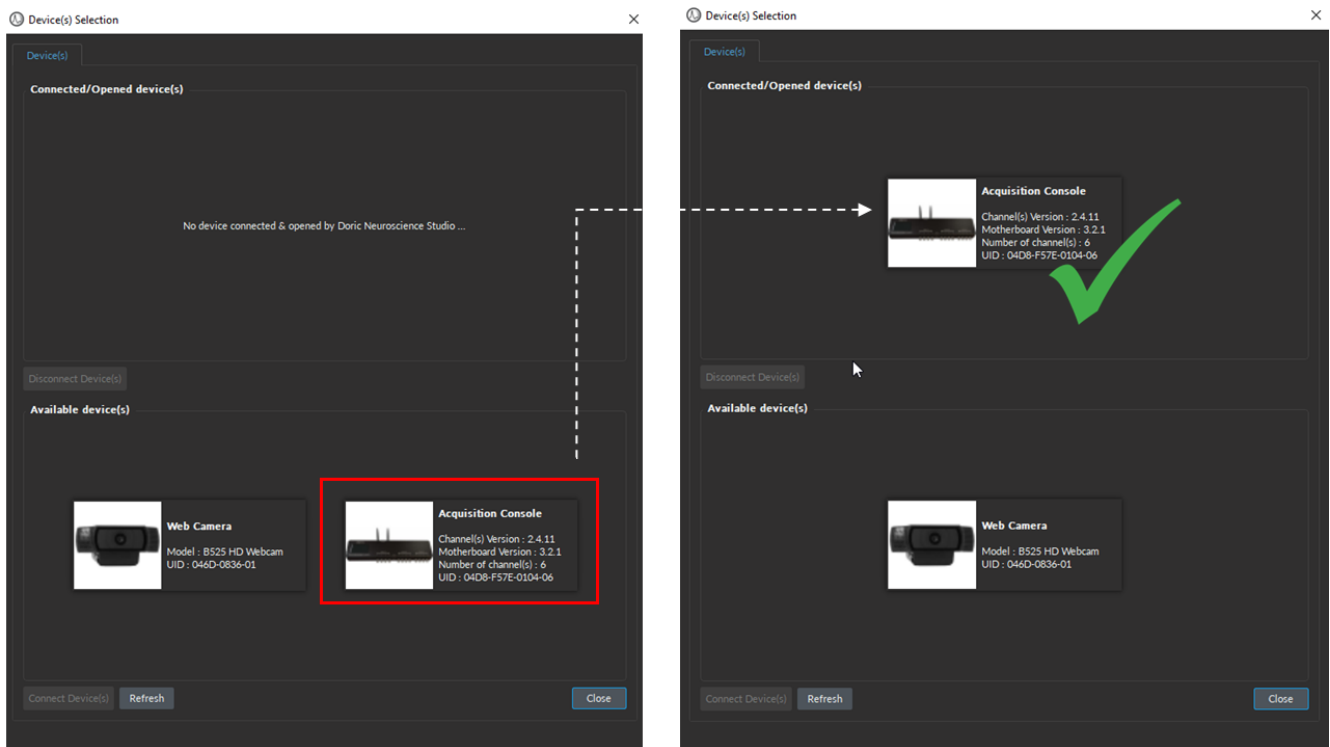
Figure 8.2: *Double click on device of choice to connect it to DNS*

NOTE: If you have switched to DNS v6, older devices will require a firmware update to be recognised by the new version of the software. This update can be easily done using *Doric Maintenance Tools (DMT)* application, and must be done one-by-one for each device. Further instructions can be found **HERE**.

Manually opening the *Device(s) Selection* window:
To manually open the *Device(s) Selection* window, select the *File*, then *Device Selection* (as per Fig. 8.3) or use the hot key: *Ctrl+N*.
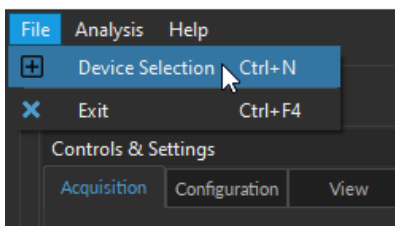
Figure 8.3: *Open Device Selection Window*

8.2 Overview

The **Acquisition Console** Module for the FiWi System of *Doric Neuroscience Studio* software is split into three sections: **(1) Control and settings tabs** (Section **??**) are used to manage different elements of the software (Acquisition, Configuration and View); **(2) Headstage Info** (Section 8.5) is used to pair the Headstage to the console; **(3) Acquisition view** (Section 8.5) displays the input and output traces for visualization.
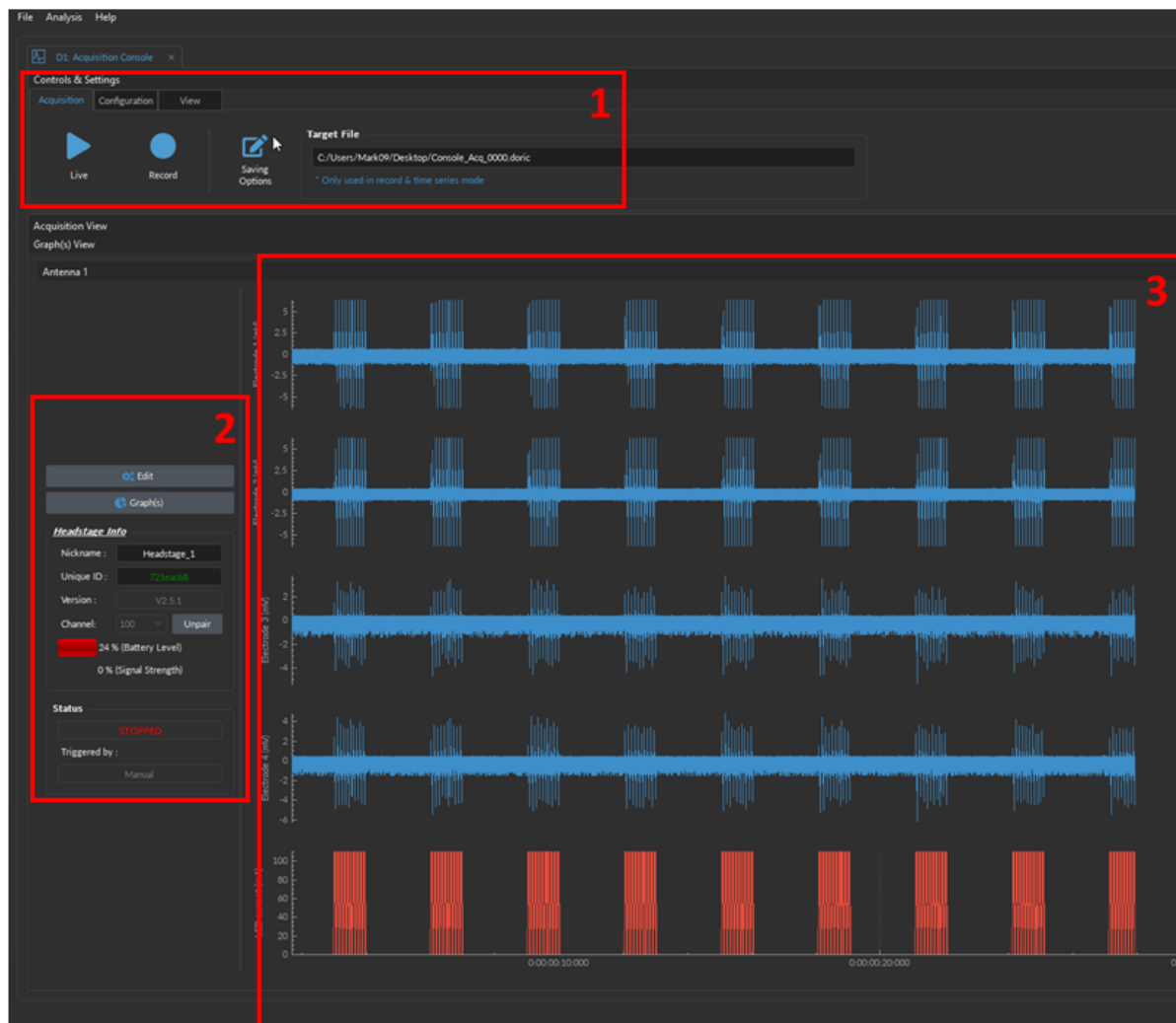
Figure 8.4: *DNS interface*

8.3   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** (Section 8.3.1), **Configuration** (Section 8.3.2), and **View** (Section 8.3.3).

8.3.1   Acquisition Tab

The **Acquisition** tab is used to start a live/recording session and set the saving parameters. The **Live** and **Record** buttons will not function if channels have yet to be set-up. See section 8.4.1 to configure channels for recording.
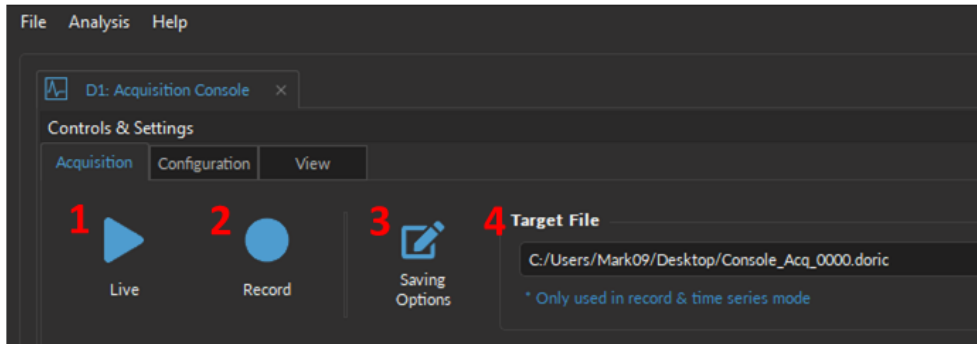
Figure 8.5: *Acquisition Tab*

1. The **Live** button (Fig. 8.5, 1) activates all prepared channels. This mode does not save data, keeping only the most recent 700 000 data points in memory. This mode is not recommended for long or critical measurement sequences. **Live** mode is useful to quickly test the recording software and to ensure that the parameters were properly set.

2. The **Record** button (Fig. 8.5, 2) activates all prepared channels while periodically saving recorded data to the disk. This mode is recommended for long measurement sequences.

3. The **Saving Options** (Fig. 8.5, 3) button opens the **Saving Parameters** window (Fig. 8.6). See section 8.3.1.1 for greater details.

4. The **Target File** (Fig. 8.5, 4) displays the path and file name where the data will be stored once the **Record** button is selected. Select the **Saving Options** button to change the path and file name.
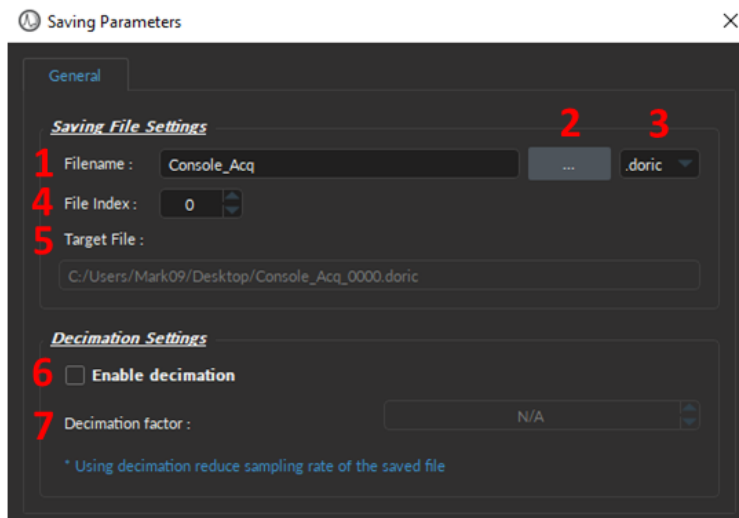
Figure 8.6: *Saving Menu Window*

8.3.1.1 Saving Parameters

The **Saving Parameter** window is used to define how and where the file is saved. This window is opened by selecting the **Saving Options** button in the Acquisition Tab (Fig. 8.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 8.6, 1).

2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 8.6, 2).

3. The **File format** (Fig. 8.6, 3) is **.doric**, an HDF5-based format that supports metadata (signal, video, images, tables, parameters, etc.). Version 6 of *Doric Neuroscience Studio* is no longer compatible with other file formats (.csv, .excel, or .tiff). We provide Matlab, Python, and Octave codes to read **.doric** files HERE. While not recommended, it is possible to export a *.doric* file into .csv format through the **Doric File Reader** module.

4. The **File Index** (Fig. 8.6, 4) box is used to define the current indexation number used for multiple files saved during the same measurement session. The suffix is incremented automatically when recording multiple files.

5. The **Target File** (Fig. 8.6, 5) displays the absolute path and filename where the data will be saved.

6. The **Enable decimation** checkbox (Fig. 8.6, 6) provides a way to reduce the file sizes. This method conserves points averaged over a number of data points equal to the **Decimation Factor**.

7. The **Decimation factor** text-box (Fig. 8.6, 7) is used to define the number of points saved.[1]

8.3.2 Configuration Tab

The **Configuration** tab is used to set the channels and the global settings (such as sampling rate and Master trigger options), as well as save and load the preset channel configurations.
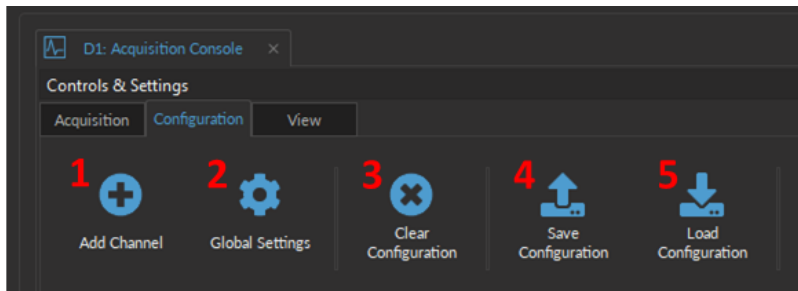
Figure 8.7: *Configuration Tab*

1. The **Add Channel** button (Fig. 8.7, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 8.4. Table 8.1 describes different types of channels available, their use cases and their individual sections.

2. The **Global Settings** (Fig. 8.7, 2) opens the **Global Options** window in Fig. 8.8, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 8.3.2.1 for more details.

3. The **Clear configuration** button (Fig. 8.7, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.

4. The **Save configuration** button (Fig. 8.7, 4) allows a console configuration to be saved in the **.doric** format. This file preserves the current channel configuration/parameters, the Acquisition View window organization, and any custom trace colors and names.

5. The **Load configuration** button (Fig. 8.7, 5) imports a pre-configured **.doric** file into the module.

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[1]For a data set of 10 points, saved with a **Decimation Factor** of 2, the first point will be saved, the third ... This produces a file of 5 points of data.

8.3.2.1 Global Settings

Through the **Global Settings**, user can set the acquisition **Sampling Rate** and specify the **Master Trigger Options** that will start recordings.

Figure 8.8: *Global Options Window*

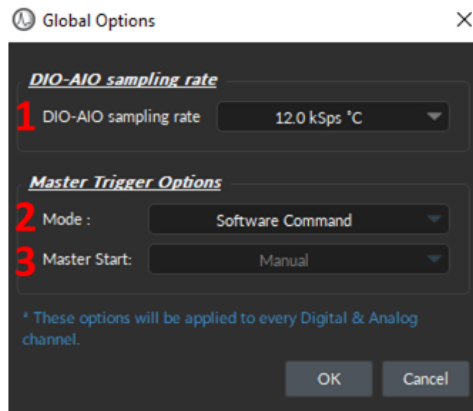1. The **DIO-AIO sampling rate** (Fig. 8.8-1) is 12 kSps°C by default. This value was selected because it is the highest value that still produced reliable data given the hardware limitations of the devices. See section 8.3.1.1 to enable the *Decimation* and effectively reduce the saving sampling rate and restrict the data file size.

2. The **Mode** (Fig. 8.8, 2) of the **Master Trigger Options** sets the origin (internal, external or time-series) of the trigger that will start the recording session and synchronize all the external and internal devices. Four options are available for different use cases:

   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 8.5, 2). The **Master Start** is, by definition, always **Manual**.

   - *Triggered* - The recording session starts when a trigger signal is received (from the **Master Start**, either manual or from an external digital source), and continues even if the trigger signal stops. Thus, the **Triggered** mode only controls the START of the recording session (and NOT the endpoint).

   - *Gated* - The recording session starts when a high TTL signal (>4 V) is detected (from the **Master Start**, either manual or from an external digital source), and will stop when a low TTL signal (<0.4 V) is detected. Thus, the **Gated** mode controls both the START and the END signals of the recording session.

   - *Timeseries* - This mode allows users to record pre-defined series over longer periods of time (that can span several days) (Fig 13.9a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 8.9b) pops up. See section 8.3.2.2 for more details.

3. The **Master Start** (Fig. 8.8, 3) defines the source that will automatically start the recording. This source can either be:

   - *Manuel* - the user ultimately starts the recording session by clicking **Record** within *Doric Neuroscience Studio*;

   - *Digital I/O Channel (1-4)* - The specified channel will automatically begin the recording session when it receives a digital trigger pulse from an external device. ***However, this mode still requires that the **Record** button is selected BEFORE the TTL trigger signal is received.***

8.3.2.2 Time Series

The **Time Series** Window (Fig 8.9b) can be opened by clicking on the **Record** button (Fig. 8.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 8.8, 2). Every **Time series** sequence is automatically saved to the *.doric* file defined in **Saving Options** (Section 8.3.1.1).
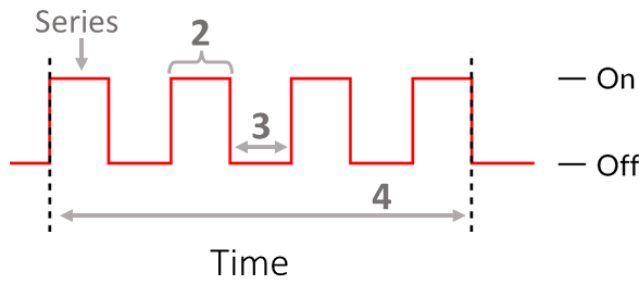
(a) *Time Series Acquisition Timing Diagram*
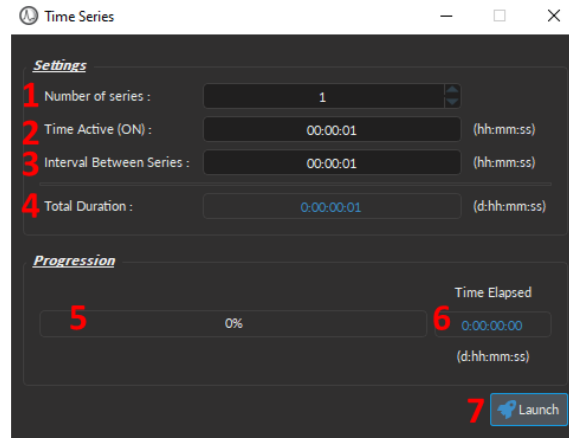
(b) *Time Series Window*

Figure 8.9: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 8.9b) sets the following parameters:

1. The **Number of series** (Fig. 8.9b, 1) defines the amount of times the series is repeated.

2. The **Time Active (ON)** (Fig. 8.9b, 2) defines the duration of the series.

3. The **Interval Between Series** (Fig. 8.9b-3) defines the amount of time between each series, if the **Number of series** is greater than 1.

4. The **Total Duration** (Fig. 8.9b, 4) displays the total amount of time that the timeseries recording will take place.

5. The **Progression bar** (Fig. 8.9b, 5) indicates the progression of the timeseries (in %).

6. The **Time Elapsed** (Fig. 8.9b-6) counter indicates the amount of time that has already passed in d:hh:mm:ss.

7. The **Launch** (Fig. 8.9b, 7) 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.

8.3.3   View Tab

The **View Tab** (Fig. 8.10) is used to modify the presentation of graphs in the **Acquisition view**.
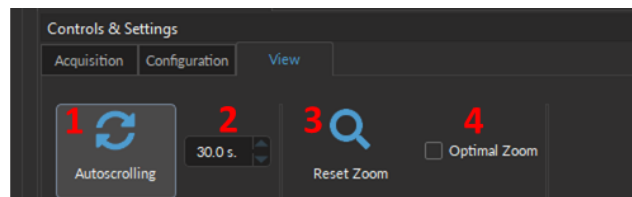
Figure 8.10: *View Tab*

The **View** parameters are as follows:

1. The **Autoscrolling** button (Fig. 8.10, 1), when selected, automatically set the graphs to scroll as new data appears.

2. the **Zoom range** (Fig. 8.10, 2) sets the graph zoom to the value of choice, specified in the text-box.

3. The **Reset Zoom** button (Fig. 8.10, 3) readjusts the graph zoom to the value specified in the zoom range text-box.

4. The **Optimal Zoom** check-box (Fig. 8.10, 4) automatically adjusts the graph range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

8.4 Channel Configurations

Before pairing the Headstage of the *FiWi electrophysiology system*, users first need to set up the required channels and specify the data acquisition parameters required for the experiment.

8.4.1 Add Channel:

To create a new channel, regardless of the input and/or output type, select the **Add Channel** button, which can be found under the **Configuration** tab (Fig. 8.11). This will open the **Channel(s) Configuration** window (Fig. 8.13).
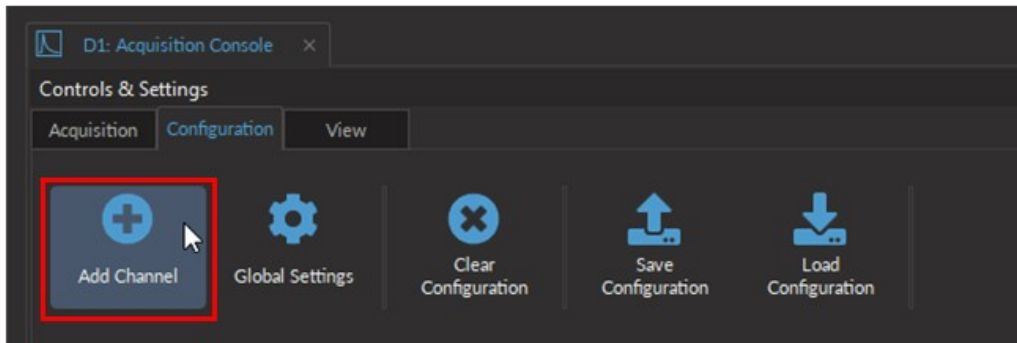
Figure 8.11: *Add Channel button opens the Channel Configuration window*

To generate a new **Channel** using the **Channel(s) configuration** window (Fig. 8.12):

1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 8.12). Table 8.1 describes the use case of each type.

2. Clicking on the icon will display the **Channel Type**-specific options on the right side of the window. Each **Channel Type** has a number of parameters which can be configured to fit the needs of the experiment(s). Details of the parameters and their options will be covered in the following sections. See Table 8.1 for hyperlinks to the relevant sections.

3. Select the **Add** button (Fig. 8.12) to generate the defined channel or to update an already configured channel, but does not automatically close the *Channel Configuration* window. This allows the user to conveniently set up all required channels one after the other.

4. Select the **Close** button to shut the window once all channels are configured.
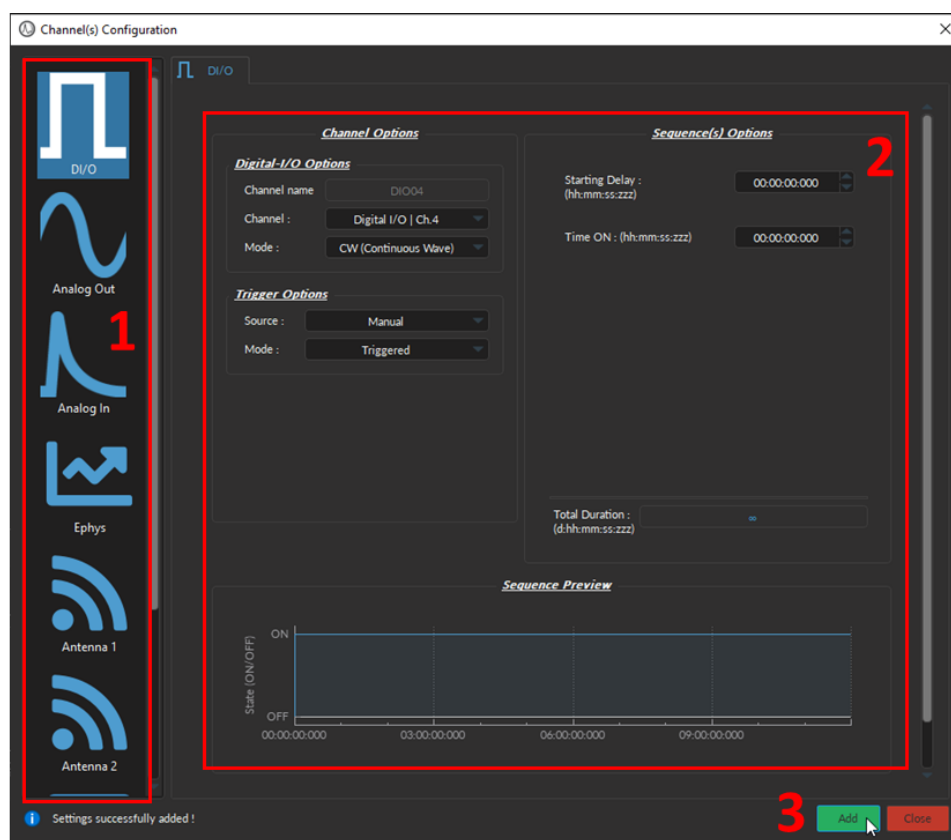
Figure 8.12: *Channel(s) configuration window, Digital I/O input*

8.4.2 Channels Types

Different types of input and output can be configured for the particular of the experiment by creating a new Channel in the Configuration tab or editing an existing one (Fig 8.11). Table 8.1 details the types of inputs and output the console and the software can handle and gives a quick access to their sections.

Table 8.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	Antenna	Pair and record electrophysiological signal from the FiWi Headstage	8.4.3
	Digital I/O	For input and output of TTL signals	8.4.4
	Analog Output	For the output of analog signals, such as sine, stair or customized	8.4.5
	Analog Input	To collect the fluorescent signal (such as GCamp, RCamp, Isosbestic or FRET)	8.4.6
	Camera(s)	To collect video for behaviour experiments	8.4.7
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	8.4.8

8.4.3   Antenna Channel

The **Antenna** Channel is required to pair the wireless Headstage to the software, to record the four electrophysiology inputs, in addition to output the digital triggers that control the LED current.

The *Channel(s) Configuration* window for the Antenna Channel is divide into four sections (Fig. 8.13): (1) the **Channel Options** (Section 8.4.3.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 8.4.3.3), and (4) the **LED Trigger Options** (Section 8.4.3.2).
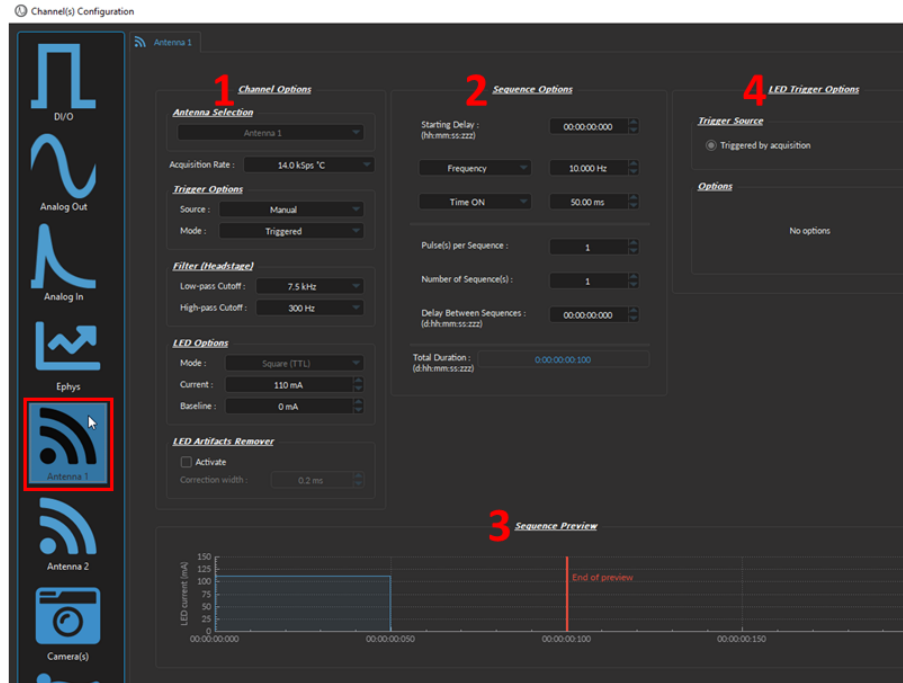
Figure 8.13: *Electrophysiology channel configuration window*

8.4.3.1   Channel Options

The **Channel Options** (Fig. 8.14) of the Antenna *Channel Configuration* window sets the trigger and recording parameters, as follows:

1. The **Antenna selection** (Fig. 8.14, 1) box shows the currently selected antenna.

    - The **Acquisition Rate** is the sampling rate of the electrophysiological data and can be a value between 0.1kSps°C and 14kSps°C. The default value is set to 14kSps°C.

2. The **Trigger options** (Fig. 8.14, 2) box allows the selection of trigger parameters for data acquisition.

    - The **Trigger source** can either be **Manual**, or can come from the **Digital I/O** channels.

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

        - **NOTE:** If the source is **Manual**, then only the **Trigger** mode is available. Whereas, if the source is one of the four **Digital I/O** channels, the mode can either be **Triggered** or **Gated**.

3. The **Filter (Headstage)** (Fig. 8.14, 3) filters define the high and low-pass frequency cutoff for electrical signal received by the headstage.

    - The **Low-pass Cutoff** (Fig. 8.14, 3) is used to attenuate the noise and smooth the waveform of the spikes. The cut-off value can be set between (1-500Hz), depending on the need of the experiment.

- The **High-pass Cutoff** (Fig. 8.14, 3) is used filter the multi-unit activity that occurs from the average / synchronized spiking of a small population of neurons in the vicinity of the electrodes. It can also removed DC noise. The cut-off value can be set between 0.1-500Hz.

4. The **LED options** (Fig. 8.14, 4) are all parameters used to control the light source of the cannula connected to the *Fi-Wi headstage*, such as:

  - The **Mode** (Fig. 8.14, 4) allows the selection of the pulse sequence mode.
  - The **Maximum current** (Fig. 8.14, 4) defines the current sent to the cannula LED. For proper function of the cannula, the current should always be greater than 10 mA.
  - The **Baseline** (Fig. 8.14, 4) 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.

Figure 8.14: *Antenna Channel Options*

5. The **LED Artifacts Remover** reduces the number of photoelectric artifacts that occurs when the electrodes pick up the electrical trigger signals of the LED. These photoelectric artifacts can often look like spikes, but their timing are locked to the initiation (Fig. 8.15a) and termination (Fig. 8.15b) of the LED.

To remove the artifacts, an algorithm detects the first spike occurring after the initiation or termination of the LED (Fig. 8.15). All the value between the time of the first spike until the specified *Correction width* are set to 0V.

To turn on the **LED Artifact Remover**:

  - Check the **Active** box (Fig. 8.14, 5);
  - Specify the size of the **Correction width** (Fig. 8.14). The minimum value is **0.1 ms**. And while the maximum value can reach 1 second, it is important to note that the larger the **Correction widths**, the more likely true spikes are removed from the signal. Thus, the value must be selected carefully, taking into account this trade-off.

⚠️ **WARNING:** Using a large *Correction width* can result in real **signal deletion**.
SELECT *Correction width* CAREFULLY! ⚠️

(a) *Initiation artifact* (b) *Termination artifact*
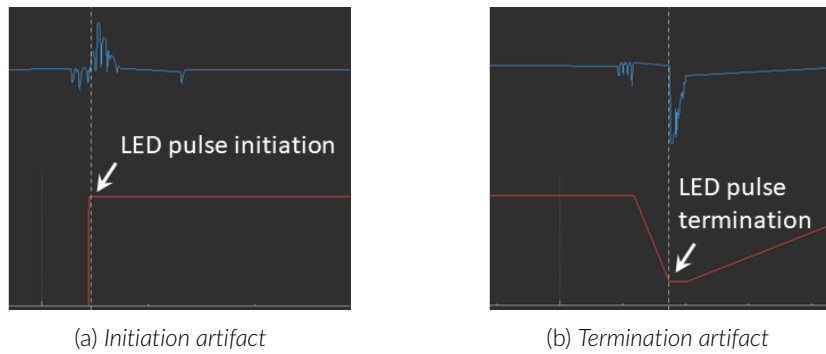
Figure 8.15: *Electrical artifacts caused by LED pulses*

(a) *LED artifacts during recording* (b) *LED artifacts removed during recording*
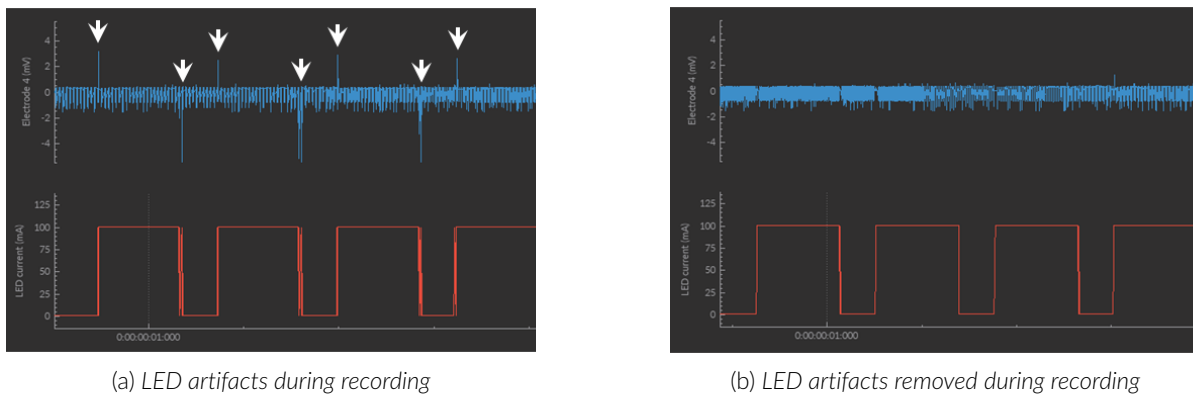
Figure 8.16: *LED Artifact Remover Option Example*

8.4.3.2   LED Trigger Options

The **LED Trigger Options** (Fig. 8.17), allow users to select what device triggers the LED source. By default the **Trigger Source** is the DNS acquisition system.
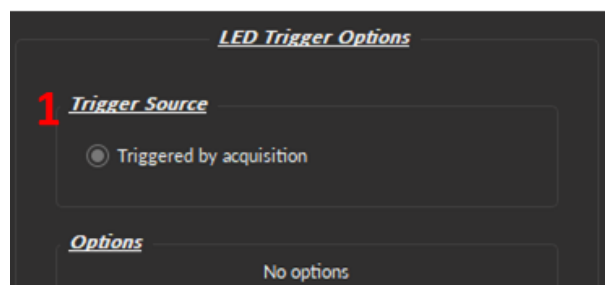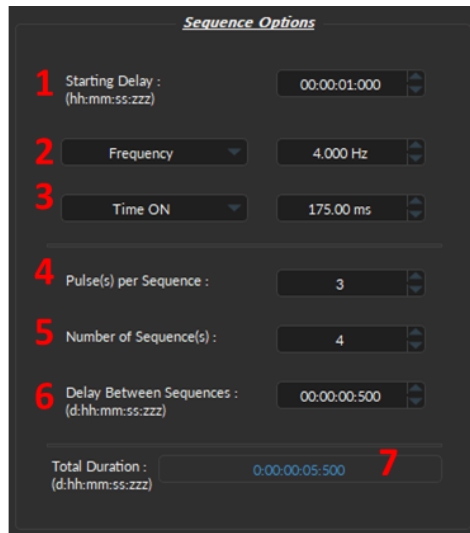

Figure 8.17: *Antenna LED Trigger Options*

8.4.3.3   Sequence Options & Preview

The **Sequence options** section (Fig. 8.18a) contains the LED pulse sequence parameters, while the **Sequence Preview** section (Fig. 8.18b) displays the corresponding shape and timing of the sequence.

1. The **Starting Delay** (Fig. 8.18) sets the delay (in hh:mm:ss:zzz format) before the first pulse.

2. The **Frequency/Period** (Fig. 8.18a) sets the frequency (in Hz) or period (in ms) for the pulse within the 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).

3. The **Time ON/Duty Cycle** (Fig. 8.18) 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.

4. The **Pulses per sequence** (Fig. 8.18a) sets the number of pulses per sequence. If it is set to 0, the pulse will be repeated indefinitely.

5. The **Number of sequences** (Fig. 8.18a) sets the number of times that the sequence will be repeated. If it is set to 0, the sequence will be repeated indefinitely.

6. The **Delay between sequences** (Fig. 8.18) sets the delay (in hh:mm:ss:zzz format) between each sequence if the **Number of Sequences** is greater than 1.

7. The **Total Duration** (Fig. 8.18) 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.
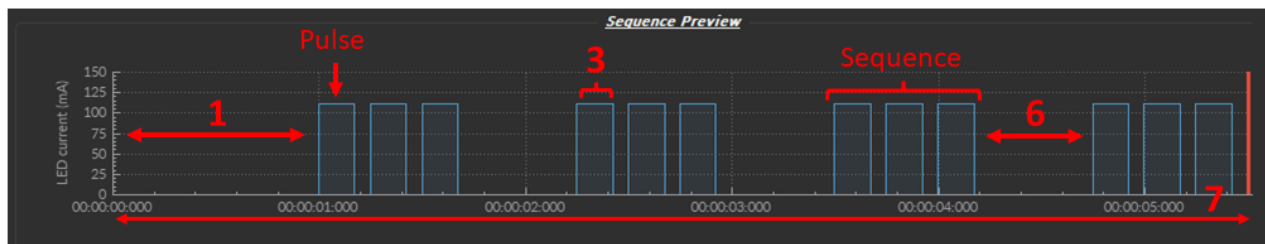
(a) *Sequence Options*

(b) *Sequence Preview*

Figure 8.18: *Channel(s) configuration window, Antenna - Sequence Options and Preview*

8.4.4 Digital I/O Channels

Each **Digital I/O** channel can be configured as an output or an input to create TTL (On/Off) pulse sequences. **Digital Outputs** can provide triggers to external devices (such as light sources) required for the experiment, while remaining synchronized with to recording system. In addition, **Digital Inputs** can record a copy of the trigger of an external driven device used during the experiment (such as the timing of a displayed stimuli or a measured behavior).

The *Channel(s) Configuration* window for the **Digital I/O** Channel is divided into three sections (Fig. 8.13): (1) the **Channel Options** (Section 8.4.4.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 8.4.4.2).

Figure 8.19: *Channel(s) configuration window, Digital I/O - CW mode*

8.4.4.1 Channel Options

The **Channel Options**/8-ScWithFChannelDig1O.png mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.
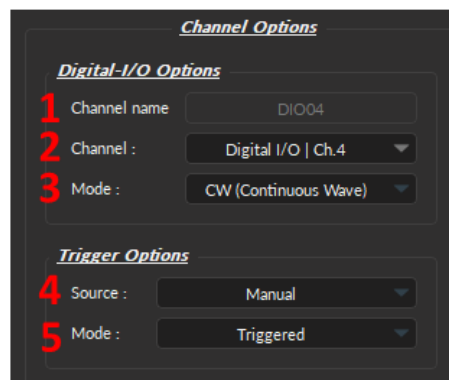
Figure 8.20: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel name** (Fig 8.20, 1) allows user to specify a label for each channel.

2. The **Channel** (Fig 8.20, 2) identifies the channels available to create a Digital I/O. The channel can be changed by selecting a new one from the drop-down list. Each numbered channel on the physical console corresponds to the same number of the digital channel within the software.

3. The **Mode** (Fig 8.20, 3) identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). Three modes are available:

   - The **Continuous wave (CW)** Mode (Fig. 8.21a);
   - The **Square (TTL)** Mode (Fig. 8.21b);
   - The **Input** mode receives a signal that are either 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the console (See Section 8.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.

(a) *Continuous* (b) *Square*
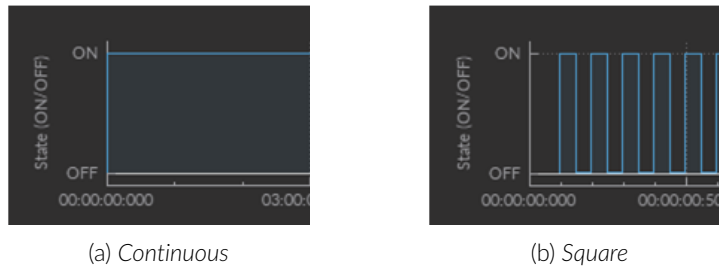
Figure 8.21: *Channel Options - Output Modes*

Trigger Options:

1. The **Source** trigger option (Fig 8.20, 4) allows the choice of a **Manual Trigger** (activated by a user) or an **Input** trigger, coming from a **Digital I/O** channel set in input mode.

2. The **Mode** (Fig 8.20, 5) defines how the trigger activates a sequence. This includes input sequences, which can be triggered/gated by an outside source.

   - In **Triggered** mode (Fig. 8.22a), 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 (Fig. 8.22b), the sequence will start once the voltage reach a high TTL signal (4 V or more) on the input modulation BNC. When the TTL signal reaches a low TTL signal (0.4 V or less), the sequence stops and waits for another high TTL signal to continue. This mode can cut pulses, once the high signal returns. ***ONLY AVAILABLE FOR SQUARE CHANNEL MODE***

(a) *Trigger* (b) *Gate*

Figure 8.22: *Trigger Options Modes*

8.4.4.2 Sequence Options & Preview

The **Sequence options** section (Fig. 8.23a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 8.23b) displays the corresponding shape and timing of the sequence. 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**.
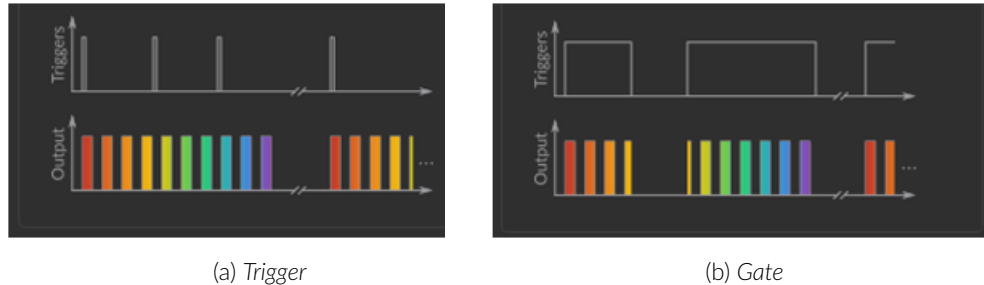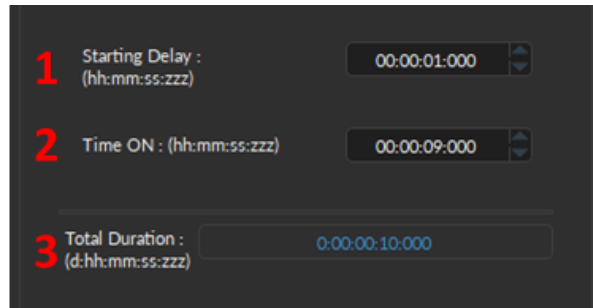
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 8.20), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 8.19) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

    1. The **Starting Delay** (Fig 8.23, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

    2. The **Time ON** (Fig 8.23, 2) 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** (Fig 8.23, 3) 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**.
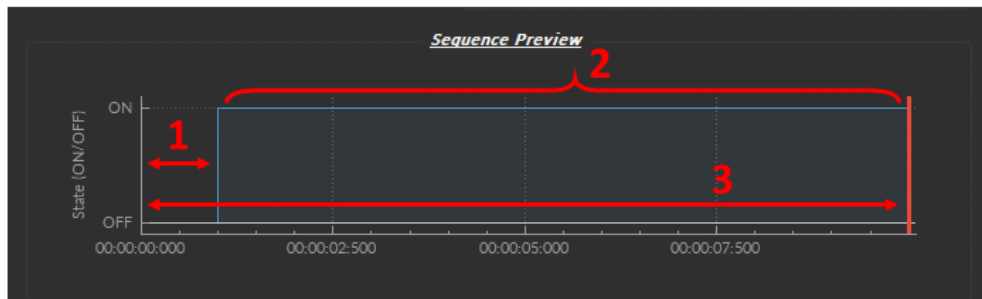
(a) *Sequence Options*

(b) *Sequence Preview*

Figure 8.23: *Channel(s) configuration window, Digital I/O - CW Mode*

- The **Square** channel mode (Fig. 8.20) allows the creation of a square TTL pulse sequence. This includes all sequence options as the **CW** mode (Fig. 8.23, 1-3), with the following additions:

    1. The **Starting Delay** (Fig 8.23, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

    2. The **Frequency** (Fig. 8.24a, 2) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period** (Fig. 8.24a, 2). 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).

3. The **Time ON** (Fig. 8.24, 3) 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.

4. The **Pulse(s) per sequence** (Fig. 8.24, 4) sets the number of pulses within a single sequence. If it is set to 0, the number of pulses will be infinite.

5. The **Number of sequence(s)** (Fig. 8.24, 5) sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** (Fig. 8.24, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).

7. The **Total Duration** (Fig 8.23, 3) 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**.

(a) *Sequence Options*

(b) *Sequence Preview*

Figure 8.24: *Channel(s) configuration window, Digital I/O - Square Mode*
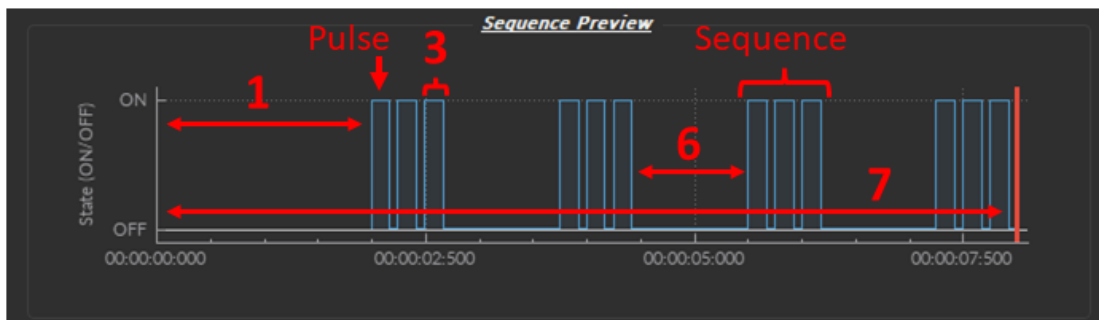
8.4.5   Analog Output
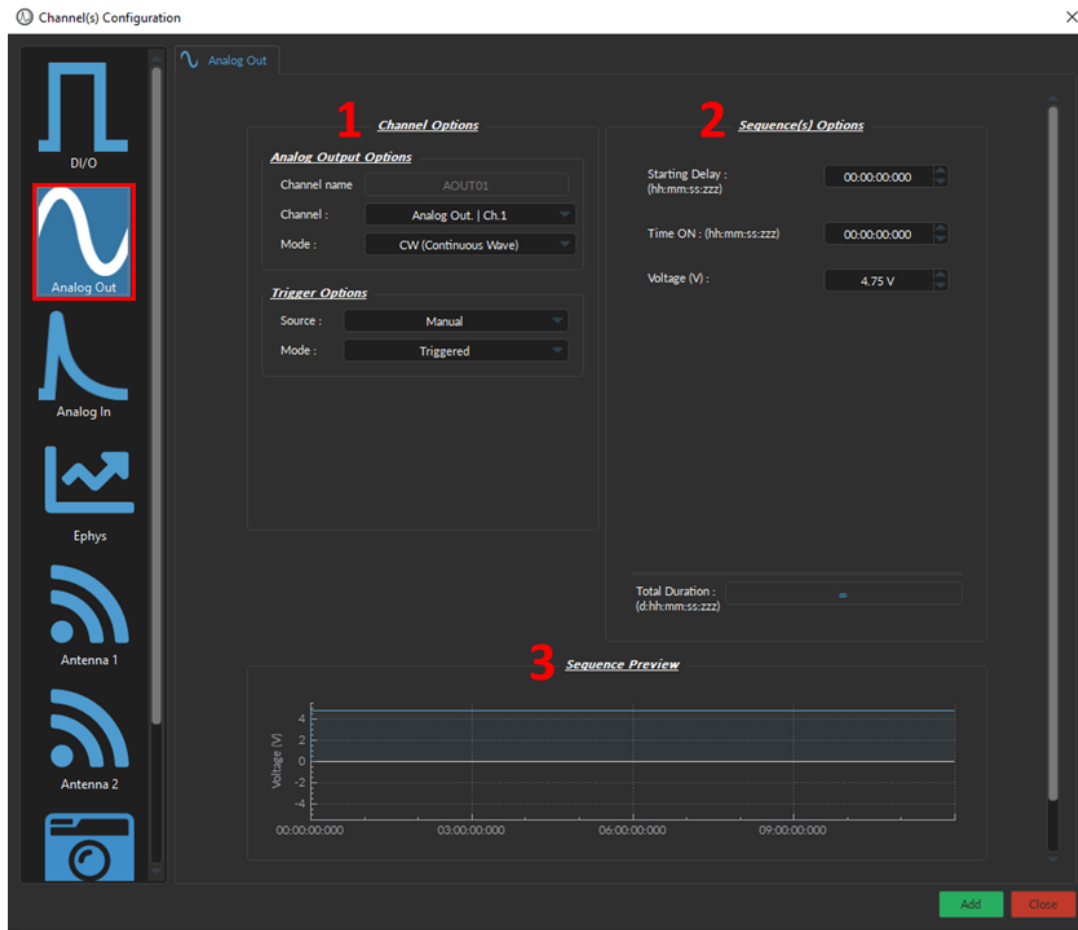
Figure 8.25: *Channel(s) configuration window, Analog Output CW*

The **Analog Output** channel type creates analog pulse sequences. Each numbered channel corresponds to the same analog channel number on the console. Pulse sequences have different parameters depending on the channel **Mode**, which can be **Continuous**, **Square**, **Sine**, **Stair** and **Custom** (Fig. 8.26).

(a) *Continuous* (b) *Square* (c) *Sine* (d) *Stair* (e) *Custom*
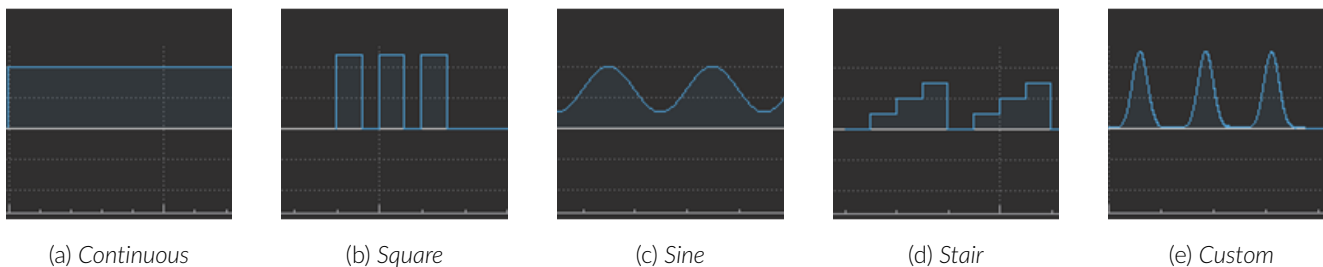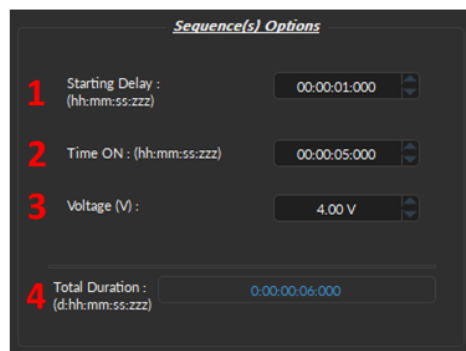
Figure 8.26: *Analog Output Modes*

8.4.5.1 Continuous Wave (CW) Mode

The **CW (Continuous wave)** channel mode (Fig. 8.27) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box (Fig. 8.27a).

1. The **Starting Delay** (Fig. 8.27) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Time ON** (Fig. 8.27) 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. 8.27) defines the voltage of the continuous signal, in volts. The signal cannot go beyond ± 4.75 V.

4. The **Total Duration** (Fig. 8.27) 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**.
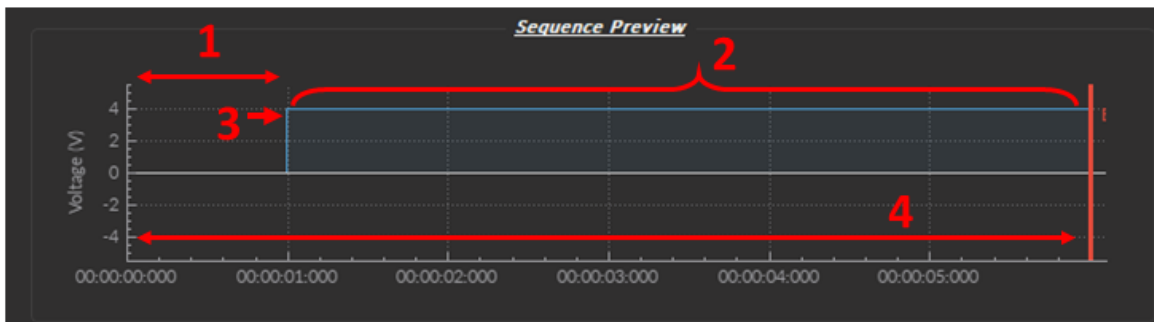
(a) *Sequence Options*

(b) *Sequence Preview*

Figure 8.27: *Channel(s) configuration window, Analog Output CW*

8.4.5.2 Square Mode

The **Square** channel mode (Fig. 8.28) creates a sequence of pulses with the minimum of the pulses at **V-** and the maximum of each pulse at **V+**.

1. The **Starting Delay** (Fig. 8.28, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Frequency** (Fig. 8.28, 2) 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).

3. The **Time ON** (Fig. 8.28, 3) 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.

4. The **Maximum Voltage (V+)** (Fig. 8.28, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.

5. The **Minimum Voltage (V-)** (Fig. 8.28, 5) defines the minimum voltage of each pulse, in volts. The signal cannot go below -4.75 V.

6. The **Pulse(s) per sequence** (Fig. 8.28, 6) set the number of pulses per sequence. If it is set to 0, the number of pulses will be infinite.

7. The **Number of sequence(s)** (Fig. 8.28, 7) sets the number of times that the sequence will be repeated.

8. The **Delay between sequences** (Fig. 8.28, 8) sets the delay between each sequence.

9. The **Total Duration** (Fig. 8.28, 9) 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**.

(a) *Sequence Options*

(b) *Sequence Preview*

Figure 8.28: *Channel(s) configuration window, Analog Output Square*

8.4.5.3 Sine Mode

The **Sine** mode (Fig. 8.29) creates a sinusoidal pulse sequence with peaks at **V+** and **V-**.

 Note: The **Starting Delay** is not available for this mode (Fig. 8.29a).

1. The **Frequency** (Fig. 8.29, 2) 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 **Phase** option (Fig. 8.29, 2) replaced **Time ON** (Fig. 8.28, 3). This allows the choice of the sine wave phase, in degrees.

3. The **Maximum Voltage (V+)** (Fig. 8.29, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.

4. The **Minimum Voltage (V-)** (Fig. 8.29, 5) defines the minimum voltage of each pulse, in volts. The signal cannot go below -4.75 V.

5. The **Period per Sequence** (Fig. 8.29, 5) is similar to the **Pulse per Sequence** parameter in Square mode (Section 8.4.5.2, Square), but where the period (a single sine wave from peak to peak, Fig. 8.29b, 1) replaces the pulse.

6. The **Number of sequence(s)** (Fig. 8.29, 7) sets the number of times that the sequence will be repeated.

7. The **Delay between sequences** (Fig. 8.29, 8) sets the delay between each sequence.

8. The **Total Duration** (Fig. 8.29, 9) 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**.

(a) *Sequence Options*
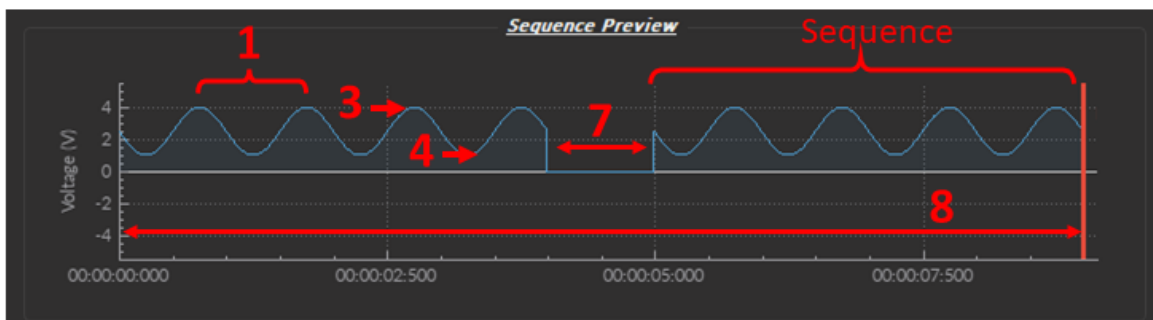
(b) *Sequence Preview*

Figure 8.29: *Channel(s) configuration window, Analog Output Sine*

8.4.5.4 Stairs Mode

The **Stairs** mode (Fig. 8.30) creates a stepwise pulse sequence with peaks at several different Voltage levels **V+**.
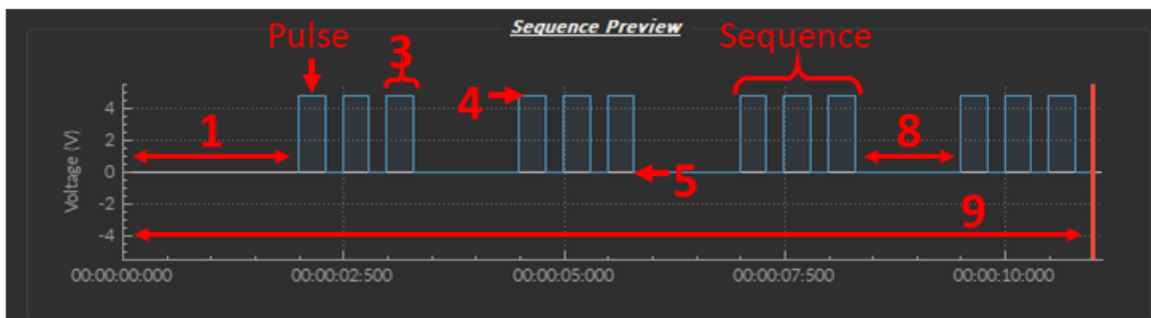
1. The **Starting Delay** (Fig. 8.30, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Frequency** (Fig. 8.30b, 2) option replaces the **Time ON** . This parameter applies to a whole pulse, which include all the voltage steps (up to a max of four).

3. The **Number of Steps** sets the amount of voltage levels of a single pulse, up to a maximum of four (Fig. 8.30b, 3). Increasing the number of steps automatically adds an additional parameter to specify the voltage of the added step below.

4. The **Step Voltage** sets the value of stair level X between **-4.75V** and **4.75V** (Fig. 8.30, 4).

5. The **Pulse(s) per sequence** (Fig. 8.30, 5) 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. 8.30, 6) sets the number of times that the sequence will be repeated.

7. The **Delay between sequences** (Fig. 8.30, 7) sets the delay between each sequence.

8. The **Total Duration** (Fig. 8.30, 8) 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**.

(a) *Sequence Options*
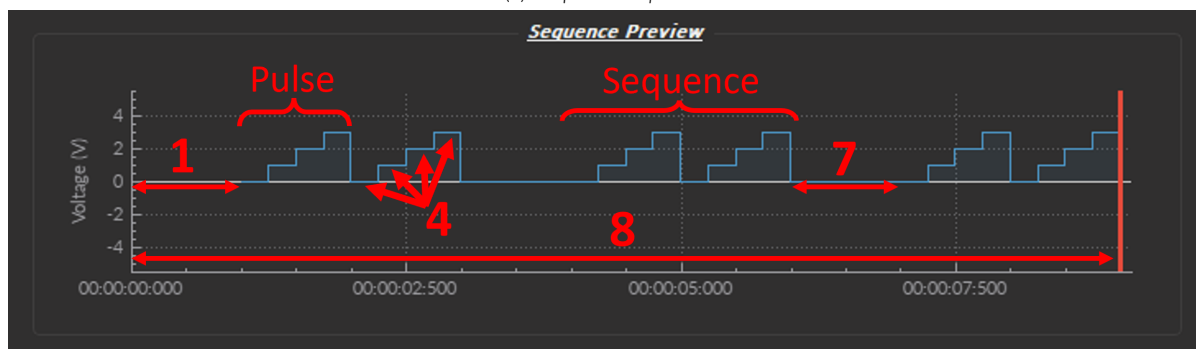
(b) *Sequence Preview*

Figure 8.30: *Channel(s) configuration window, Analog Output Stairs*

8.4.5.5 Custom Mode

The **Custom** mode (Fig. 8.31) provides users with the ability to design a pulse with non-standard shape to fit experimental needs.

1. The **Select File** button (Fig. 8.31a, 1) is used to input a custom .csv file containing the data for the pulse sequence. This must be a .csv format and requires 2500 values in column vector format (i.e. with *line break* between values), as in Fig. 8.31b. The values can be any value between **-4.75V** and **+4.75V**.

2. The **Starting Delay** (Fig. 8.31, 2) defines the time between the activation of the pulse sequence and the beginning of the signal.

3. The **Period** option (Fig. 8.31, 3) replaces the **Time ON** option (Fig. 8.28, 3). This option will stretch or shrink the 2500 value sequence to fit the specified amount of time.

4. The **Period per Sequence** (Fig. 8.31, 4) is similar to the **Pulse per Sequence** found in **Square** modes (Fig. 8.4.5.2, 6), where the pulse is replaced by the period sequence (Fig. 8.31c, Sequence).

5. The **Number of sequence(s)** (Fig. 8.31a, 5) sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** (Fig. 8.31, 6) sets the delay between each sequence.

7. The **Total Duration** (Fig. 8.31, 7) 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**.

8.4.6 Analog Input

The **Analog Input** channel type acquires signal from the **Analog Input** BNC connector ports. Each numbered channel corresponds to the same analog channel number on the console.

The *Channel(s) Configuration* window for the **Analog Input** is divided into two sections (Fig. 8.32): (1) the **Channel Options** (Section 8.4.6.1) and (2) the **Mode-specific Options** (*Linear*, Section 8.4.6.2; *Interleaved*, Section 8.4.6.3; *Lock-In*, Section 8.4.6.4).
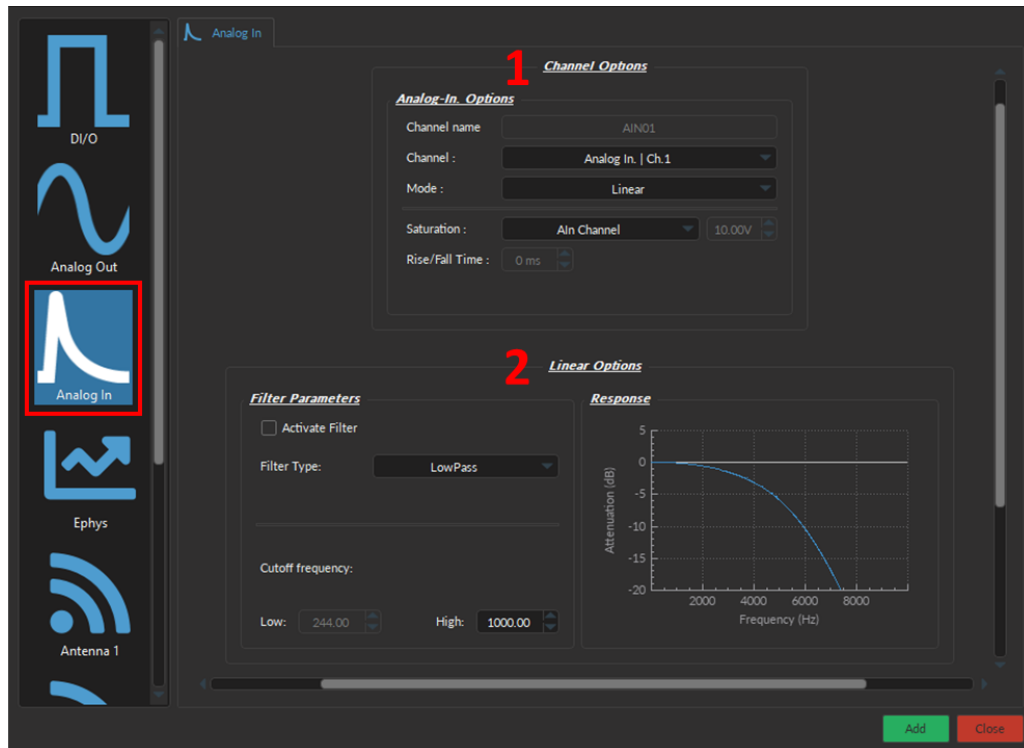
Figure 8.32: *Channel(s) configuration window, Analog Input*
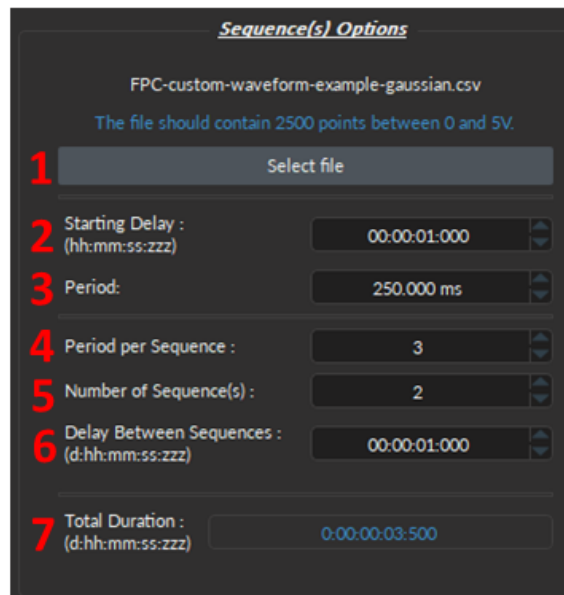
(a) *Sequence Options*

(b) *Example .csv file*
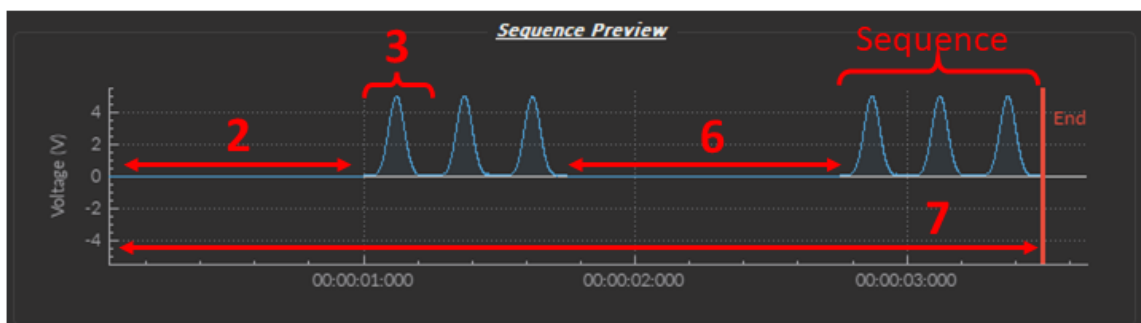
(c) *Sequence Preview*

Figure 8.31: *Channel(s) configuration window, Analog Output Custom*

8.4.6.1 Channel Options

The **Channel Options** (Fig. 8.33) defines the channel, source and mode of the digital signal, as following:
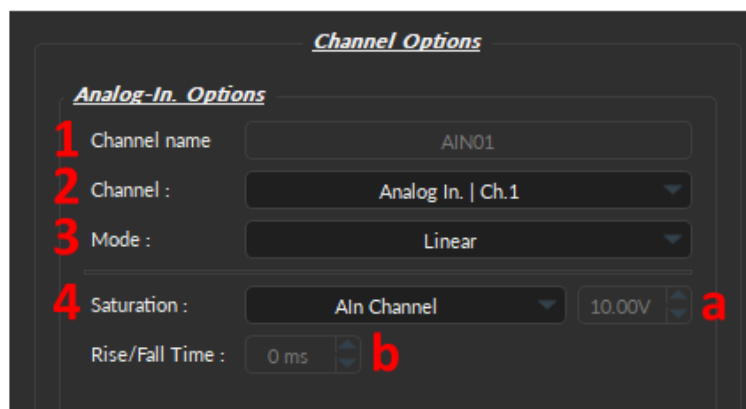
Figure 8.33: *Channel(s) configuration window, Analog Input - Channel Options*

1. The **Channel name** (Fig 8.33, 1) allows user to specify a label for each channel.

2. The **Channel** (Fig 8.33, 2) identifies which of the 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. Each numbered channel on the physical console corresponds to the same number of the digital channel within the software.

3. Three **Mode** are available to record the input signal, each of which have their own defined parameters in the Option box below **Channel Options** (Fig. 8.32, 3):

    - *Linear* - Section 8.4.6.2;
    - *Interleaved* - Section 8.4.6.3;
    - *Lock-In* - Section 8.4.6.4.

4. The **Saturation** (Fig. 8.33, 4) automatically sets the following parameters and depends on the detector acquiring the data (Detectors: *Doric detector*, *Newport Detector*, *Hamamatsu C10709*, and *AIn Channel*):

    a) The **Maximum Voltage** (Fig. 8.33, 4a)

    b) The **Rise/Fall Time** (Fig. 8.33, 4b)

    To manually set either parameter, select **Custom** in the drop-down menu.

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

8.4.6.2   Linear

The **Linear** channel mode (Fig. 8.34) allows the direct measurement of signal received by a channel. The linear mode-specific parameters are as follows:
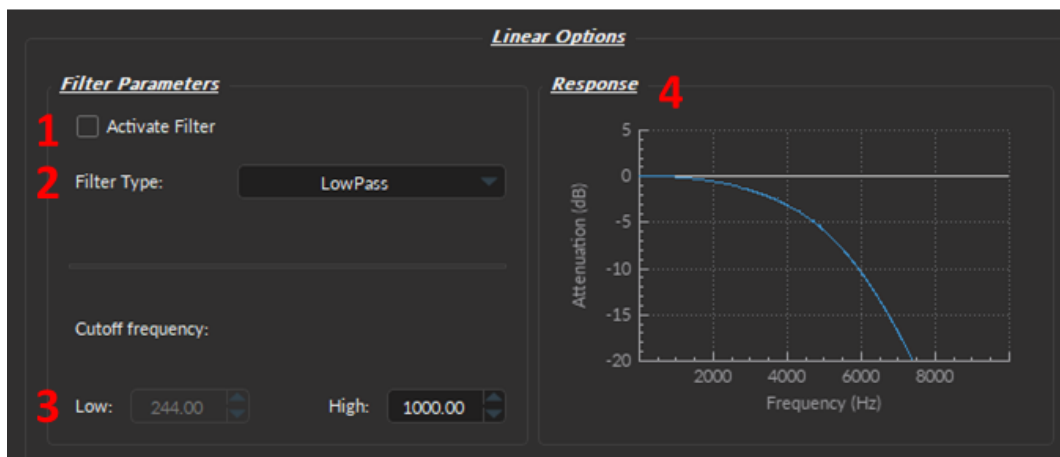
Figure 8.34: *Channel(s) configuration window, Analog Input Linear*

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 Type** drop-down list allows the choice of a filter type from **High-Pass**, **Low-Pass**, **Band-Pass** and **Band-Stop**.

3. 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. Note: the true cut-off value is, by definition, always 3 dB below (Low Cutoff) or above (High Cut-off) the specified value.

4. The **Response** box displays Frequency (Hz) vs Attenuation (dB) trace of the filter according to both the filter type and the cut-off values.

8.4.6.3 Interleaved

The **Interleaved** channel mode allows two 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. 8.35).
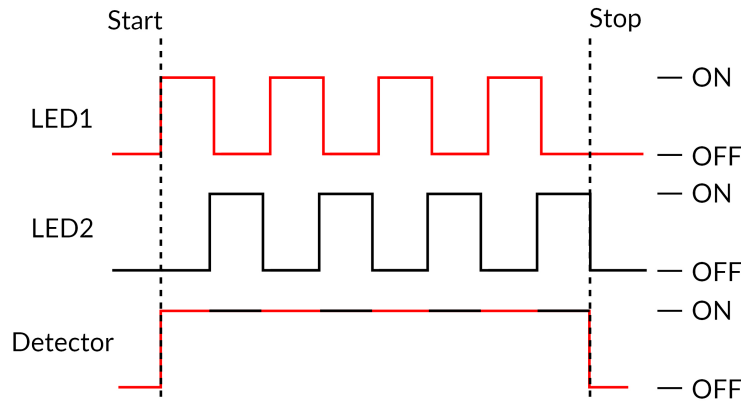
Figure 8.35: *Interleaved Acquisition Timing Diagram*

The interleave preset is using 50% duty cycle for each LED, without delay between them (Fig. 8.35). Thus, depending on the Rise/Fall time of the detector in use (Fig. 8.33, 4b, Detector Rise/Fall Time), there will be more or less crosstalk between the interleaved channels (Fig. 8.36).

> **WARNING:**
> **Crosstalk** occurs between **two interleaved** Digital I/O channels. If possible, use **Lock-In mode** instead, or **switch to a detector** will smaller Rise/Fall Time.

Specifically, when one of the digital channels is ON, it will pick up when the other is turn ON or OFF (Fig. 8.36). Figure 8.36 shows how the Digital Output channel of LED 1 has a small increase in voltage when the LED 2 is turned ON. And, conversely, there is a small dip in voltage in the LED 2 channel when LED 1 is turned OFF (Fig. 8.36).
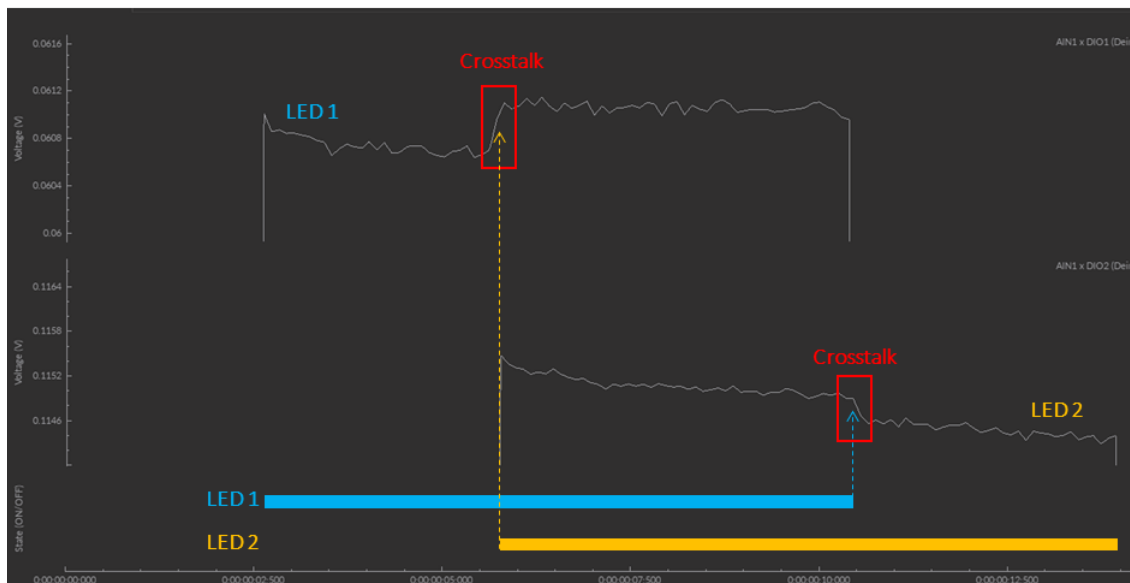
Figure 8.36: *Interleaved Cross-talk*

Strategies to Mitigate Crosstalk:

1. If the sampling rate of the triggered device(s) is high enough (>120Hz), use the **Lock-In mode** (Section 8.4.6.4) instead of the **Interleaved mode**;

2. Switching to a detector with a smaller Rise/Fall Time will reduce the crosstalk. For instance, the *Doric* and *Newport Detectors* have a Rise/Fall Time of 15 ms, while Hamamatsu C10709 has one of 1 ms.

Regardless of the Detector in use, **care should be taken not to misinterpret crosstalk as real signal during data analysis.**
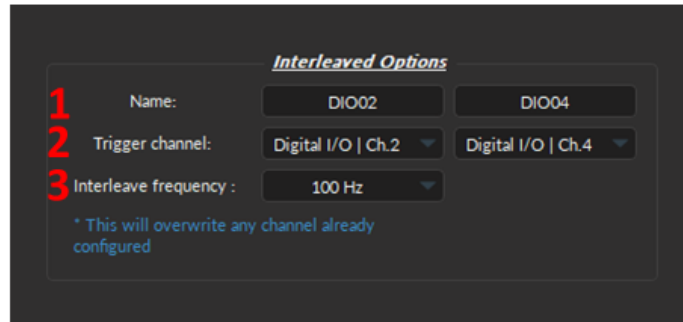
Figure 8.37: *Channel(s) configuration window, Analog Input Interleaved*

To use the **interleaved mode**, specify the parameters in the **Interleaved Options** section of the *Channel Configuration* window (Fig. 8.37):

1. The **Name** (Fig. 8.37, 1) lets users customize the label of the channel to increase clarity of the acquisition system.

2. The **Trigger channel** (Fig. 8.37, 2) drop-down list allows the choice of interleaved outputs (can be either digital or analog). However, once the first channel is selected, the user will only be allowed to select the same type of output (analog or digital) for the second channel.

3. The **Interleave frequency** (Fig. 8.37, 3) drop-down list allows the choice of a pre-configured frequency (either 10, 20, 50 or 100Hz) for the interleaved channels. The two selected trigger channels will be configured to function at the chosen frequency.

WARNING:
Specifying the interleave frequency will **overwrite** any channel already configured.

8.4.6.4 Lock-In

The **Lock-In** mode can be used to detect fluorescence signals embedded in strong noise (e.g. Isosbestic and a fluorophore) or to separate multiple signals (e.g. Green and red fluorophores) during fiber photometry.

Each *LED light source* emits a sinusoidal illumination at a given frequency (Fig. 8.38a & 8.38b). The detector collects the fluorescent data at a frequency corresponding to the summation of the LED frequencies (Fig. 8.38c).

(a) *LED 1 frequency*

(b) *LED 2 frequency*
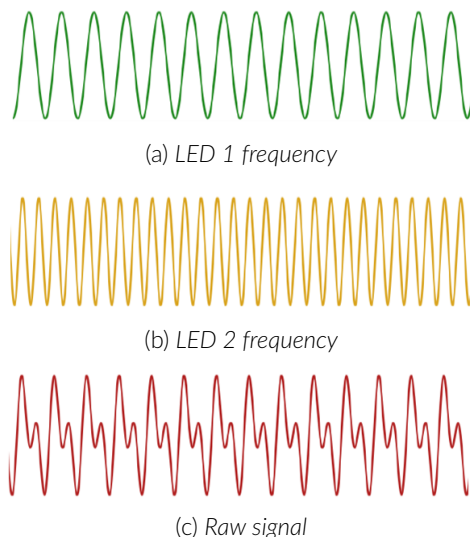
(c) *Raw signal*

Figure 8.38: *Lock-In Acquisition Timing Diagram*

The amplitude changes of the raw signal are due to the collected fluorescence and are dependent of the frequency (Fig. 8.39a). By targeting the known LED frequencies in the raw signal using filters, it is possible to demodulate the fluorescence based on the emission wavelength (Fig. 8.39). The result is separated from the ambient noise that occurred at different frequencies (Fig. 8.39b). The same principle can be applied to demodulate two fluorescent signals.

(a) *Amplitude changes (signal) are frequency dependent*

(b) *Separated signals*
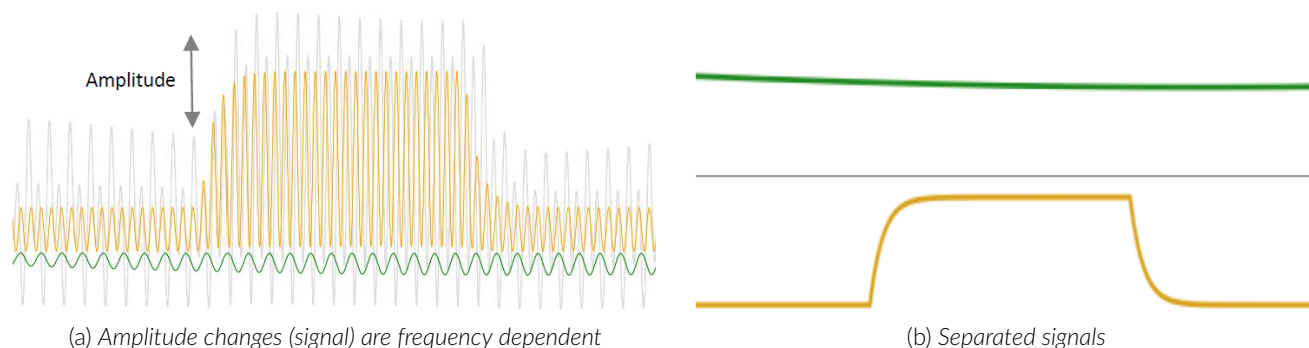
Figure 8.39: *Demodulation separates noise from signal or two signals from each other*

WARNING:
To properly set-up the Lock-In mode, users must have a complete understanding of the wiring of inputs and outputs of they photometry system.

The **Lock-In Mode** parameters are as follows (Fig. 8.40):

1. The **Enable** (Fig. 8.40, 1) row lets users select which output channel to include in the Lock-In settings by clicking the respective check boxes. Each column corresponds to an Analog Out channel of the console (in order, such

that left most column = AOUT1). Users should enabled the output(s) channels that will be driving the input specified in Fig. 8.33, 2.

2. **Trace Name** (Fig. 8.40, 2) is the identity of the Input and Output Channel(s) enabled for this Lock-In configuration.

   - The **AIN #** corresponds the console Analog In port number that receives the raw (non-demodulated) signal from the detector. To change the **AIN #**, select a different **Channel** number from the **Analog In Options** box (Fig. 8.33).

   - The **AOUT #** number corresponds the the Analog Out port on the console that sends eletrical information (including the reference frequency) to the *LED Driver*. While you cannot change the **AOUT #** since it is native to each column of the **Carrier Frequency Options** (Fig. 8.40), changing which port is enabled using the checkbox (Fig. 8.40) or physically moving the cable to a different port on the console allows user to specify the connection of the output.

3. **Reference Frequency** (Fig. 8.40, 4) is the oscillating trigger signal that drives the LED (or device(s) of choice). We recommend using the default values since they are optimised for fiber photometry. But, if modified, frequencies will be re-adjusted in steps of 5.96 Hz. In addition, the reference frequency should not be a multiple a of known noise frequency (e.g. 50 and 60 Hz), or a multiple of another reference frequency.

4. **LED maximum current** (Fig. 8.40, 5) is the largest current that the LED can handle. This value should be set either in low power mode (recommend) or based on the intrinsic maximum current of the LED in use (500 mA or 1000 mA, depending of the type of LED).

   - **Low Power Mode (200 mA)** - allows reduced power for the same voltage. This allows low-power signals to be more stable in time. The **maximal current** is reduced to one tenth of light source normal maximal current. 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).
   *Recommended for Fiber Photometry using *Doric FMC or RFMC* systems*

   - **500 mA** - the **LED maximum current** for the following LEDs: 365 nm, 385 nm & 405 nm.

   - **1000 mA** - the **LED maximum current** for most *Doric* LEDs, except the three mentioned above.

   - **Custom** - this setting allows users to manually adjust the Vmax and Vmin of the LED, regardless of LED maximum current. Care should be taken to remain below the maximum voltage, or the excitation signal will be cropped at the true maximum value (see Fig. 8.41).

Figure 8.40: *Channel(s) configuration window, Analog Input Lock-In*
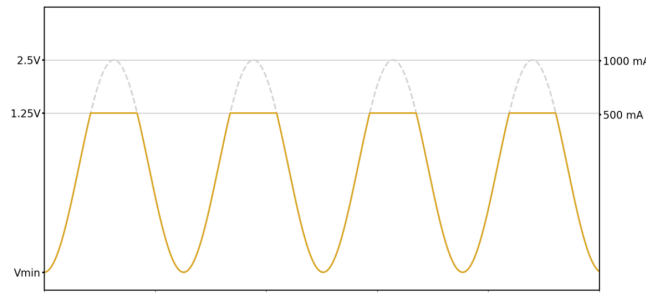
Figure 8.41: *Cropped LED excitation signal*

5. **LED power** (Fig. 8.40, 6) is the percentage of maximum current (converted to voltage) that will be used as **Vmax** and in **External Mode** during the recording, since the LED driver outputs a current proportional to the voltage with a conversion factor of 400 mA/V in standard operation mode, and 40 mA/V in low-power mode.

   - **Note:** The **LED current** should always be set to its maximum on the *LED driver* (and in **External Mode**), while increasing or decreasing **Vmax** should always be done by changing the *FP console* **LED power**.

6. **Vmax Preview** - automatically displays the maximum voltage based on the **LED maximum current** and the **LED power** selected above (Fig. 8.40, 7). **Vmax** can be changed if the **Custom** LED maxiumum current mode is selected. The **Vmax** should never be below 0.3 V, nor above 4.7 V.

   - **Note:** If you are using GCaMP and its isosbestic, we recommend that the isosbestic demodulated trace be about half the power of the GCaMP demodulated trace to reduce the risk of photobleaching (as in Fig. 8.40, 6).

7. **Vmin Preview** (Fig. 8.40, 8) - the default value is set to 0.2 V, but can be changed if the **Custom** LED maxiumum current mode is selected. The **Vmin** should never be below 0.1 V.

8. **Lock-in LPF Frequency** (Fig. 8.40, 9) define the **Cutoff Frequency** of the low-pass filter that extracts the signal and is set to 12 ksps by default. This value was selected because in photometry experiments, the greatest source of noise to the filter is around the carrier frequency above 200 Hz. Thus, with the current filtering algorithm, a cutoff frequency of 12 Hz (corresponding to a decimation factor of 200x) gives the best filtering results.

   - **Note 1:** The saved file **effective sampling rate** is set to 5x the lowpass filter frequency, using a decimation factor (which can be disabled in Saving Options, see Fig. 8.42).

   - **Note 2:** The **Cutoff Frequency** (the frequency at which a -3 dB attenuation will occur) should be chosen as a value close to that of the phenomena observed. A lower cutoff frequency may not result in smaller noise figures.
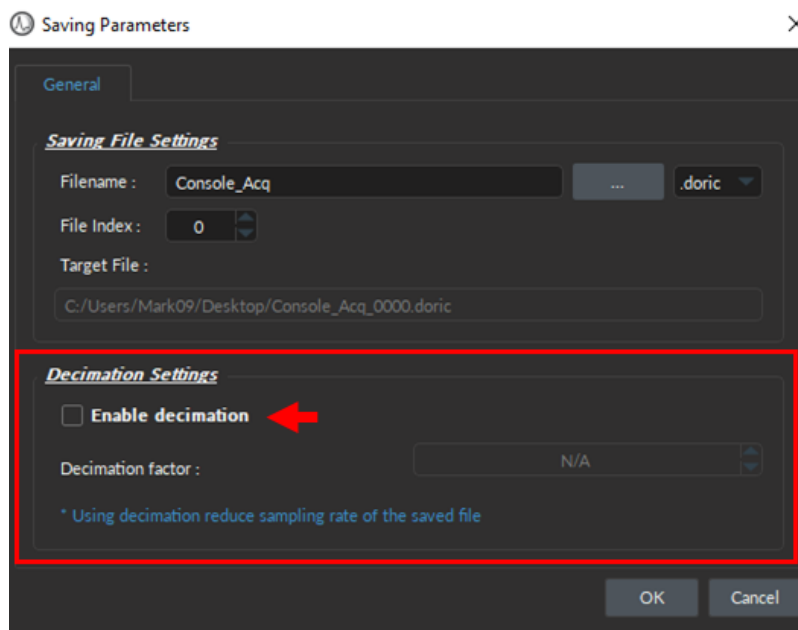
Figure 8.42: *Enable/Disable the decimation factor that reduces Sampling Rate of the saved files*

8.4.7   Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for its measurement.
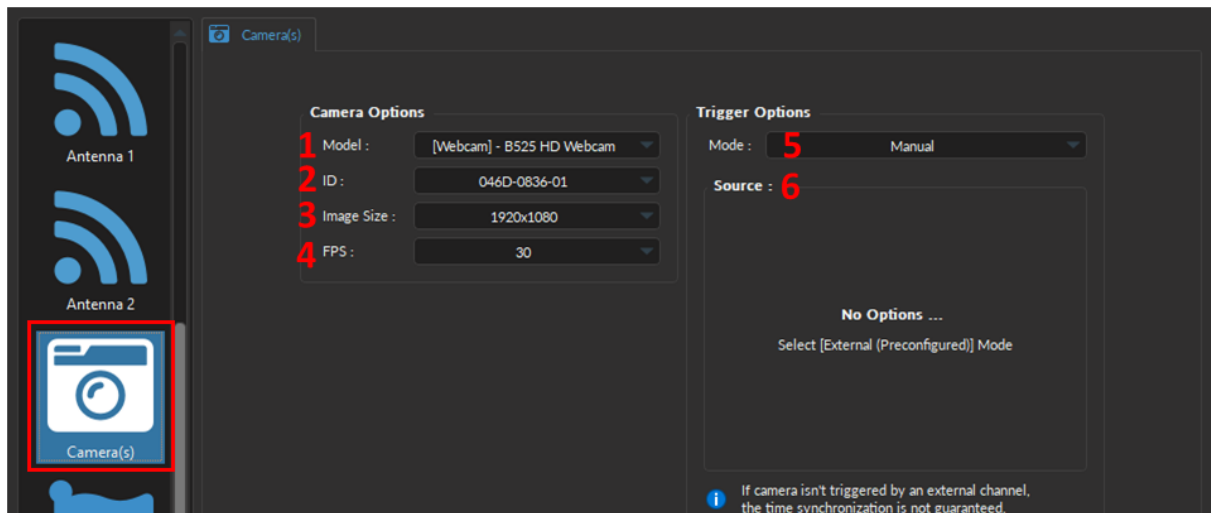
Figure 8.43: *Channel(s) configuration window, Camera*

WARNING:
A camera cannot be used for <u>BOTH</u> **Acquisition Console** and **Camera** modules.
When creating a Camera Channel, if *"No available camera detected..."*, <u>disconnect</u>
the camera in the **Device Selection** window to close the extra module.

Camera Options:

1. The **Model** (Fig. 8.43, 1) allows you to select the camera of choice based on the type of camera.

2. **ID** drop-down list (Fig. 8.43, 2) is used to select a camera based on its unique ID. The ID is particularly useful if multiple cameras of the same model are required for the experiment.

3. The **Image Size** (Fig. 8.43, 3) is used to set the resolution of the image. The large the number of pixels used for width x height, the better the resolution. Currently, image size can ranges between 160x120 to 1920x1080 pixels.

4. The **FPS** (Fig. 8.43, 4) is used to specify the frame rate of the camera (i.e. the number of images displayed per second). FPS can be any value between from 5-30 for web cameras and up to 60 FPS for the *Doric Behavior Camera*.

Trigger Options:

5. The **Mode** (Fig. 8.43, 5) sets the type of trigger that will control the camera. Depending on the type of camera, at most three modes are available:

WARNING:
If the camera isn't triggered by an external channel,
the **time synchronization is NOT guaranteed**.

- **Manual** - Selecting the *Live* or *Record* buttons located in the Acquisition Tab will the trigger the start of the camera recording. ***The time difference between the actual start time and when the first frame is received depends on the camera itself.*** Around a 1 second delay is pretty common for web cameras.

The time delay (in ms) between the photometry and video data is recorded in the *DifferenceMasterStart-ToFirstImage* attribute, located in .*doric* file under the **Web Camera ID** folder (Fig. 8.44). This attribute can be used to retroactively align the video and fiber photometry data during analysis.
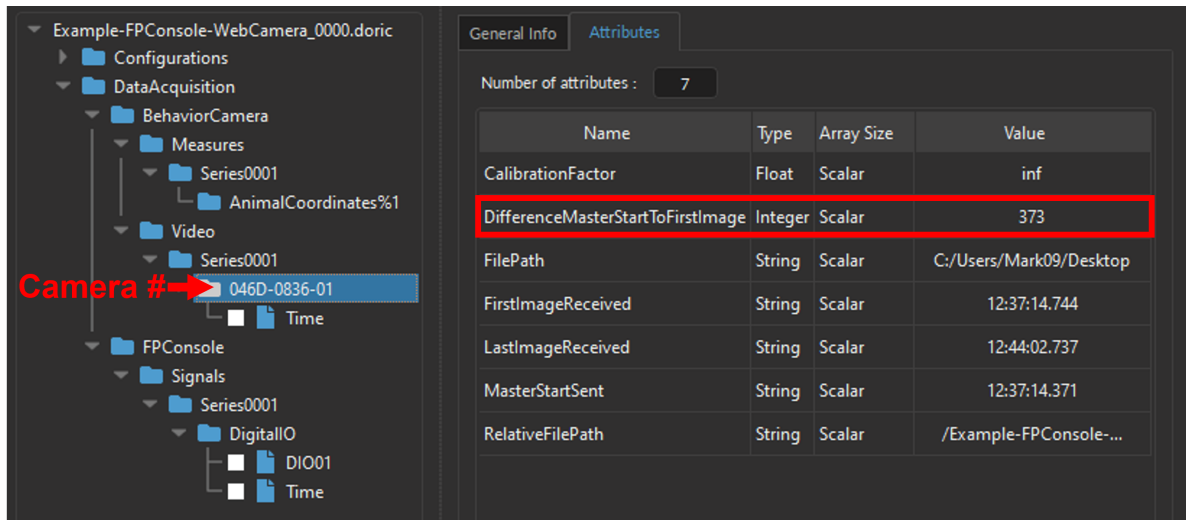
Figure 8.44: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **External** - Will drive the camera using external TTL signal through the trigger cable (Frequency: 30 Hz (or camera FPS); Time ON: 5 ms). This signal can come from any external device connected to the opposite end of the trigger cable. If using *Doric Neuroscience Studio* to synchronize the recording, use *External (Preconfigured)* mode below instead. ***ONLY offered for the *Doric Behavior Camera*.***

- **External (Preconfigured)** - This is the recommended mode to synchronize the camera with the rest of the Acquisition system. This mode automatically creates an additional Digital I/O channel configured to drive the camera at the proper frequency and Time ON. ***ONLY offered for the *Doric Behavior Camera*.***

6. The **Source** (Fig. 8.43, 6 & Fig. 8.45) is only used for the **External (Preconfigured)** mode, and displays the **Digital I/O** channel with the preconfigured parameters that will be created at the same time as the **Camera Channel** (Fig. 8.45). For detailed description of each Digital I/O parameter see the corresponding section in the Fiber Photometry System Manuel. Briefly, key parameters include:

a) The **Channel** (Fig. 8.45, a) corresponds to the physical Digital I/O channel number on the Console that is connected to the trigger cable of the *Doric Behavior Camera*.

b) The **Mode** (Fig. 8.45, b) is by default set to the *Square (TTL)* which provides the external trigger signal to the camera. This parameter cannot be changed.

c) The **Frequency** (Fig. 8.45, c) corresponds to the **FPS** set in the **Camera Options**. Changing the **FPS** will automatically change the **Frequency** in the **Sequence(s) Options**.

d) The **Duty Cycle** (Fig. 8.45, d) is by default 50%. The frame will be taken at the start of each square pulse.

Figure 8.45: *Channel(s) configuration window, Camera - External (Preconfigured)*

8.4.8 KeyPress Event(s)

Keypress Event(s) are ideal when manually labelling or annotating events during experiments. Specifically, selecting any keyboard key during a recording will save the output synchronized to other measurements. Keypress events can be used to:

- Flag disruptions during the experiment, such as lights on, door opened, construction noise, etc.

- Record experimentally relevant events/stimuli, such as airpuff, licks or any other behavior.

WARNING:
Keyboard event(s) timing are **accurate within 1 second** due to variations in Windows priority management and buffering of the signals.

8.4.8.1 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the botton of the window (Fig. 8.46, left). To remove a KeyPress, use - button (Fig. 8.46, right).

- **NOTE:** Selecting the + button (without clicking the *Add* button or the *Close* of the *Channel Configuration* window) will **automatically** add the Keypress Event channel at the **bottom** of the Acquisition View window, below any pre-existing channels (Fig.8.46).
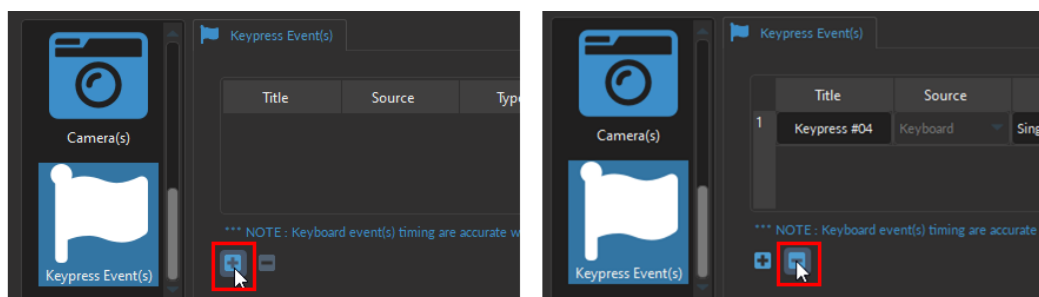
Figure 8.46: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 8.47) in the **Acquisition View**.
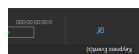
Figure 8.47: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 8.49:

1. The **Title** allows you to give a name for the Keypress event.

2. The **Source** is by default *Keyboard*.

3. Three **Types** of Keypress Event(s) can be specified with the drop-down list:

   - **Single** - Records single event at the touch of a key (Fig. 8.48a).

- **Toggled** - Records the start and end of an event using the same key. First press denotes the start of the event while a second press denotes the end of it (Fig. 8.48b).
- **Timed** - Records an event for a predetermined duration of time (Fig. 8.48c). Every keypress is a new event, with the start of the event occuring when the key was depressed.

(a) *Single*

(b) *Toggled*

(c) *Timed*

Figure 8.48: *Three types of Keypress Event(s)*

4. The **Duration** is only used for the **Timed** Keypress type to specify the predetermined amount of time a Keypress Event will span. The duration is set in hh:mm:ss:zzz.

5. Select the **Color** field to open the **Select Color** window. Basic colors are provided, in addition to custom colors can be created and stored.

6. The **Shortcut Key(s)** can be any keyboard key, including space bar, enter, backspace, any letters, number and special characters (*, !, ? etc.). To specify the key, click inside the *Shortcut Key(s)* cell, then press the keyboard key of choice. If a key was properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 8.49, column 6).

7. The **Information** column provides space to make notes or write a short description of the Keypress Event.

	1 Title	2 Source	3 Type	4 Duration	5 Color	6 Shorcut Key(s)	7 Information
1	Event	Keyboard	Single	N/A		Return	
2	Disruption	Keyboard	Toggled	N/A		Space	Loud sounds, lights on, etc.
3	Airpuff	Keyboard	Timed	00:00:01:000		A	

*** NOTE : Keyboard event(s) timing are accurate within 1 second due to variations in Windows priority management & buffering of the signals.

Keypress Event(s)

0:00:00:00:000   0:00:00:05:000   0:00:00:10:000   0:00:00:15:000

Figure 8.49: *Channel(s) configuration window, KeyPress Event(s)*

#2   #3 #4

ss #1   #2   #3

0:00:00:05:000
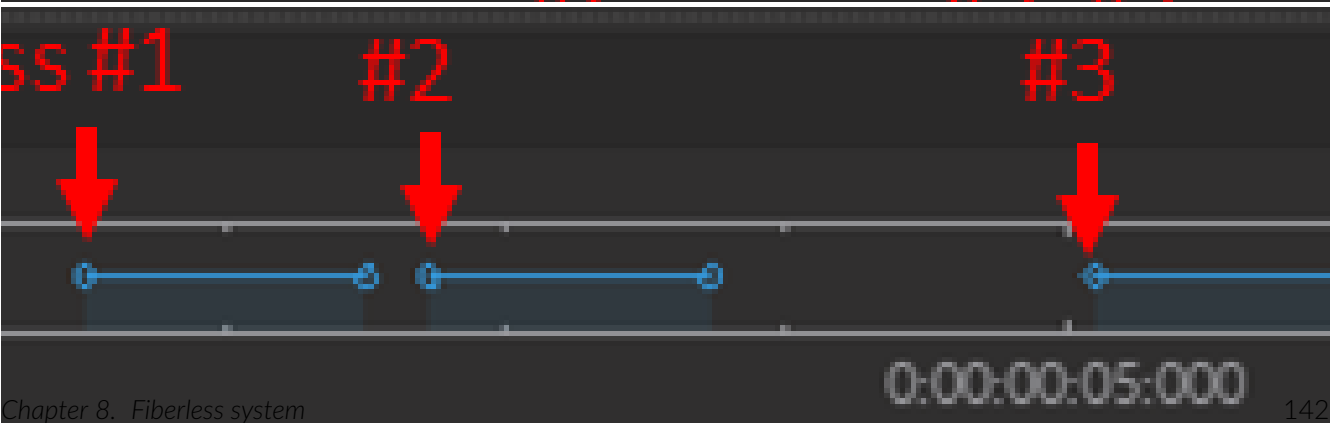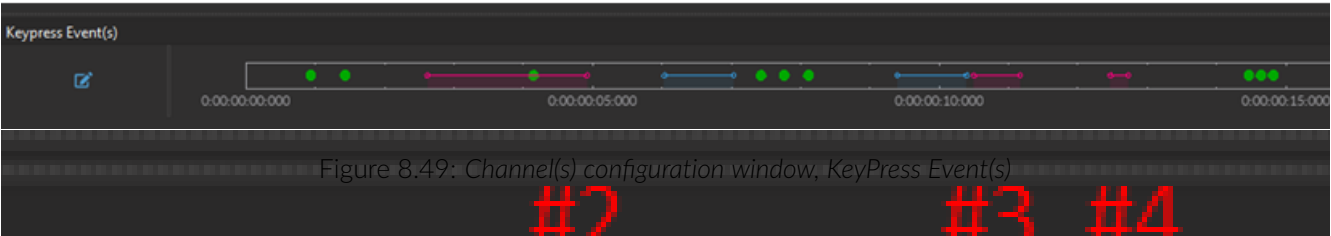
8.5   Acquisition View

The **Acquisition View** displays all the information concerning active channels: **Control box** (Fig.8.50a) and the **Graphs** (Fig.8.50b).

If neither **Control Box** nor **Graphs** are displayed in the **Acquisition View**, this means channels have yet to be configured. User can either use the **Load Configuration** button (see Section 8.3.2) to load a *.doric* file with previously saved channel parameters, or user can manually add channels using the **Add Channel** button (see Section 8.3.2).

(a) *Channels*   (b) *Common parameters*
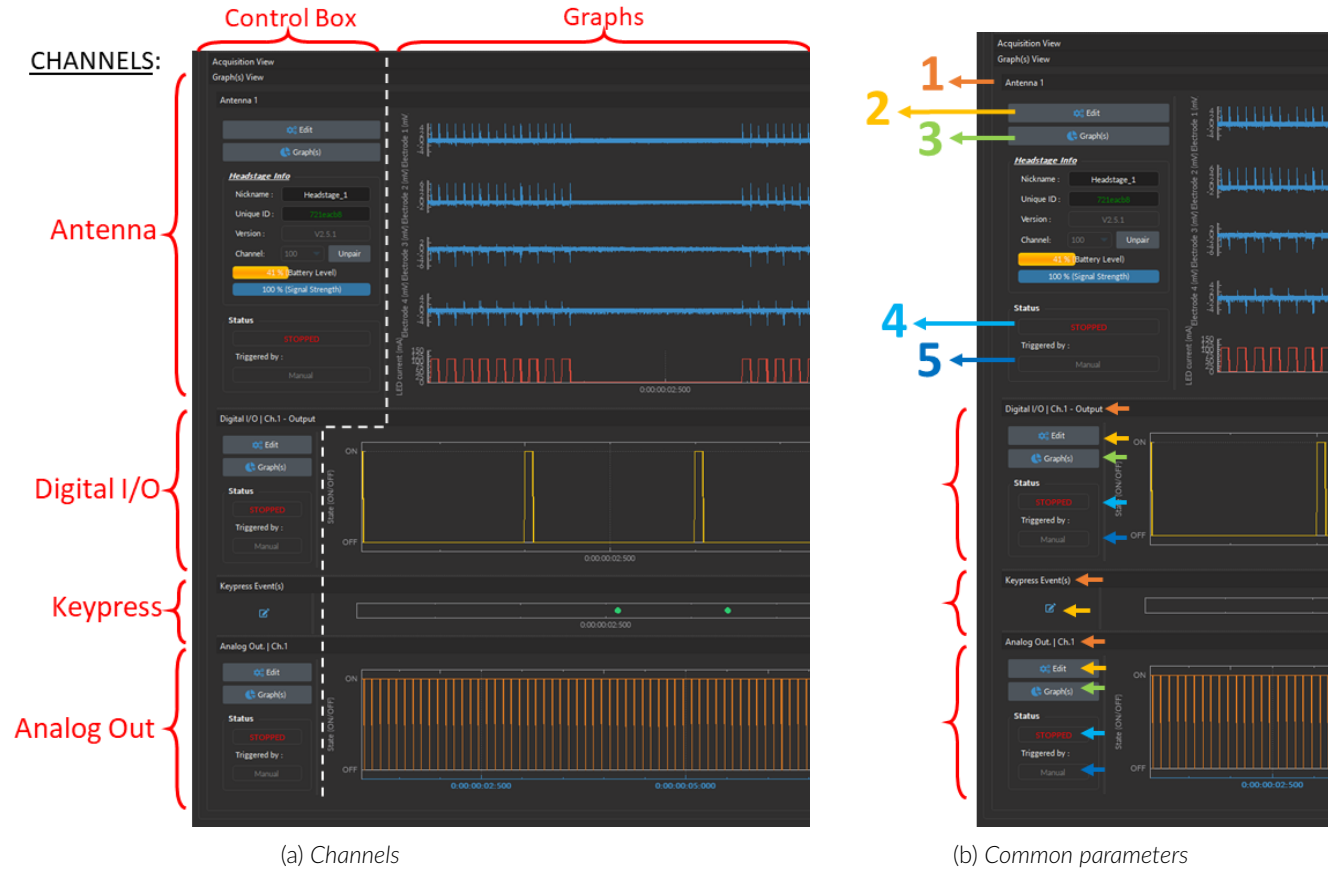
Figure 8.50: *Acquisition View*

8.5.1   Channel Control Box

Each channel **Control box** shows the following basic elements (Fig.8.50b), with additional elements available for specific channel types:

1. The **Channel name** (Fig. 8.50b, 1) is located on the upper left of the **Control box**, 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 (Fig. 8.51).

2. The **Edit** button (Fig. 8.50b, 2) opens the **Channel Configuration** window, were channel parameters can be modified (See section 8.4.1).

3. The **Graph(s)** (Fig. 8.50b, 3) button opens the **Graph Options** window (Fig. 8.51) corresponding to the channel whose graph will be modified. This window allows users to configure visualization and naming parameters of each channel graph (Fig. 8.51). If a channel has multiple traces, parameters to configure each trace individually will appear automatically on different rows (Fig. 8.51). **Graph(s) Options** parameters (Fig. 8.51) are as follows:
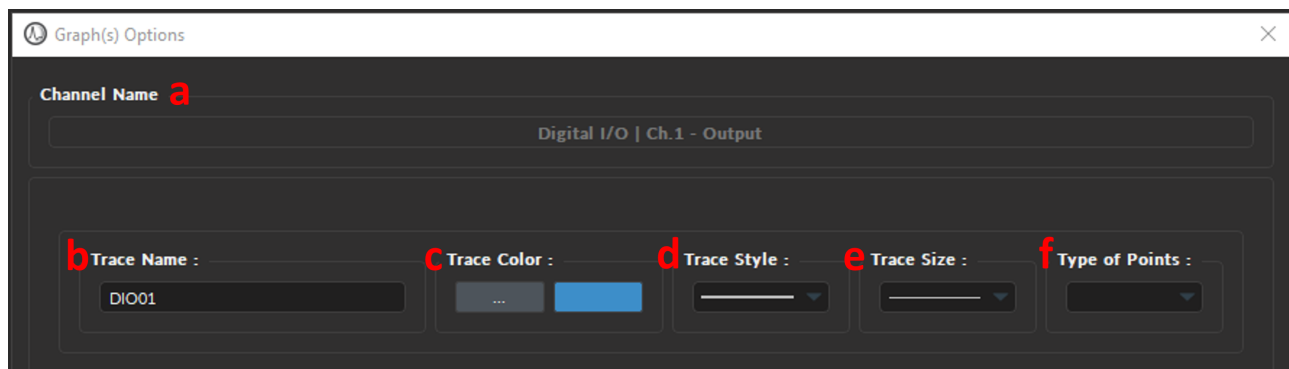
Figure 8.51: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 8.51, 1) is the default name assigned by the software, which includes the type of channel (Digital / Analog In or Out) and the location of said channel on the console (BNC connector 1-4).

b) The **Trace Name** text-box (Fig. 8.51, 2) allows users to specify a name for the trace, instead of the default name generated by the software.

c) The **Trace Color** button (**...**) (Fig. 8.51, 3) opens the **Color Select** window (Fig. 8.52), 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.

d) The **Trace style** drop-down list (Fig. 8.51, 4) 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.

e) The **Trace size** drop-down list (Fig. 8.51, 5) allows the selection of the trace size. Using a bigger **Trace size** than the default may result in slower display and performance degradation.

f) The **Type of points** drop-down list (Fig. 8.51, 6) 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.
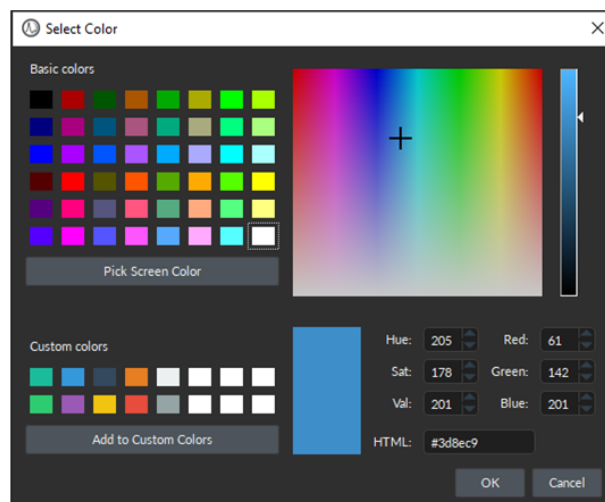
Figure 8.52: *Select Color Window*

4. The **Status** bar (Fig. 8.50b, 4) displays acquisition status. **STOPPED** is displayed when the acquisition is inactive, and **STARTED** when acquisition is active.

5. The **Triggered by:** (Fig. 8.50b, 5) text-box displays the source of the trigger for that channel, which can either be Manuel (i.e. selecting the **Record**/**Live** button) or a specific channel that provides external trigger signal.

The **Headstage Info** box (Fig. 8.53) displays the *EP Console*-specific elements which are important to pair, control and monitor the wireless headstage. The following elements are included
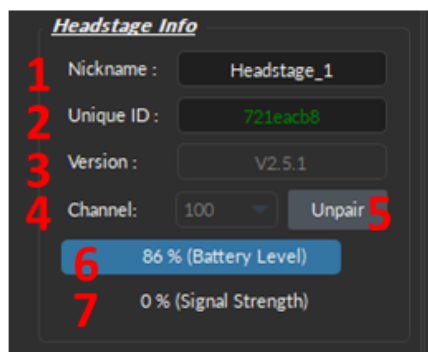
Figure 8.53: *Acquisition View, Controls*

1. The **Nickname** text-box (Fig. 8.53, 1) allows the user to change the name used for the connected headstage.

2. The **Unique ID** box (Fig. 8.53, 2) displays the unique ID sequence associated with the headstage currently in use.

3. The **Version** box (Fig. 8.53, 3) displays the headstage firmware version.

4. The **Channel** drop-down list (Fig. 8.53, 4) shows the channel currently in use by the headstage. When using two headstages, the channel must be different for each headstage.

5. The **Pair** button (Fig. 8.53, 5) 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.

6. The **Battery Level** bar (Fig. 8.53, 6) displays the headstage battery level, in %, at all times.

7. The **Signal strength** bar (Fig. 8.53, 7) displays the percentage of data packets lost during the WiFi transmission. 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. Note that the signal will weaken if the animal/headstage is too far from the antenna or if there are obstructions between them.

To pair the headstage (Fig. 8.54), (1) click **Pair** and (2) unique ID in green will appear if correctly paired.
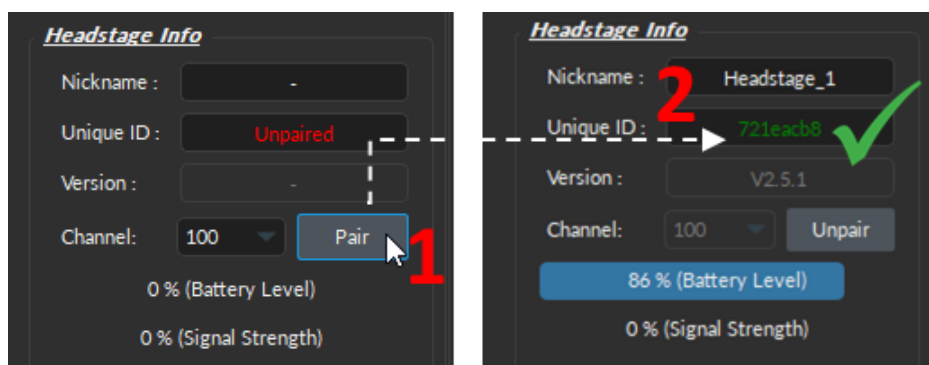
Figure 8.54: *Pairing Headstage*

8.5.2   Channel Graph Visualisation

Besides editing the trace of the channel **Graph**, which can be done through the **Edit** button of the **Control box** (section 8.5.1), other features of **Graph** view can be directly manipulated by selecting elements of the **Graph** itself. This section includes changing axis properties, manual zoom, and determining instantaneous values.
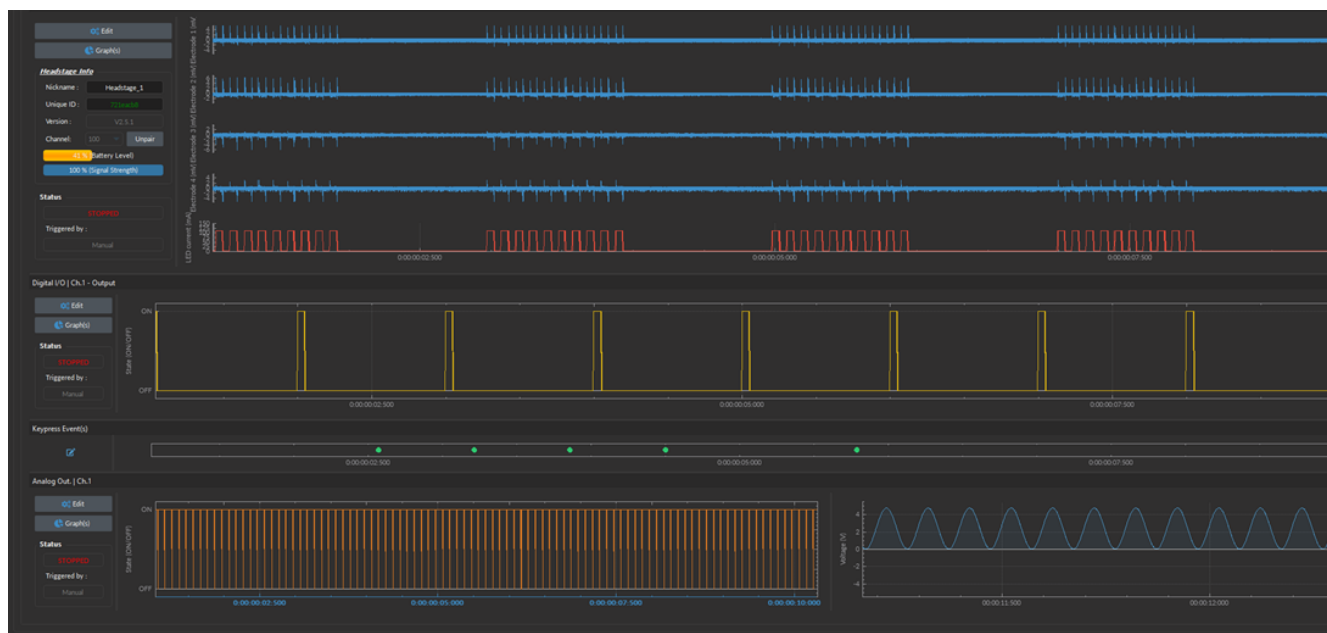
Figure 8.55: *Acquisition View - Graph*

- *Axis Options* - Each **Graph** (Fig. 8.51) has both a **Voltage** or **State** as the vertical axis and **Time** as the horizontal axis. Double-clicking either axis will open an **Axis Options** window where the axis limits can be set, similar to the **Zooming Range** in the **View Tab**. Any changes done on a horizontal axis will change the axis limits for every channel.
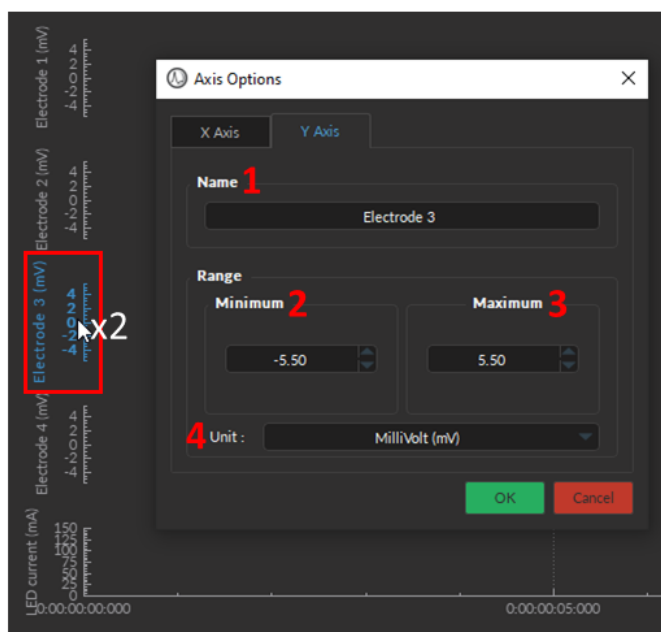
Figure 8.56: *Double click on any axis to open its Axis Options window*

- By clicking and **dragging the graph sideways or upwards**, one can scroll through nearby values on either axis, keeping the zoom range constant. Any changes done on a horizontal axis will change the axis limits for every channel.

- Using the **Mouse Scroll Wheel**, one can change the zoom range of the graph. Any changes done on a horizontal axis will change the axis limits for every channel.

- The **Instant values** box can be activated by double-clicking the **Input graph** box and selecting **Show instant values** (Fig. 8.57). 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**. To remove instantaneous value, double click on the dot.
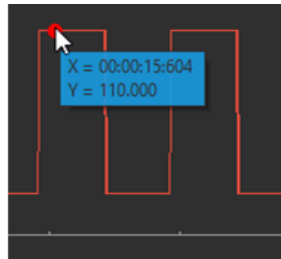
Figure 8.57: *Acquisition View - Instant values*

- The **Channel tabs** 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.

- Analog output channels display an **Active state** graph (Fig. 8.58, left panel). This graph displays whether the channel is outputting a signal (On, V≠0) or not (Off, V=0).

- Output channels display a **Preview** graph (Fig.8.58, right panel), showing a preview of the pulse sequence.
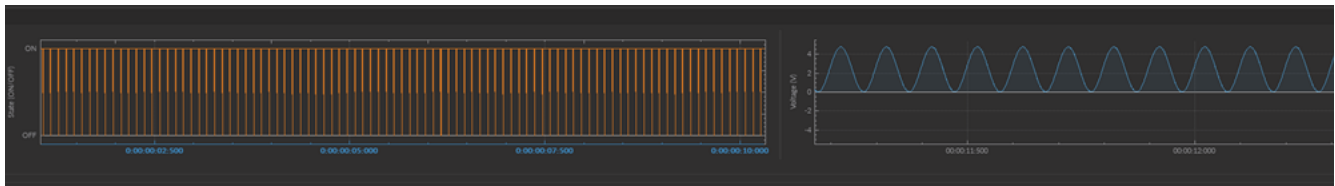
Figure 8.58: *Acquisition View - Output graph*

Optpgenetics TTL Pulse Generator

The *Optogenetics TTL Pulse Generator* (OTPG; 4 or 8 channels) are controlled by the *Doric Neuroscience Studio*. Various pulses train sequences can be designed for any type of experiment.

The *OTPG* user interface (Fig. 9.1) is split into two main sections: the **Controls & Settings** (Section 9.2) and the **Acquisition view** (Section 9.3). From these sections, the **Channel(s) configuration** (Section 9.1) window can be accessed to add and configure channels.
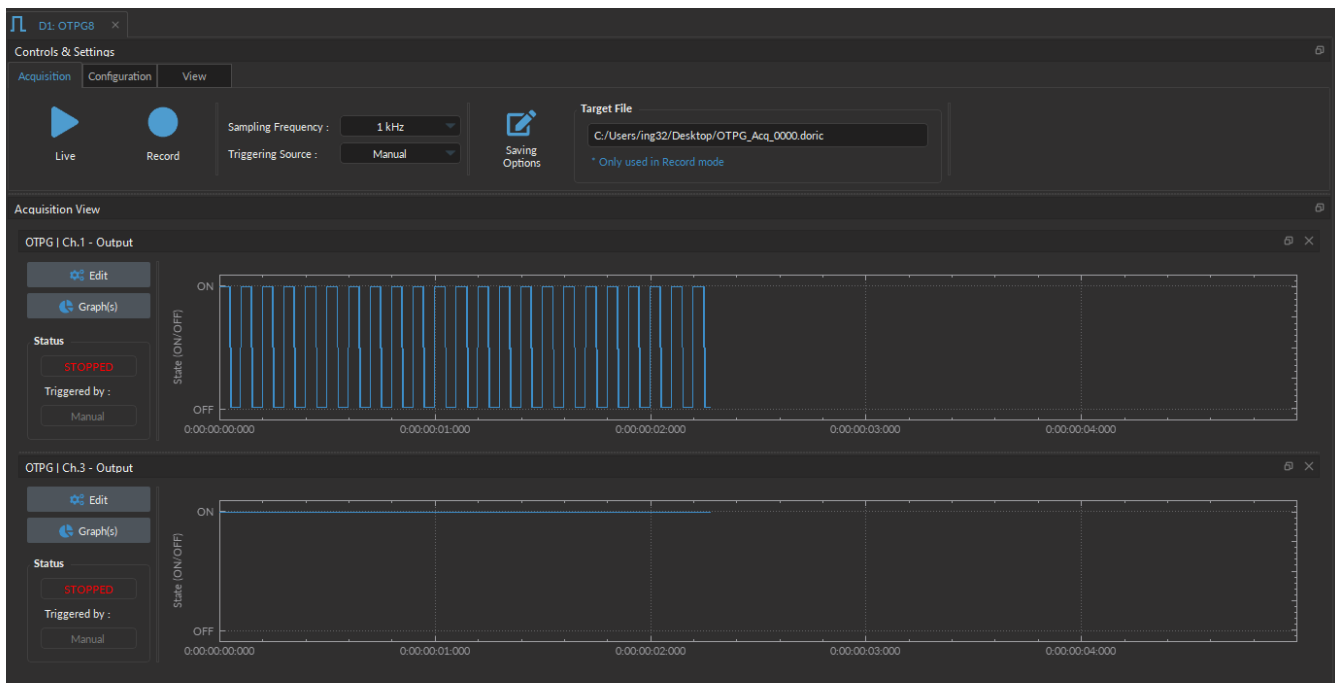
Figure 9.1: *Optogenetics TTL Pulse Generator Interface.*

9.1 Channel Configuration

9.1.1 Channel Configuration Window Overview
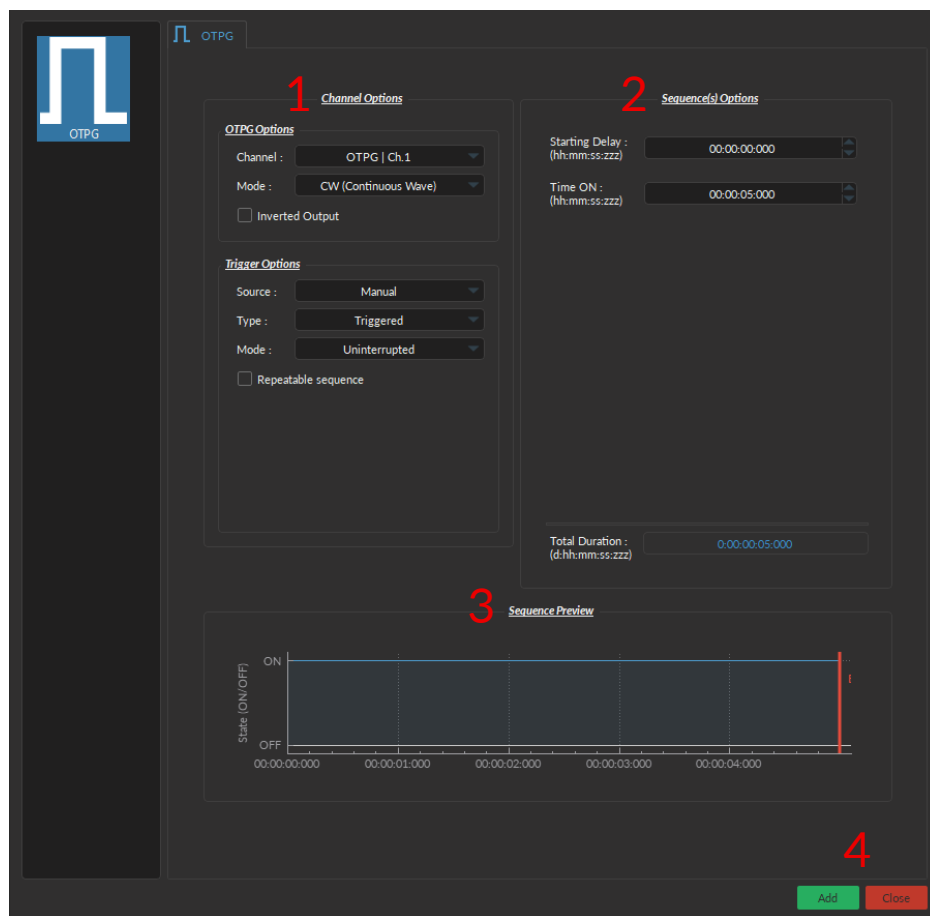
Figure 9.2: *Channels Configuration Main Interface.*

The **Channels configuration** window is used to configure each channel. The window can be accessed by using either the **Add channel** or **Edit** buttons. This window is separated into multiple sections shown in Figure 9.2 that are defined below.

1. The **Channel Options** section allows you to define the Channels Type and the Triggering Options. The different fields of this section are explained in more detail in section 9.1.2.

2. The **Sequence Options** defines the parameters of each pulse sequence for the channel. These parameters are different for each Channel Code. 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**. The different fields for the different Channel Mode are explained in more detail in section 9.1.3

3. The **Sequence Preview** section shows a visualization of the output sequence that will be generated by the current configuration.

4. The **Add** button will save the current channel configuration and enables a new channel to be configured. The **Close** button will close the window without saving the current channel configuration.

9.1.2   Channel Options Section
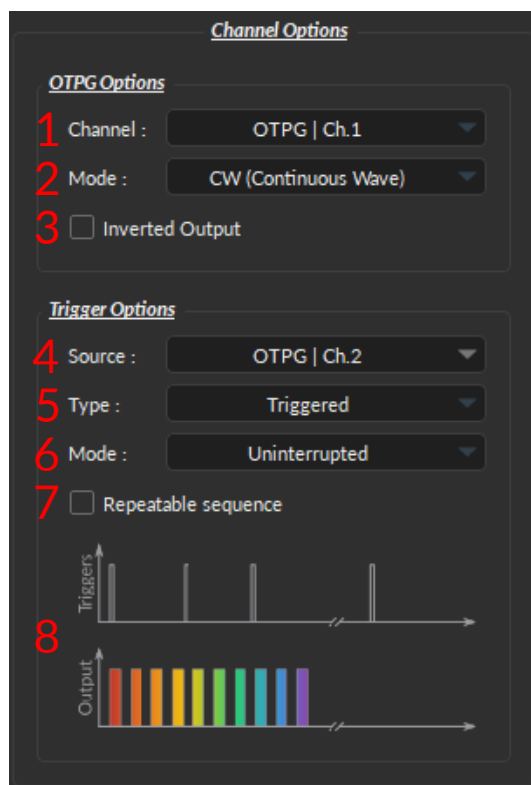
Figure 9.3: *Channel Options of the Channel Configuration Window.*

The Channel Option section is separated in 2 sub-section, the **OTPG Options** section that defines the channel and its mode and the **Trigger Options** section that control the trigger method of the selected channel. The Trigger Options are not available when the channel is in Input mode.

9.1.2.1   OTPG Options

1. The **Channel** field identifies which of the available channels is currently being modified. The channel can be changed by selecting a new one from the drop-down list.

2. The **Mode** field identifies the type of signal sent. Three modes are available, **CW (Continuous Wave)**, **Square**, and **Input** mode. Each mode enables different options of the Sequence Option section that are explained in more detail in section 9.1.3.

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

9.1.2.2   Trigger Options

4. The **Source** trigger option allows to chose between a **Manual Trigger** (activated by the user) or an **Input** trigger, which is coming from a channel input.

5. The **Type** defines the type of trigger that is used to start/stop a sequence. The **Triggered** type can starts and stops a sequence at a rising edge while the **Gated** type can starts the sequence at a rising edge and stops it at a falling edge. A more refined interaction of the trigger with the defined sequence can be set up using the **Mode** field. Not all Trigger Type are available for each combination of Trigger Mode and Repeatability. The different combinations are shown in Figure 9.8.

6. The **Mode** field defines how the trigger activates a sequence. Each mode are not compatible with each combination of trigger type and repeatability. Figure 9.8 shows the different available combinations for the different Trigger Modes. Four Modes are available and are the following:

- **Uninterrupted**: This mode activates the channel sequence when an input greater than 3.3 V is detected by the BNC input. Following input pulses will be ignored while the sequence is running (Fig. 9.4). When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Fig. 9.4b). This mode is available for *Triggered* pulse only.

(a) *Triggered Non-Repeatable Sequence*   (b) *Triggered Repeatable Sequence*
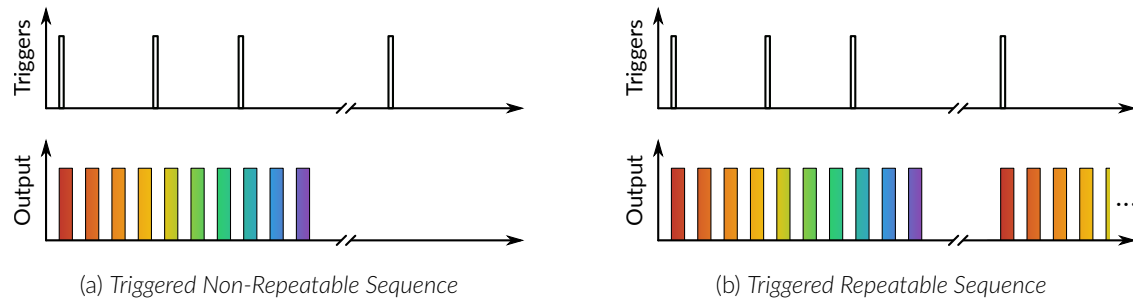
Figure 9.4: *Uninterrupted Sequence Mode.*

- **Pause**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 9.5). Following input pulses (when *Triggered*, Fig. 9.5a) or falling edge (when *Gated*, Fig. 9.5c) will pause the sequence and the sequence will continue when the next rising edge is received. When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Figs. 9.5b and 9.5d).

(a) *Triggered Non-Repeatable Sequence*   (b) *Triggered Repeatable Sequence*

(c) *Gated Non-Repeatable Sequence*   (d) *Gated Repeatable Sequence*
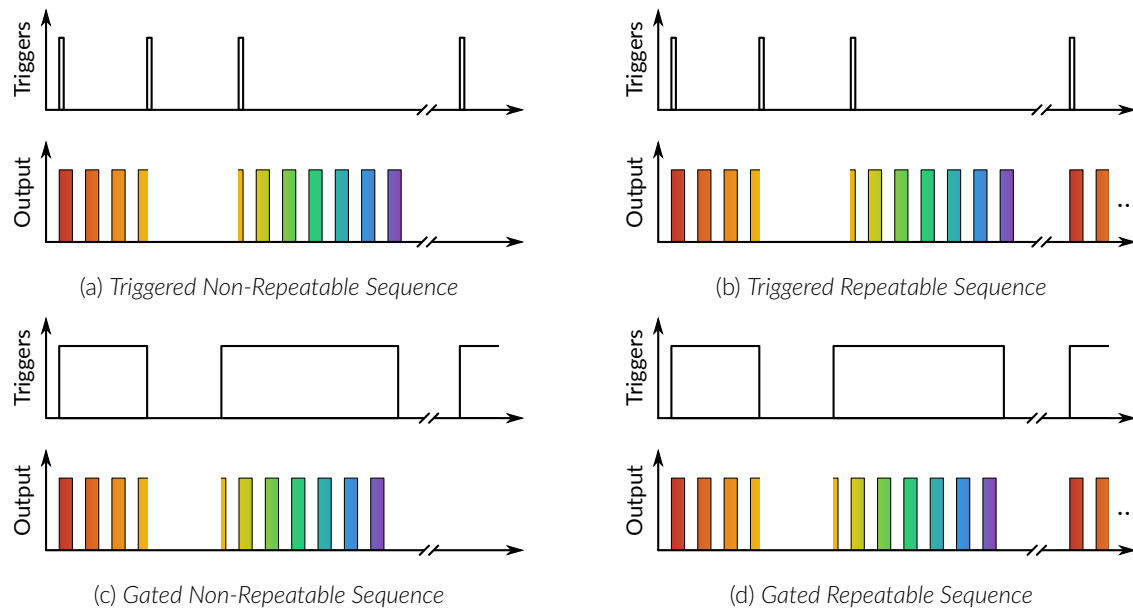
Figure 9.5: *Pause Sequence Mode.*

- **Continue**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 9.6). The following input pulse (when *Triggered*, Fig. 9.6a) or a falling edge (when *Gated*, Fig. 9.6c) will turn off the output, but the sequence will continue. The output will be turned back on at the reception of the following rising edge. Triggers only affect the output voltage value. When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Figs. 9.6b and 9.6d).

- **Restart**: This mode activates the channel sequence when a rising edge higher than 3.3 V is detected on the BNC input. The following input pulse (when *Triggered*, Fig. 9.7a) or falling edge (when i, Fig. 9.7b) will

(a) *Triggered Non-Repeatable Sequence*

(b) *Triggered Repeatable Sequence*

(c) *Gated Non-Repeatable Sequence*

(d) *Gated Repeatable Sequence*
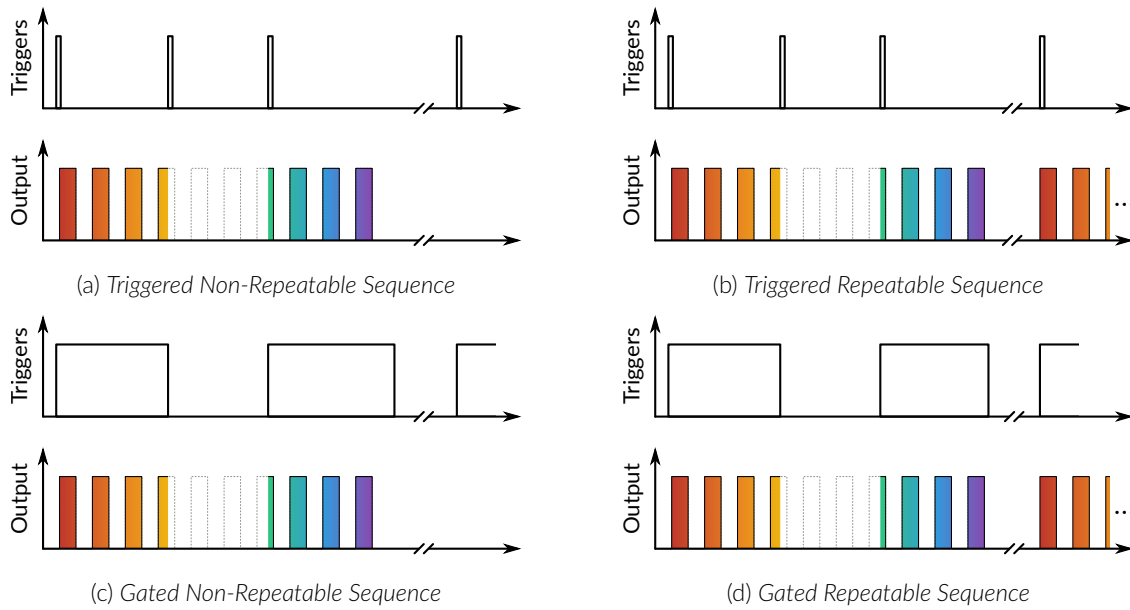
Figure 9.6: *Continue Sequence Mode.*

stop the sequence and the sequence will restart from the beginning when the next rising edge is received. When the sequence is completed, it will restart with the next input pulse.

(a) *Triggered Non-Repeatable Sequence*

(b) *Triggered Repeatable Sequence*
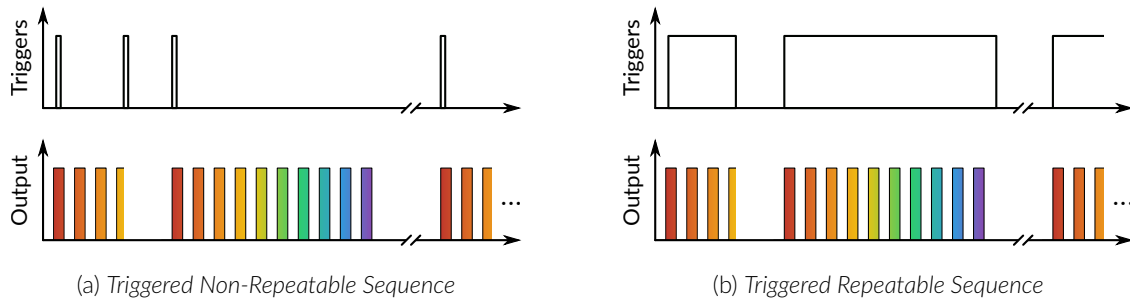
Figure 9.7: *Restart Sequence Mode.*

	Triggered		Gated	
	Non-repeatable sequence	Repeatable sequence	Non-repeatable sequence	Repeatable sequence
Uninterrupted	✔	✔		
Pause	✔	✔	✔	✔
Continue	✔	✔	✔	✔
Restart		✔		✔

Figure 9.8: *Trigger options possibilities.*

7. The **Repeatable sequence** checkbox, when selected, allows a sequence to be repeated. Not all modes and trigger types can be repeated. Please refer to the Figure 9.8 to know the repeatable sequence combinations.

8. The **Sequence Visualisation** shows a graphical representation of the behavior of the selected Trigger Option Type, Mode and Repeatability.

9.1.3   Sequence Options Section

9.1.3.1   CW (Continuous Wave) Mode

Figure 9.9: *Sequence Options of the CW Channel Mode.*

The **CW (Continuous Wave)** channel mode allows the creation of a continuous TTL signal. The following elements appear in the **Sequence Options** section (Fig. 9.9).

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 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**.

9.1.3.2 Square Mode

Figure 9.10: *Sequence Options of the Square Channel Mode.*

The **Square** channel mode allows the creation of a square TTL pulse sequence. The Sequence Options of this mode are shown in Figure 9.10 and are explained below.

1. The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the signal.

2. 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).

3. The **Time ON** 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.

4. 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.

5. The **Number of sequences** sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** sets the delay between each sequence.

7. 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**.

9.1.3.3 Input Mode

The **Input** mode (Fig 9.11) 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 OTPG. No **Sequence Options** or **Sequence Previews** are available for this mode.
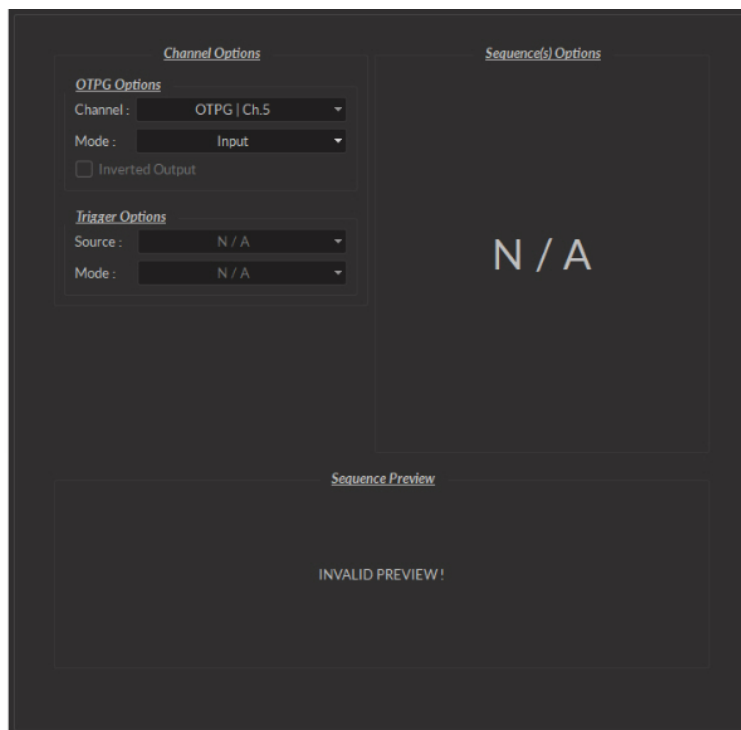
Figure 9.11: *Channels Configuration of the Input Mode Interface*

9.2 Control and Settings

The **Control and Settings** box is used to manage the different parts of the software. It contains three tabs, the **Acquisition**, **Configuration**, and **View** Tabs.

9.2.1 Acquisition Tab

Figure 9.12: *Acquisition Tab*

The different buttons and fields of the **Acquisition Tab** are shown in Figure 9.12 and their functions are explained below.

1. The **Live** button starts all the configured channels without recording their signal.

2. The **Record** button starts all the configured channels and record the signal for each of them at the defined **Sampling Frequency**. The recorded datas are saved in a .doric file (hdf5 based file) where the saved path and filename can be defined through the **Saving Options** button.

3. The **Stampling Frequency** field defines the frequency at which the recorded signal (using the Record button) is sampled. Note: In Doric Studio version 6 and later, the record button also saves the configuration of the OTPG (channel parameters, sampling frequency, ...) in the same .doric file. Use the Load config button (see section 9.2.2) to load this parameters.

4. The **Triggering Source** field defines if the sampling will be triggered manually (by clicking Live or Record) or will be triggered by one of the input channel.

5. The **Saving Options** button opens an external window that allows you to configure the saving path and filename of the channels signal file.

6. The **Target File** field indicates the saving path and filename of the channels signal file.

9.2.2   Configuration Tab
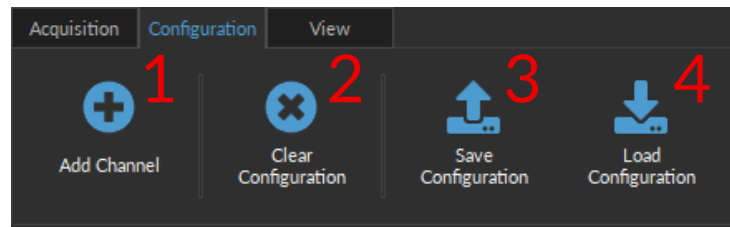
Figure 9.13: *Configuration Tab*

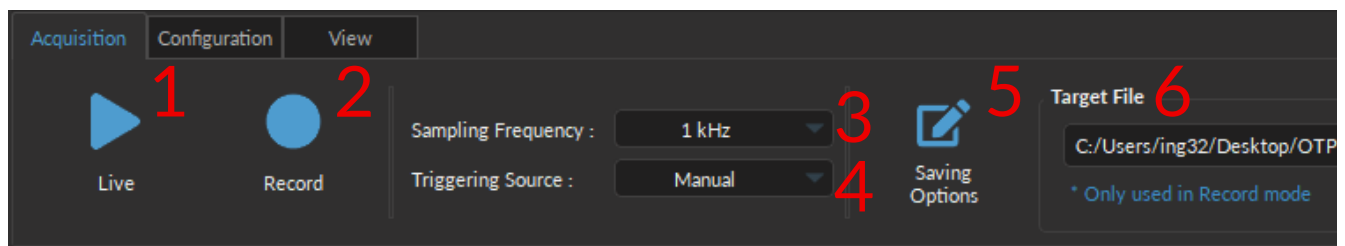The different buttons of the **Configuration Tab** are shown in Figure 9.13 and their functions are explained below.

1. The **Add Channel** button opens the **Channels configuration** window to setup the channels. This window is detailed in section 9.1.

2. The **Clear Configuration** button resets the acquisition view and all other parameters set. Any configurations already set will be lost.

3. The **Save Configuration** button is used to save the OTPG configuration in a **.doric** format.

4. The **Load Configuration** button allows an OTPG configuration in **.doric** format to be loaded. Recorded data files also contains the configuration used during the experiment and this configuration can be loaded using this button.

9.2.3   View Tab
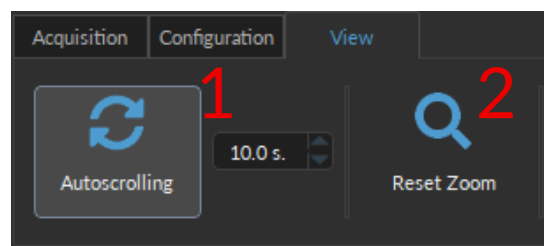
Figure 9.14: *View Tab*

The different buttons and fields of the **View Tab** are shown in Figure 9.14.

1. The **Autoscrolling** button, when clicked, makes the graphs scroll as new data appears. The duration (in seconds) kept on display is controlled by the field beside the button.

2. The **Reset Zoom** button resets the horizontal axis of all graphs displayed in the **Acquisition View** to the duration chosen in the **Autoscrolling** field.

9.3   Acquisition View
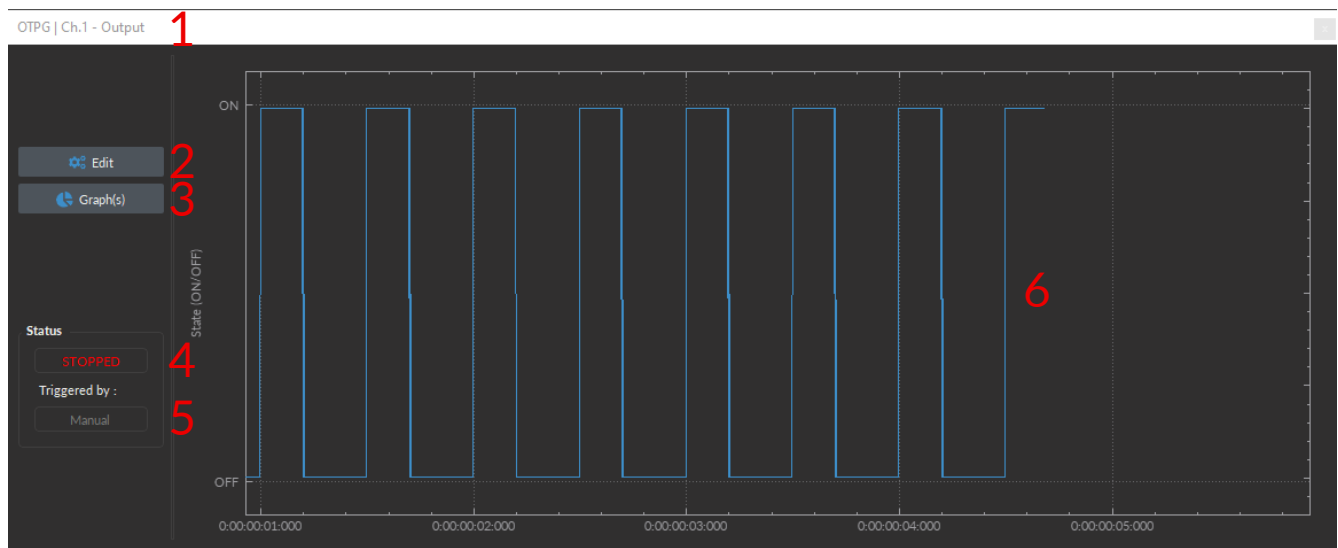
Figure 9.15: *Acquisition View Box*

The **Acquisition View** box displays all the information concerning active channels. Each channel chosen using the **Add Channel** button is displayed in this section, occupying a rectangular box. Each **Channel box** shows the following basic elements shown in Figure 9.15.

1. The **Channel name** is located on the upper left of the **Channel box**, identifying the type of channel and it's number, corresponding to the one displayed on the *OTPG*. The channel name can be modified in the Graph(s) menu.

2. The **Edit** button allows the editing of channel parameters, opening the **Channel Configuration** window. For additional information, see section 9.1.

3. The **Graph(s)** button opens an external window with options to change the displayed parameters of the graphic as well as the possibility to change the graphic name.

4. The **Status** box shows whether the channel is active, displaying **STOPPED** when inactive and **RUNNING...** when active.

5. The **Triggered By** box shows the current trigger source of the channel sequence.

6. The **Sequence Display** box shows a graphic of the recorded channel. It shows the signal as it is generated by an output channel or as it is received by an input channel. The sampling rate of the displayed graphic is controlled by the **Sampling Frequency** field in the Acquisition Tab.

Signal Analyzer

Doric Neuroscience Studio includes data processing modules for both basic fiber photometry and electrophysiology. This module provides an easy means to process data from the data acquired by the Doric's *Acquisition Console*. The software loads data in .doric format, implements signal processing functions, and saves the traces in .doric format.

Note that users that have purchased **DANSE**, Doric's specialized data analysis software, should load raw data directly into **DANSE** (skipping **Signal Analyzer** module) since all the data processing functionalities offered in this module are also included in **DANSE**, in addition to extended data analysis functionalities that can handle simultaneous video and neural activity. Download **DANSE** HERE.
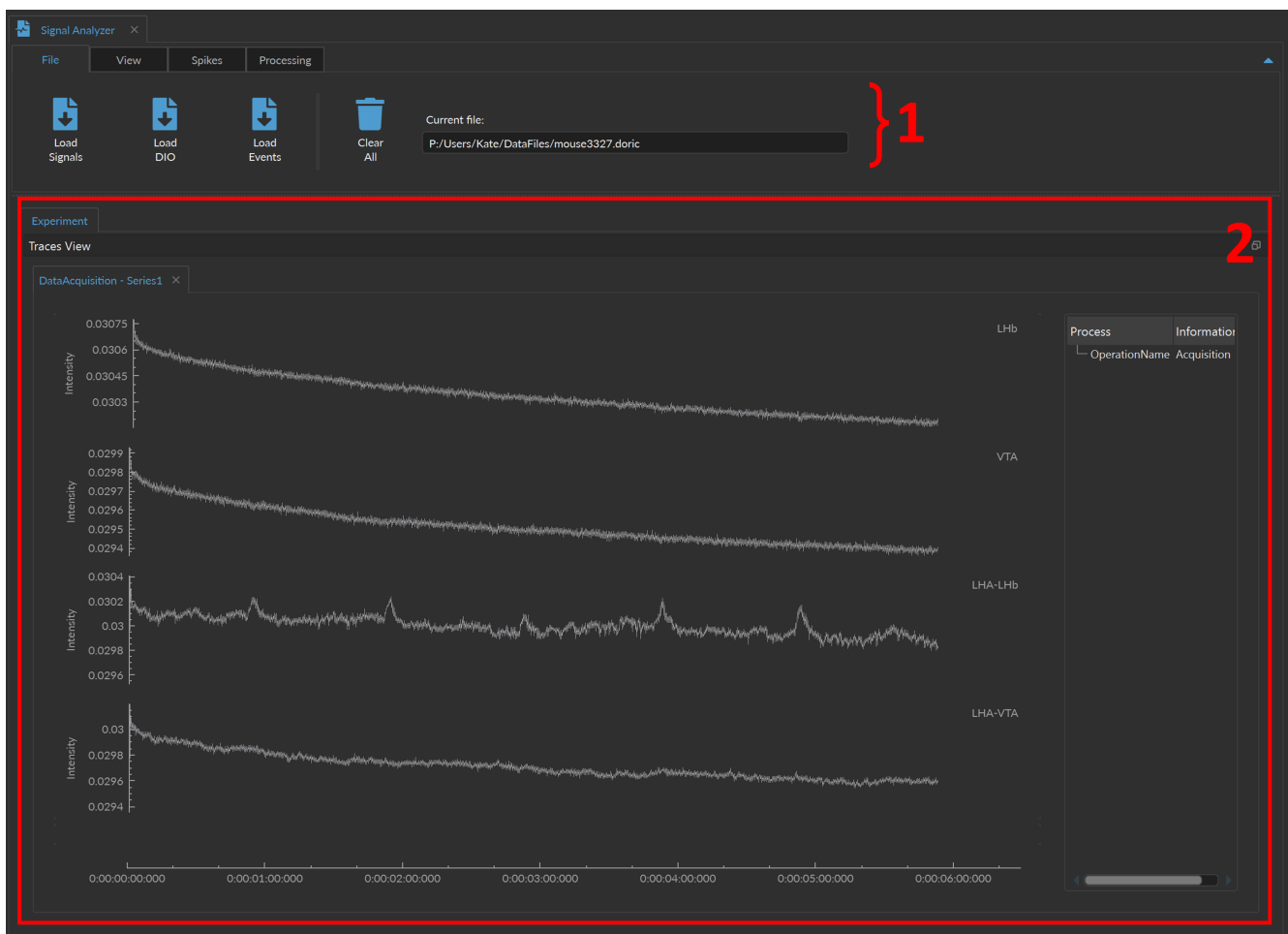

Figure 10.1: *Signal Analyzer Module*

The **Signal Analyzer** module can be accessed through the **Analyse** Tab at the top right of the main window (Fig. 10.2).
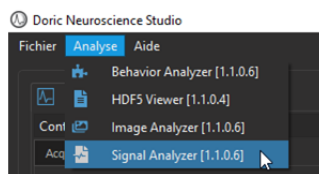
Figure 10.2: *Open the Signal Analyzer module*

The **Signal Analyzer module** (Fig 10.1) is separated into two main sections.

1. The **Control section** (Fig. 10.1, 1) contains all controls, separated into the **File**, **View**, **Spikes** and **Processing** tabs.

2. The **Trace View** (Fig. 10.1, 2) contains all currently displayed graphs as well as timestamped notes.

10.1   File Tab

The **File** tab (Fig. 10.3) is primarily used to load and save the data. The following details the specific features of each button, as per Fig. 10.3:
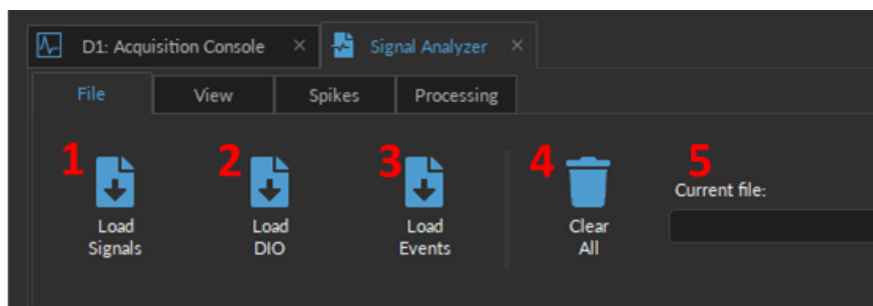
Figure 10.3: *File Tab*

1. The **Load Signals** button (Fig. 10.3, 1) opens a File Selection Window where users can select the *.doric* file of a previous recording and import it into the module. The file must contain both time and signal data. Once the file is selected, a second window will pop up (Fig. 10.4), allowing user to specify which channels to include for data processing. Only the selected channels will be displayed in the graph box. Multiple channels can be selected at once. If channels were renamed before data acquisition, then user-defined names will appear in the **Load selected Channels** window.

   Note: Additional *.doric* files can be loaded into the module for comparison between recordings.
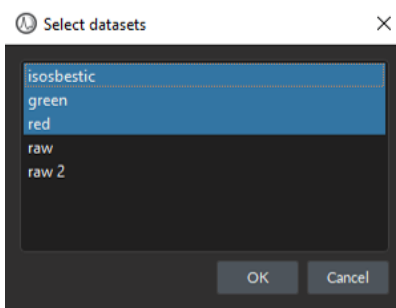
Figure 10.4: *Load selected channels*

2. The **Load DIO** button (Fig. 10.3, 2) allows users to import Digital Input/Output signals from a .doric file into *Trace View*.

3. The **Load Events** (Fig. 10.3, 3) allows users to display **Keypress Events** from a .doric file.

4. The **Clear all** button (Fig. 10.3, 4) deletes all data currently loaded in the module. This data cannot be recovered, so ensure the data is properly saved before clearing it.

5. The **Current File** box (Fig. 10.3, 5) displays the name and path of the most recent file loaded into the analysis module. If no file has been imported, the box will be blank.

Notes:

- The **Signal Analyzer** module can display data from multiple files simultaneously. If time values are missing, they will be left blank in the **Graph** window.

- To merge several data files together (to use the *Doric's* **DANSE** data analysis software) use the **Doric File Editor** module.

10.2   View Tab

The **View** tab (Fig. 10.5) is used to adjust the view in the **Graphs box**. The following details the specific features of each button, as per Fig. 10.5:
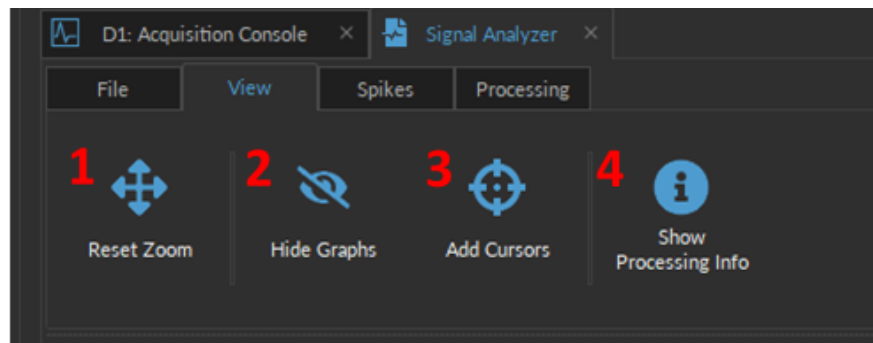

Figure 10.5: *View Tab*

1. The **Reset zoom** button (Fig. 10.5, 1) resets the axis so that the entire recording is visible.

2. The **Hide graph** button (Fig. 10.5, 2) opens the **Show/hide graphs** window (Fig. 10.6). Any checked data sets will be displayed in the **Graph box**.
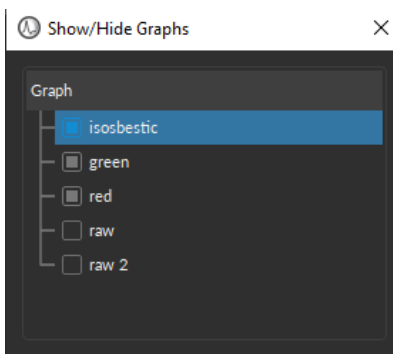
Figure 10.6: *Show/hide Graphs Window*

3. The **Add cursors** button (Fig. 10.5, 3), when selected, allows users to add 1-2 markers on the graph and displays the coordinates of the chosen point (Fig. 10.8). A left click will activate a blue cursor, while a right click will activate an orange cursor. If both cursors are used, the **Time Difference** between the two cursors will be displayed at the top of the graph (Fig. 10.8, red box). To remove the cursors, click the **Remove cursors** button (Fig. 10.7) (previously the **Add cursors** button).
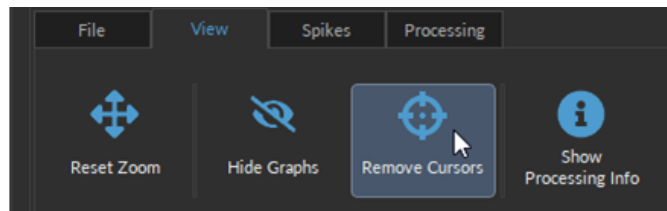
Figure 10.7: *Remove Cursors Button*

Figure 10.8: *Add Cursors*

4. The **Show Processing Info** button (Fig. 10.5, 4) opens a box on the right side of **Traces View** (Fig. 10.9). This box records all the data manipulation and parameters that a user has performed on the raw data. This includes the processing algorithms used (Operation Name), the device that collected the data (Source), and the parameter(s) specified for each algorithm.
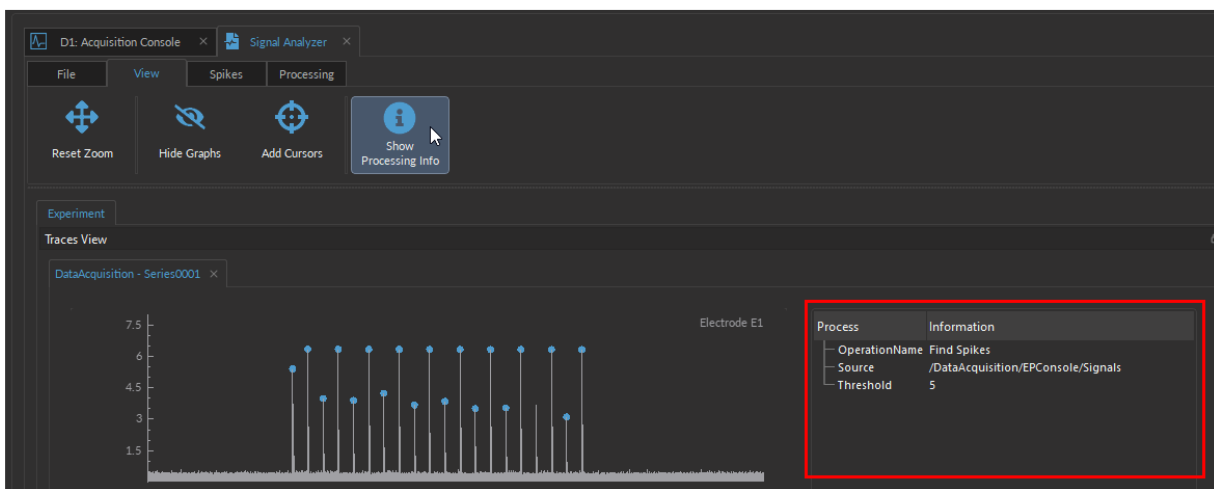
Figure 10.9: *Processing Info Box*

10.3   Spikes Tab

The **Spikes** tab (Fig. 10.10) is used to save, load, and edit data files containing process data within the module.

Note: For electrophysiological data, all detected spikes correspond to multi-unit activity since the algorithm used does not differentiate between different spike shapes.
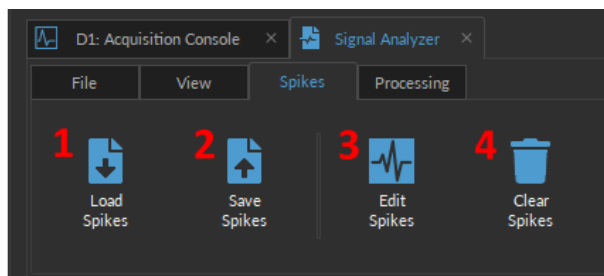
Figure 10.10: *Spikes Tab*

1. The **Load Spikes** button (Fig. 10.10, 1) will open a file selector window where the user can pick which _Spikes#.doric data set to import into the module.

2. The **Save Spikes** button (Fig. 10.10, 2) will automatically output a new file containing the processed data in the same folder as the raw data. This new file will share the name of the raw data file, plus a _Spikes between the old name and the .doric. If a spike file of that name already exists, an additional Spikes file is generated as _Spikes#.doric, instead of overriding the data.

3. The **Edit Spikes** button (Fig. 10.10, 3) allows users to update the spikes information in the current _Spikes.doric file without generating a new Spikes file. Instead, the new and old processed data are combined into a folder of the same name.

4. The **Clear Spikes** button (Fig. 10.10, 4) will erase the Spike file currently loaded in the module.

10.4   Processing Tab

The **Processing** tab (Fig. 10.11) contains all the operations that can be run over the raw data. This module can be used to process electrophysiology and fiber photometry data. All the functions offered in version 5 of the software remain in addition to several new additions.

Note: There are now TWO available $\Delta F/F_0$ functions (Fig. 10.11, 6 & 7). The original $\Delta F/F_0$ from version 5 was conserved and is found in no. 6 of the **Processing** tab. This function is a general purpose $\Delta F/F_0$ calculation, while no. 7 (**Photometry $\Delta F/F_0$**) is a function specifically designed for calcium-dependent signals.

The following **Processing** functions are available:
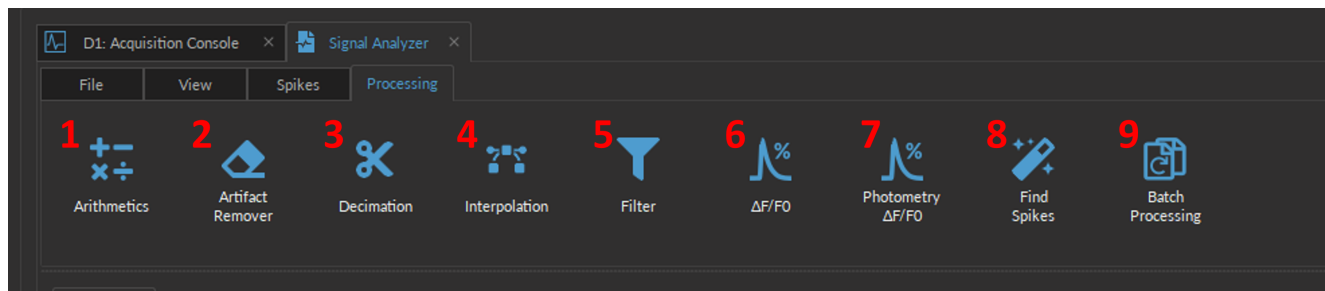

Figure 10.11: *Processing Tab*

1. The **Arithmetics** button (Fig. 10.11, 1) opens the arithmetic window. From this window, simple arithmetic operations (+,-,×,÷) can be performed on any two data sets currently in the module. This function is useful to subtract the isosbestic control from the signal of interest to control motion artifacts. It is recommended to first

convert fluorescent dataset into $\Delta F/F_0$ signals before using **Arithmetic**. If using the **Photometry $\Delta F/F_0$** function to subtract isosbestic from the calcium-dependent trace, the **Arithmetic** function is no longer necessary since similar functionality is already included into the **Photometry $\Delta F/F_0$**.

    a) The **Dataset** drop-down list specifies which two traces will the operation be applied upon.

    b) The **Multiplication factor** will scale the trace by the designated value to optimize the subtraction of two traces.

    c) The **Operations** specifies which of the four arithmetic operations (+,-,× or ÷) will be performed on the datasets.
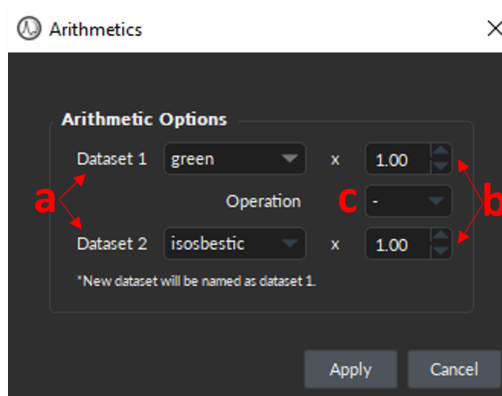
Figure 10.12: *Arithmetic Window*

2. The **Artifact Remover** button (Fig. 10.11, 2) opens the **Artifact Remover** window (Fig. 10.13). This window enables users to delete any LED artifacts from electrophysiology data by finding the first "spike" (artifact) that occurs around LED onset or offset and forcing all the data points associated with the artifact to zero. To use this operation, the LED Exc. trace must be loaded into the module (using the **Load signals** button from the File Tab, see Fig. 10.3-1 and Fig. 10.4). For more information on this algorithm and the principles behind the artifacts, see section 8.4.3.1, no. 5, LED Artifacts Remover).

    a) Using the electrode selector box (Fig. 10.13, a), select one or more **Electrode** channel(s) on which the artifact remover algorithm will be applied.

    b) Select the *LED Exc.* signal for the **LED trace** (Fig. 10.13, b). This should be a Digital Output signal and NOT electrophysiological data. This trace tells the algorithm when the LED was on and off so that onset and offset artifacts can be appropriately detected.

    c) Specify the **Artifact Width** (Fig. 10.13, c), which can be any value between 0.1 ms and 1 sec. However, since spikes of a neuron are on the order of 1-2 ms, we do not recommend values larger than 2 ms, as it will likely remove real spikes.
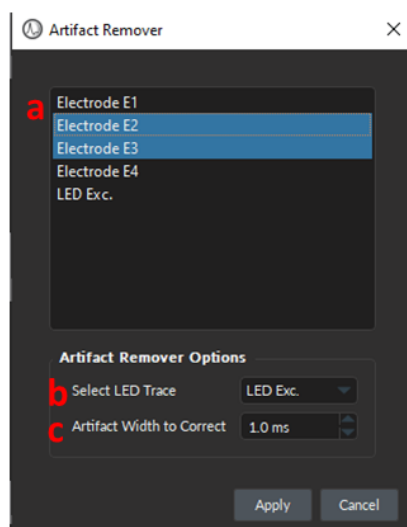
Figure 10.13: *Artifact Remover Window*

3. The **Decimation** button (Fig. 10.11, 3) opens the decimation window (Fig. 10.14). From this window, the data can be decimated to reduce the size of a photometry data file. ***BE CAREFUL not to re-decimate the data if it was already decimated during acquisition.*** (For Lock-In data, a decimation factor of 200x is set by default.)

   a) The **Trace name** (Fig. 10.14, a) specifies the channel that will be decimated. Multiple channels can be decimated at once.

   b) The **Decimation Factor** (Fig. 10.14, b) defines the number of points saved. One point is conserved over a number of data points equal to the **Decimation Factor**.
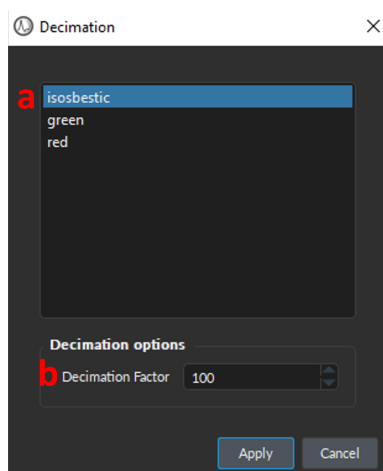
Figure 10.14: *Decimation Window*

4. The **Interpolation** button (Fig. 10.11, 4) opens the interpolation window (Fig. 10.15). This function will fill in missing data points using the existing neighboring data points. This function is useful when other data processing/analysis algorithms require datasets to have an identical number of data points.

   a) The **Trace name** (Fig. 10.15, a) specifies the channel where the function will be applied. Multiple channels can be selected at once.

   b) The **Interpolation Type** (Fig. 10.15, b) is linear by default. Do not use interpolations for data loss with large gaps, as the linear function will not be sufficient. No other options are available.
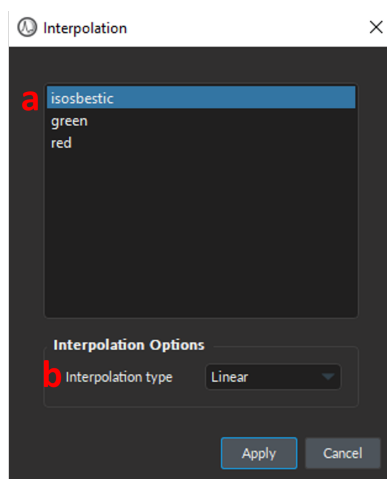
Figure 10.15: *Interpolation Window*

5. The **Filter** button (Fig. 10.11, 5) is used to filter out specific frequencies from the data. This can be used to either smooth the data or remove noise. Selecting the button opens the **Filter window** (Fig. 10.16) where users can choose the filter parameters. By default, the Butterworth Filter (order 10) is used to process the data.

NOTE: If using the **Photometry ΔF/F$_0$** function to process calcium-dependent signal, the **Filter** function is no longer necessary since similar functionality is already included.

The **Filter** parameters are as follows:

a) Using the electrode selector box, select one or more channel(s) on which the filter will be applied (Fig. 10.16, a).

b) The **Filter type** (Fig. 10.16, b) defines whether the filter is low-pass, high-pass or bandwidth.

c) The **Cutoff frequency** (Fig. 10.16, c) defines which frequencies are filtered. Which values are accessible depends on the **Filter type**. Either / or both **Low** and **High** cutoff values can be defined. The low pass value must always be smaller than the high pass value.

d) The **Filter Response Graph** (Fig. 10.16, d) displays a visualization of the filter in use, with the specified low and/or high pass filter cutoffs.
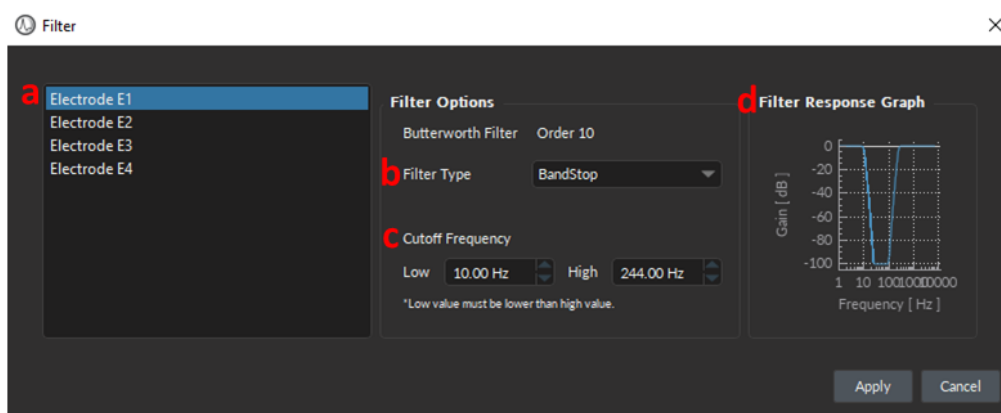
Figure 10.16: *Filter Window*

6. The **ΔF/F$_0$** button (Fig. 10.11, 6) opens the simple ΔF/F$_0$ window (Fig. 10.17). This is a general purpose calculation and is identical to the one offered in version 5 of the software. This function takes as input raw data and outputs the change in relative fluorescent as a ratio. 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.

a) The **Trace name** (Fig. 10.17, a) specifies the channel on which the function will be applied. Multiple channels can be selected at once.

b) The **F0 Calculation Method** drop-down list (Fig. 10.17, b) include:

- *Least mean squares* - is an adaptive filter that continuously re-estimates and updates filter weight when calculating the change in fluorescence. The algorithm is inspired by 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.

- *Running average* - the algorithm is inspired by 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.

c) The **Time Window** (Fig. 10.17, c) (for Running Average ONLY) specifies the amount of time the algorithm will be performed during each iteration. ***Running average is SLOW if the time window contains too many points.***
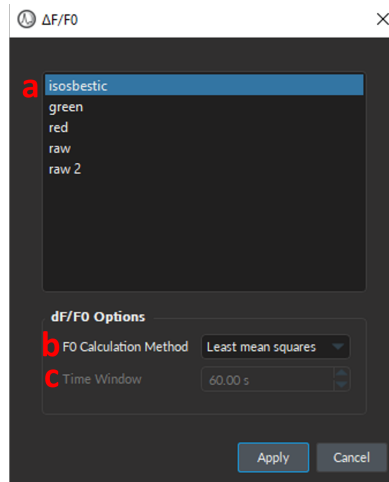

Figure 10.17: $\Delta F/F_0$ window

7. The **Photometry ΔF/F$_0$** button (Fig. 10.11, 7) opens the Photometry ΔF/F$_0$ window (Fig. 10.19), and is a specialized function that calculates the calcium-dependent fluorescent fluctuations. This function is a new addition and did not exist in version 5 of the software and is based on the paper from E. Martianova, S. Aronson, and C. D. Proulx, *Multi-Fiber Photometry to Recrod Neural Activity in Freely-Moving Animals*, J. Vis. Exp. (152), e60278, doi:10.3791/60278 (2019). You can also access the Github repository with source codes in Python, Matlab, and R used in the paper HERE .

a) The **Signal** (Fig. 10.19, a) specifies the input channel to the function. Both the calcium independent and calcium dependent signals must be specified using the drop-down menus.

b) The **Smooth Signal** (Fig. 10.19, b) option specifies which algorithm will be used to smooth the data. Three options are available, including:

- *None* - no smoothing function will be applied to the data.
- *Low-pass Butterworth Filter* - smooths the data using a Fourier transforms-based algorithm to filter the frequency response across its bandpass.
- *Running Average* - smooths the data by taking a rolling mean over many small time windows through the entire data set.

c) The **Correction Baseline** (Fig. 10.19, c) function uses an adaptive iterative re-weighted Penalized Least Squares algorithm (airPLS; Github) to remove the slope and low-frequency fluctuations within the signal. The **Lambda** value is the baseline (calcium-independent) slope used to fit the calcium-independent data.

Make sure that the line fits the calcium-independent trace very strongly (Fig. 10.19, h), but does not pick up the peaks in the calcium-dependent trace (Fig. 10.19, i). A lambda value that is too low will overfit the data and prevent the detection of real calcium-dependent peaks, while a lambda value that is too large will not fit the baseline trace appropriately. Typically a value around 10 (+/- 1) is appropriate for most fiber photometry data.

d) The **Discard Signal Onset** (Fig. 10.19, d) specifies a time window where the data will be ignored for the $\Delta F/F_0$ calculations. This parameter is useful to remove the steep drop common at the beginning of photometry recordings (Fig. 10.18), which often messes up the fitting algorithm. It is recommended to discard the first 1-3 seconds of data. If the value is 0, no data will be discarded.
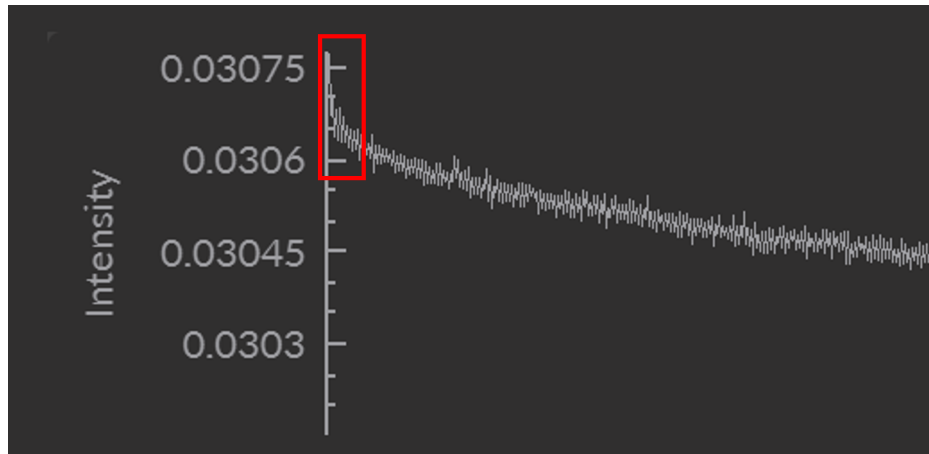

Figure 10.18: *Discard steep drop in the signal*

e) The **Fit Signals** (Fig. 10.19, e) specified the residual threshold used when fitting the line between calcium-dependent and calcium-independent signal (Fig. 10.19, k). Changing the value of the residual threshold will change the slope of the line of best fit. For most fiber photometry experiments, a residual threshold of 1 is adequate.

f) The **Channel Drop-down** (Fig. 10.19, f) specifies the channel on which the $\Delta F/F_0$ algorithm will be applied.

g) The **Update Plots** button will recalculate the data for the Example View when new parameters are specified and display the new smoothed traces in Fig. 10.19, h and the new line of best fit in Fig. 10.19, i.

h) The **Processing Example View Graphs** (Fig. 10.19, g) displays the original raw trace and smoothed curve for both the *Calcium independent* (Fig. 10.19, h) and *Calcium dependent* signal (Fig. 10.19, i). The bottom graph displays the output $\Delta F/F_0$ trace (Fig. 10.19, j).

i) The **Signal Fit Line Graph** (Fig. 10.19, k) displays the line of best fit between calcium-dependent and independent signals. Changing the value of the **Residual Threshold** will modify the slope of the line of best fit. This line should represent the correlation between the two sets of data.
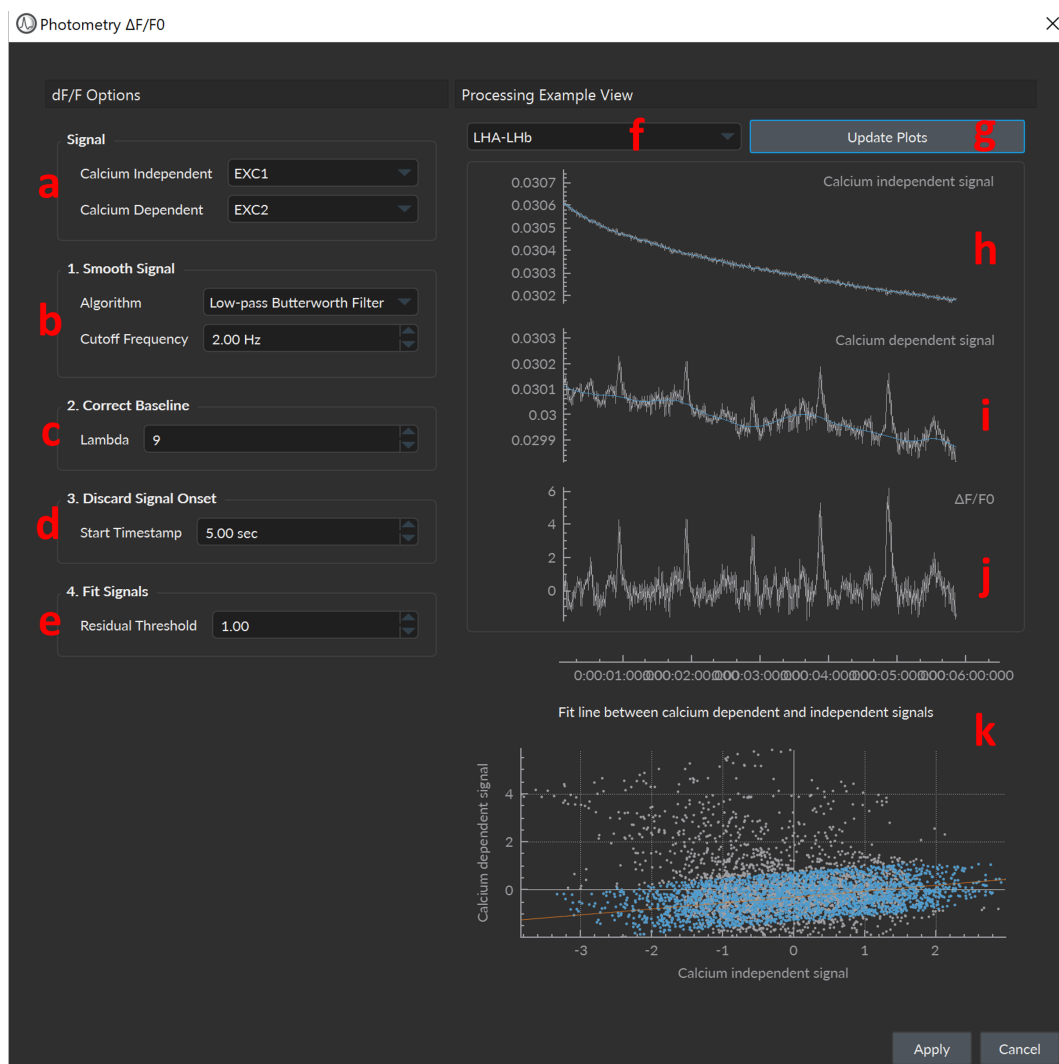
Figure 10.19: *Photometry ΔF/F₀ Window*

8. The **Find spikes** button (Fig. 10.11, 8) identifies peaks in the data over one or multiple electrode/channels (Fig. 10.20). This operation can be used for both electrophysiology and fiber photometry to identify peaks in the data above a threshold value. Peaks will be identified as spikes if they cross a certain **Threshold** value. The **Threshold** value must be specified by the user at the bottom of the **Find Spikes** window (Fig. 10.20). This value represents the number of standard deviations over the mean baseline activity. Once detected, the spikes are displayed as blue dots over the traces in **Trace View** (Fig. 10.21).

Note: For electrophysiological data, all detected spikes correspond to multi-unit activity since the algorithm used does not differentiate between different spike shapes.

Figure 10.20: *Find Spikes Window*

Figure 10.21: *Spikes are identified on the signal trace with blue dots*

9. The **Batch Processing** button (Fig. 10.11, 9) allows data from multiple recordings to be automatically processed with the same operations in a specified sequence and without user inputs.

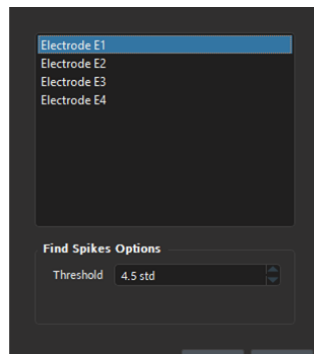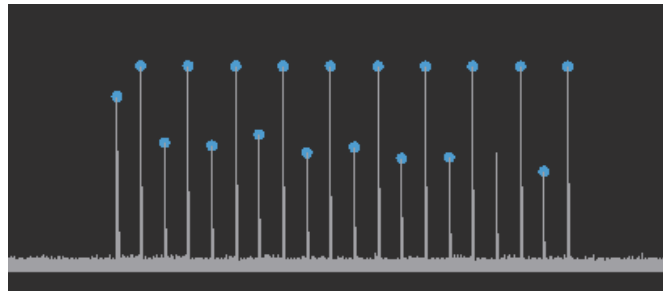   a) The **Select Folder** button (Fig. 10.23, a) will open a file explorer window where users can specify the folder that contains the data for batch processing.

        • *Batch Processing will not run* if other files (such as FILENAME_Spikes.doric files are included in the folder). Make sure only raw data file are contained in the selected folder.

    b) The **Select Datasets** button (Fig. 10.23, b) is not required for FiWi data since each file should contain the same four electrode channels and LED excitation signals. If the automatic selection occurred, a path will be displayed as per Fig. 10.22. However, if other inputs/outputs are included during the recordings, make sure each file has identical channels, in order for the batch processing to work.
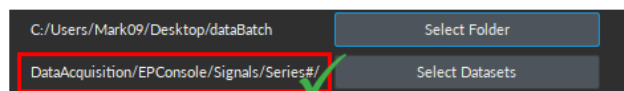
Figure 10.22: *Automatic FiWi Dataset selection*

    c) If the **Save Intermediate Files** options (Fig. 10.23, c) is selected (**Yes**) batch processing will generate a file after every operation, and for each recording (Fig. 10.24). Select **No** if intermediate files are not required.

    d) The **Available Operations** box (Fig. 10.23, d) contains all the possible operations that run in batches over the data. These are the same operations that can be manually selected over a single recording using the **Processing** Tab (Fig. 10.11).

    e) The **Workflow** box (Fig. 10.23, e) displays the operations that will be run over each recording during batch processing, following the order of the operations. To add an operation to the **workflow**, click on the operation of choice in the **Available Operations** box. Note that the order that operations are added to

the **Workflow** is the order they will be run during batch processing. To remove an operation from the **Workflow** click on that operation and it will return to the **Available Operations** box.

f) The **Options** section (Fig. 10.23, f) displays a box to specify the parameters for each operation. Details concerning the parameters of each operation can be found in the non-batch processing section of the operation in question (Section 10.4: 1-8).

NOTE: Some operations use data-set names. Make sure that the names are the same across all the files.
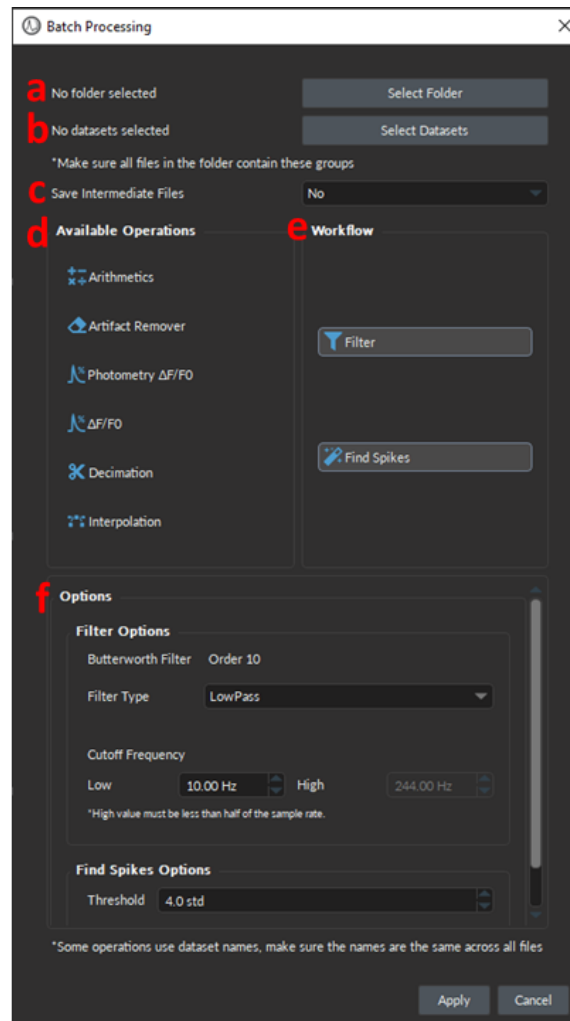
Figure 10.23: *Batch Processing Window*
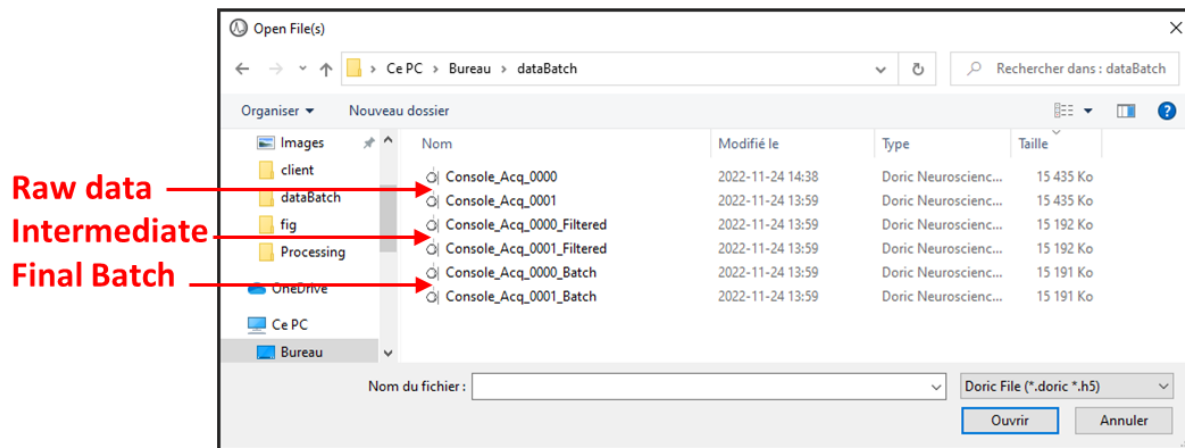
Figure 10.24: *Batch Processing Output*

Behavior Analyzer

The **Behavior Analyzer** module allows simultaneous observation of behavior video with traces from experimental measurements. Note that video data must be in **.avi** format, while trace data is received in **.doric** format.

Note that users that have purchased **DANSE**, Doric's specialized data analysis software, should load raw data directly into **DANSE** (skipping **Behavior Analyzer** module) since all the data processing functionalities offered in this module are also included in **DANSE**, in addition to extended data analysis functionalities that can handle simultaneous video and neural activity. More information on **DANSE** software is AVAILABLE HERE.

Figure 11.1: *Behavior Analyzer Module Interface*

To open the **Behavior Analyzer** module, select **Analysis** and the module name from the drop-down menu, as per Fig. 11.2.

Figure 11.2: *Open Behavior Analyzer module*

The interface can be separated into three major sections (Fig 11.1):

1. The **Tabs** (Fig 11.1, 1) are used to access the controls, settings and functions of the module. These controls and settings are split into three tabs, which will be treated in the following sections:

   - *Files Tab* - Section 11.1
   - *View Tab* - Section 11.2
   - *Processing Tab* - Section 11.3

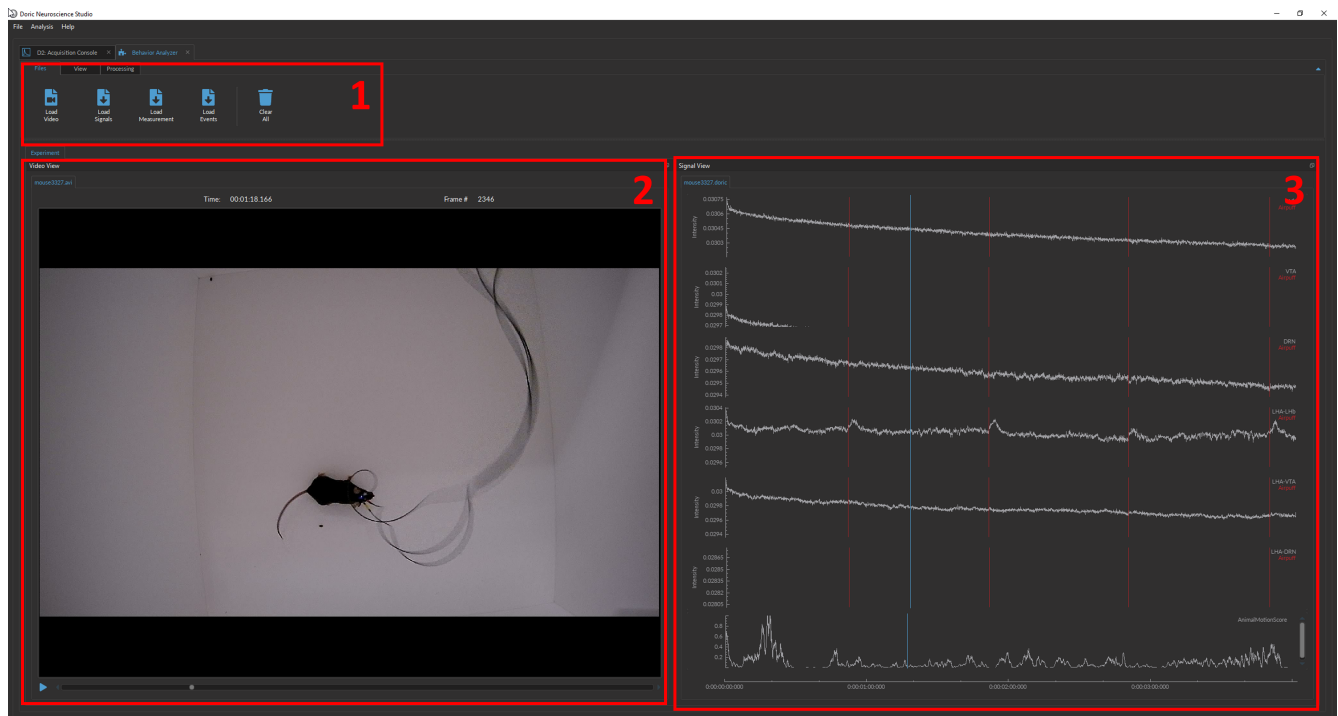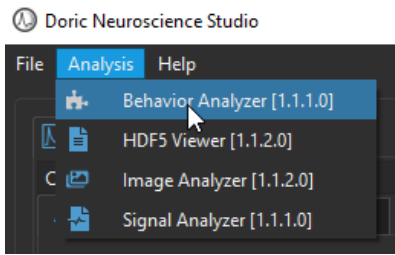2. The **Video View** (Fig 11.1, 2) displays the recorded footage and controls the frames displayed. The **Play** button on the bottom left runs the video. The scroll bar beside it can be used to choose a frame while the video is paused. See Section 11.4 for more details.

3. The **Signal View** (Fig 11.1) displays the raw traces associated with the video. The red bar over the traces corresponds to the timestamp of the associated frame of the video. See Section 11.5 for more details.

11.1   Files

The **File** tab (Fig. 11.3) is primarily used to load and save the data. The following details the specific features of each button, as per Fig. 11.3:
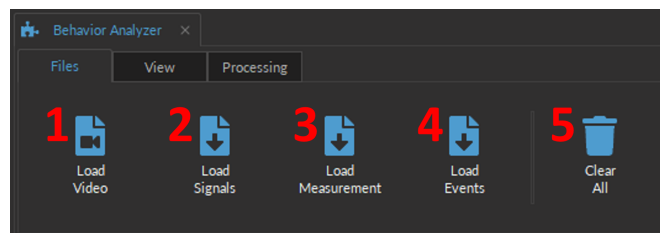
Figure 11.3: *Behavior Analyzer, Files tab*

1. The **Load Video** button (Fig. 11.3, 1) will open File Explorer window where users can select any saved video that are in either .avi or .mp4 format. This does NOT necessarily need to be video data recorded with *Doric Neuroscience Studio*. Note that a warning will pop up if Video and Signal data are not the same lengths since this could affect data alignment.

2. The **Load Signals** button (Fig. 11.3, 2) opens a File Selection Window where users can select the *.doric* file of a previous recording and import it into the module. The file must contain both time and signal data. Once the file is selected, a **Select Dataset** window will pop up (Fig. 11.4), allowing user to specify which device (Fig. 11.4a), signal type (Fig. 11.4b), and the channels (Fig. 11.4c) to include for data processing. Only the selected channels will be displayed within the **Signal View**. Multiple channels can be selected at once.

3. The **Load Measurement** button (Fig. 11.3, 3) allows users to import previous measurements (Animal Tracking or Motion Score) from a .doric file into **Signal View**. Data will be pulled from *DataBehavior/Measurement* folder within .doric file.

(a) *Device*　　　　　　(b) *Type*　　　　　　(c) *Channels*

Figure 11.4: *Select Datasets*

4. The **Load Events** (Fig. 11.3, 4) allows users to display **Keypress Events** from the a .doric file and overlay it on the signal data in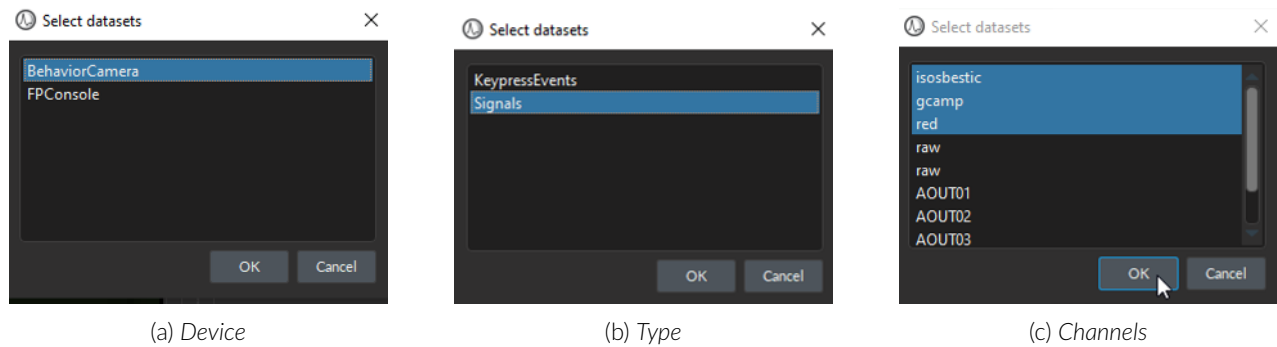 **Signal View** as vertical lines, at the appropriate time points. Select **KeyPressEvents** within the **Select Dataset** window (Fig. 11.4b) to import them into the module.

5. The **Clear all** button (Fig. 11.3, 5) deletes all data currently loaded in the module. This data cannot be recovered, so ensure the data is properly saved before clearing it.

11.2   View
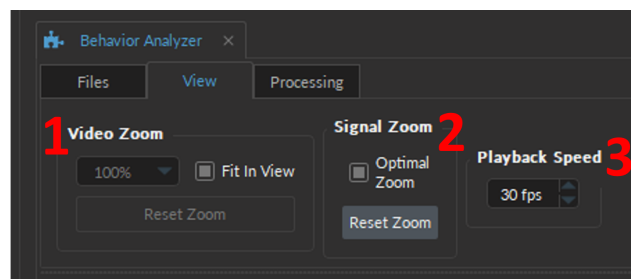
Figure 11.5: *Behavior Analyzer, View tab*

The **View** tab (Fig. 11.5) sets the visualization parameters for the **Video View** and the **Signal View**. The following parameters are included within the tab:

1. The **Video Zoom** (Fig. 11.5, 1) sets the image magnification factor.

    a) The **Zoom %** drop-down list specifies the zoom factor for the image display, which ranges between 10%-500%.

    b) The **Reset Zoom** button returns the zoom factor to 100%.

    c) The **Fit Image** checkbox automatically adjusts the image to fit the entire Acquisition View.

2. The **Signal Zoom** (Fig. 11.5, 2)

    d) The **Optimal Zoom** checkbox automatically adjusts the y-axis of the **Signal View** graphs based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

    e) The **Reset Zoom** button readjusts the y-axis of the **Signal View** graph zoom to a default value.

3. The **Playback Speed** (Fig. 11.5, 3) sets the frame rate (in FPS) that the video and signal data will be run once user selected **Play** from the **Video View**.

11.3 Processing

The **Processing** tab (Fig. 11.6) includes two behavior measurements, which will be treated in the following sections:

- *Animal Tracking* (Fig. 11.6, 1) - Section 11.3.1
- *Motion Score* (Fig. 11.6, 2) - Section 11.3.2
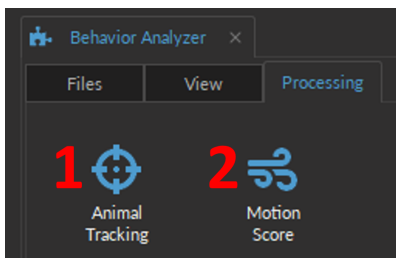
Figure 11.6: *Behavior Analyzer, Processing tab*

11.3.1 Animal Tracking

The animal tracking algorithm uses changes in contrast, hue, or saturation to differentiate the animal from the background. The center of the mouse is calculated for every frame. The change in coordinate displacement of this central animal point between each frame is calculated. If the calibration was done, this pixel number can be converted into real distance.
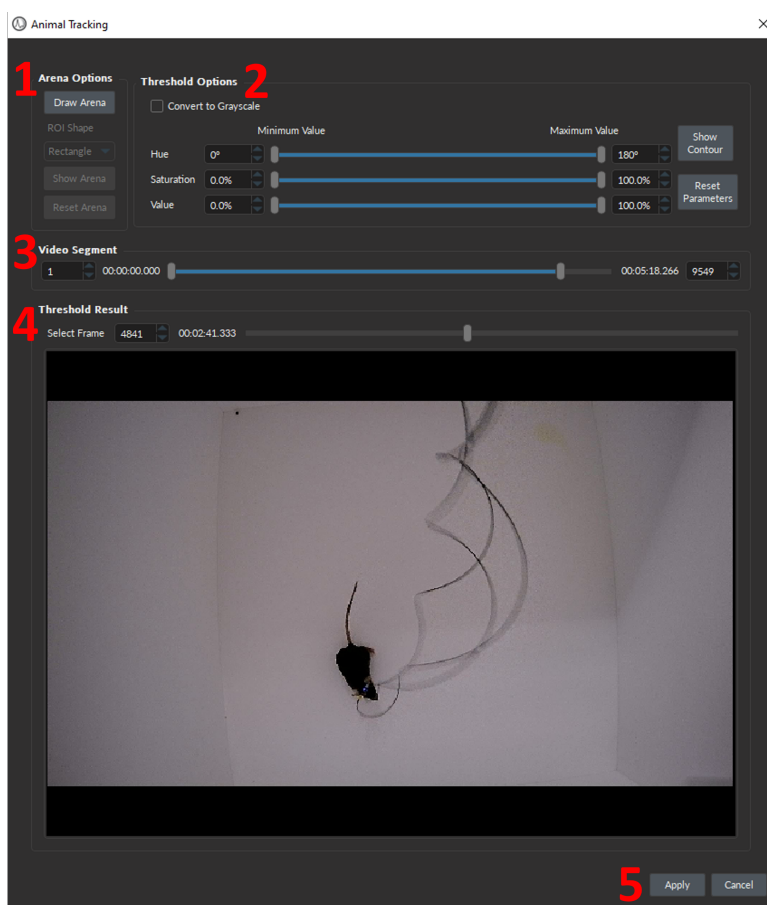
Figure 11.7: *Animal Tracking window*

1. The **Arena Options** (Fig. 11.7, 1) set the area within the video where the algorithm will be applied. If no arena is defined, the entire image will be set at the arena. To define the arena area use the following parameters:

   a) The **Draw Arena** button (Fig. 11.8, a) when selecting will allow users to draw a shape on a still frame of the video to define the part of the image that corresponds to the animal arena. This selected element will serve as the background for the moving animal. Once selected the **Draw Arena** button become a **Done**, which must be selected to save the current arena area for the algorithm.

   b) The **ROI shape** drop-down menu (Fig. 11.8, b) set the geometrical shape that will be used when drawing the arena area. User can select between: *Freehand, Rectangle, Circle or Square*.

   c) The **Show Area** button (Fig. 11.8, c) will blackout the part of the video that isn't contained within the user-defined arena.

   d) The **Reset Area** button (Fig. 11.8, d) will remove the video blackout that designates the arena in order to redraw the shape.
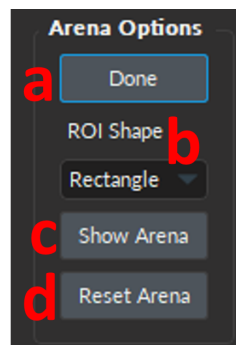

Figure 11.8: *Animal Tracking window, Arena Options*

2. The **Threshold Options** (Fig. 11.7, 2) allows users to set the range values (either **Hue**, **Saturation** or **Value**) that correspond to the moving animal in order to differentiate it from the background (Fig. 11.10). *** There should be high contrast between the animal and the background arena for best results.***
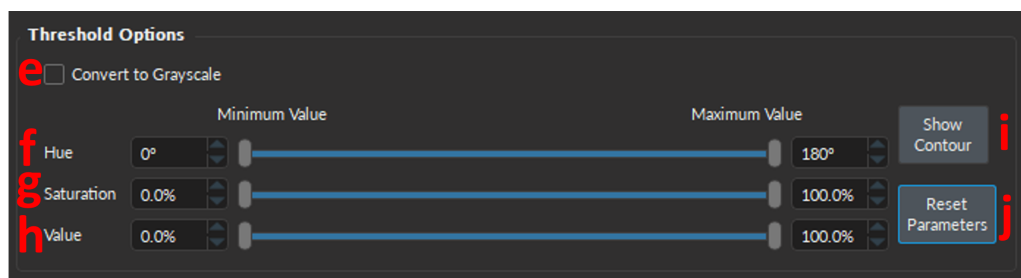
Figure 11.9: *Animal Tracking window, Threshold Options*

   e) The **Convert to Grayscale** checkbox (Fig. 11.10, e) will convert color pixels to a binary, back & white pixels with corresponding intensity level. This setting is ideal if the animal and background do not differ in color (e.g. black animal on white background, or white animal on a black background).

   f) The **Hue** (Fig. 11.10, f) sets an absolute color as threshold values. The hue is a number between 0 and 180 degrees, where red: 0-30°; yellow: 30-60°; green: 60-90°; cyan: 90-120°; blue: 120-150°; magenta: 150-180°. *Useful if using thermal camera*.

   g) The **Saturation** (Fig. 11.10, g) describes the intensity of the pixel. Saturation is a percentage ranging from 0% (grayscale) to 100% (pure color).

   h) The **Value** (Fig. 11.10, h) set the pixel value as threshold. The value is a percentage that ranges from 0% (black) to 100% (white).

i) The **Show Contour** button (Fig. 11.10, i) will apply the minimum and maximum values of the **Hue**, **Saturation** and/or **Value** as a threshold to detect the animal within the still frame and display the result within the **Threshold Results** view. A blue outline will surround the detected animal and the small blue circle will be computed as the center at the shape.

j) The **Reset Parameters** button (Fig. 11.10, j) will erase the contour displayed in the **Threshold Results** view and reset all the parameters within the entire **Animal Tracking** window to their default values.
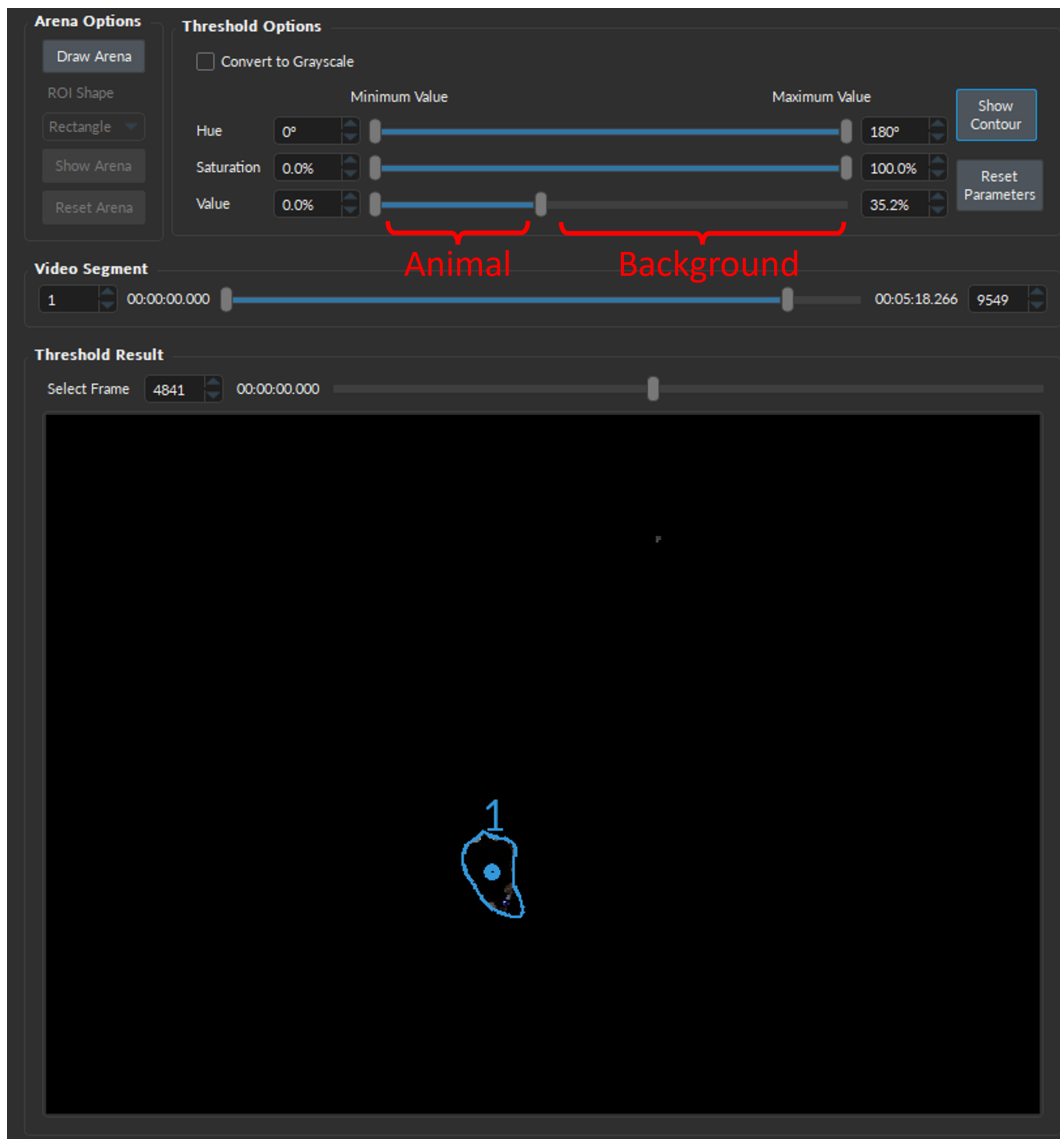
Figure 11.10: *Animal Tracking window, Threshold Options - Show Contour*

3. The **Video Segment** sliding scale (Fig. 11.7, 3) is used to define a time window within the video where the **Animal Tracking** algorithm will be applied. *This feature is useful to test the parameters on very small chunks of the video at a time to verify if those parameters are satisfactory.* Users can define the time window using either by specifying a starting and ending frame (Fig. 11.11, k & n respectively) or by using the two slidding scale pointers (Fig. 11.11, l & m respectively).
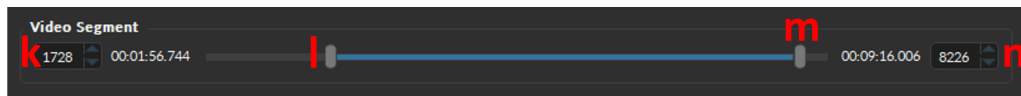
Figure 11.11: *Animal Tracking window, Video Segment*

4. The **Threshold Results** (Fig. 11.7, 4) view displays a still frame of the video where the user will define the arena and view the animal tracking contour. User can **Select Frame** from the video footage (wither with the text box or using the sliding scale) to test the parameter at multiple time points within the video.

5. The **Apply** button (Fig. 11.7, 5) will use the parameters set within the **Animal Tracking** window to track the animal within the segment of the video and calculate the animal's speed. The Output will be displayed at the bottom of the **Signal View** as *Animal Speed* graph (Fig. 11.14).

11.3.2 Motion Score

The **Motion Score** algorithm calculates the number of pixels whose intensity increased/decreased by a specified amount (the **Freezing Threshold**). An animal that moves a lot will cause the intensity of pixels to change frequently between frames (and thus lead to a large Motion Score) while an immobile animal will not affect pixel intensities will change (and thus lead to a small Motion Score).
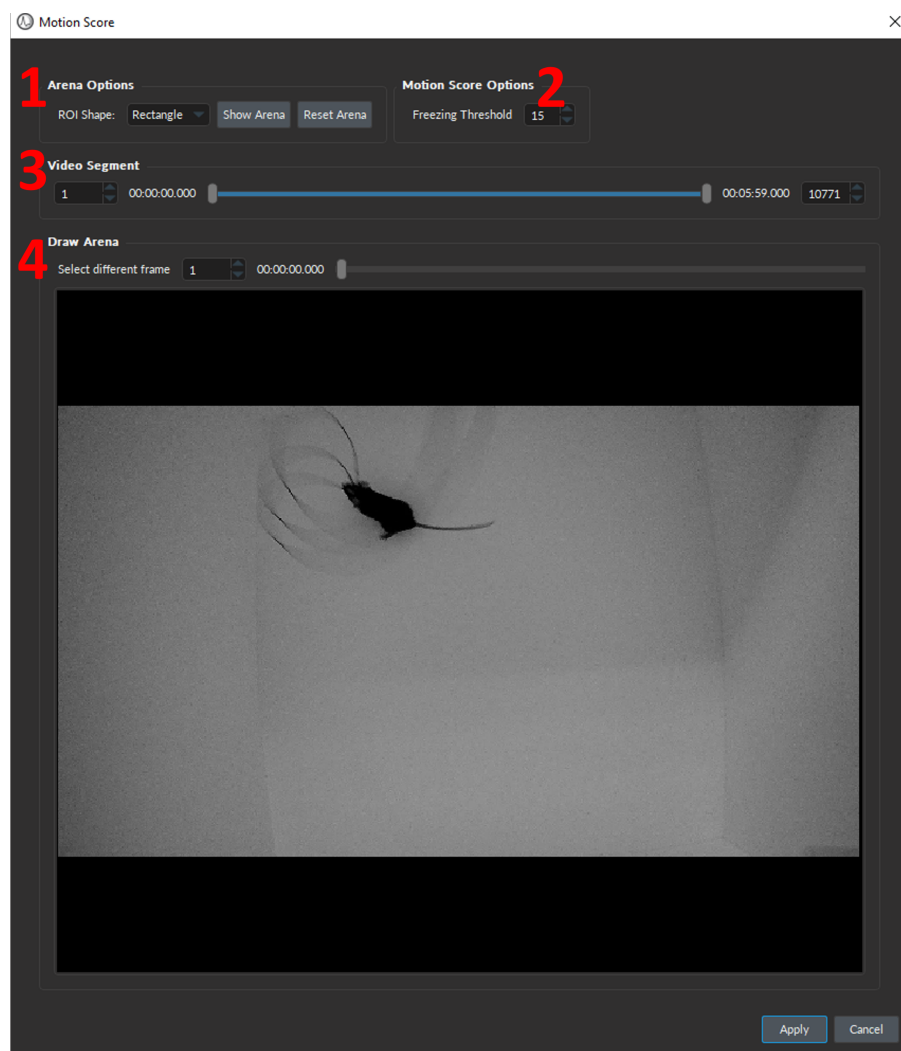
Figure 11.12: *Motion Score window*

1. The **Arena Options** (Fig. 11.12, 1) allows users to draw a shape on a still frame of the video to define the part of the image that corresponds to the animal arena. If no arena is defined, the entire image will be set at the arena. To define the arena area use the following parameters:

    a) The **ROI shape** drop-down menu set the geometrical shape that will be used when drawing the arena area. Users can select between *Freehand*, *Rectangle*, *Circle or Square*.

    b) The **Show Area** button will blackout the part of the video that isn't contained within the user-defined arena.

    c) The **Reset Area** button will remove the video blackout that designates the arena in order to redraw the shape.

2. The **Motion Score Options** defines the **Freezing Threshold** pixel value. This value must be between 0 and 255. This is a relative value over which change large that **Freezing Threshold** value will count as movement.

3. The **Video Segment** sliding scale (Fig. 11.7, 3) is used to define a time window within the video where the **Animal Tracking** algorithm will be applied. *This feature is useful to test the parameters on small video increments at a time to verify whether those parameters are satisfactory.* Users can define the time window using either by specifying a starting and ending frame or by using the two sliding scale pointers.

4. The **Draw Arena** view displays a still frame of the video where the user will define the arena and view the animal tracking contour. Users can **Select Frame** from the video footage (whether with the text box or using the sliding scale) to test the parameter at multiple time points within the video.

11.4   Video View

The **Video View** (Fig. 11.13) displays the video feed once a video is loaded into the module. Users can scroll through the video frame-by-frame or play the footage at a set frame rate.
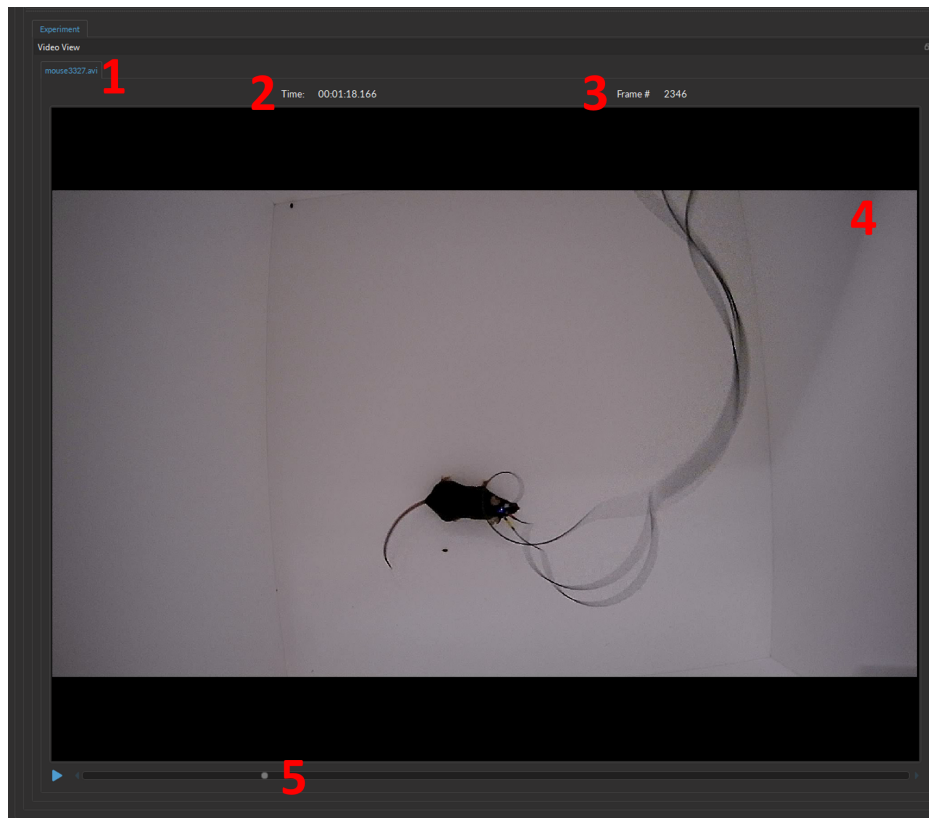

Figure 11.13: *Video View*

1. The **Video tab** (Fig. 11.13, 1) allows multiple video files to be simultaneously loaded into the module. Each video file will have its own tab, labeled with the filename (e.g. *mouse3322.avi*). Only one video can be viewed at a time. To switch between loaded videos, select the tab of choice.

2. The **Time** (Fig. 11.13, 2) displays the timestamp associated with the current frame (in hh:mm:ss:zzz).

3. The **Fame #** (Fig. 11.13, 3) displays the index of the current frame within the entire video.

4. The **Current frame** (Fig. 11.13, 4) is where the image associated with the **Frame #** of the video is displayed.

5. The **Current Time Bar** (Fig. 11.13, 5) allows users to quickly hop between frames. Use the play button (on the left of the bar) to run the video at a normal frame rate. The **Time** and **Frame #** will be automatically updated when moving the cursor within the **Time Bar**. Note that moving

11.5   Signal View

The **Signal View** (Fig. 11.14) displays the raw signal data from .doric files, in addition to keypress events. When either **Motion Score** or **Animal Tracking** measurements are taken, these will be displayed at the bottom of the signal view.
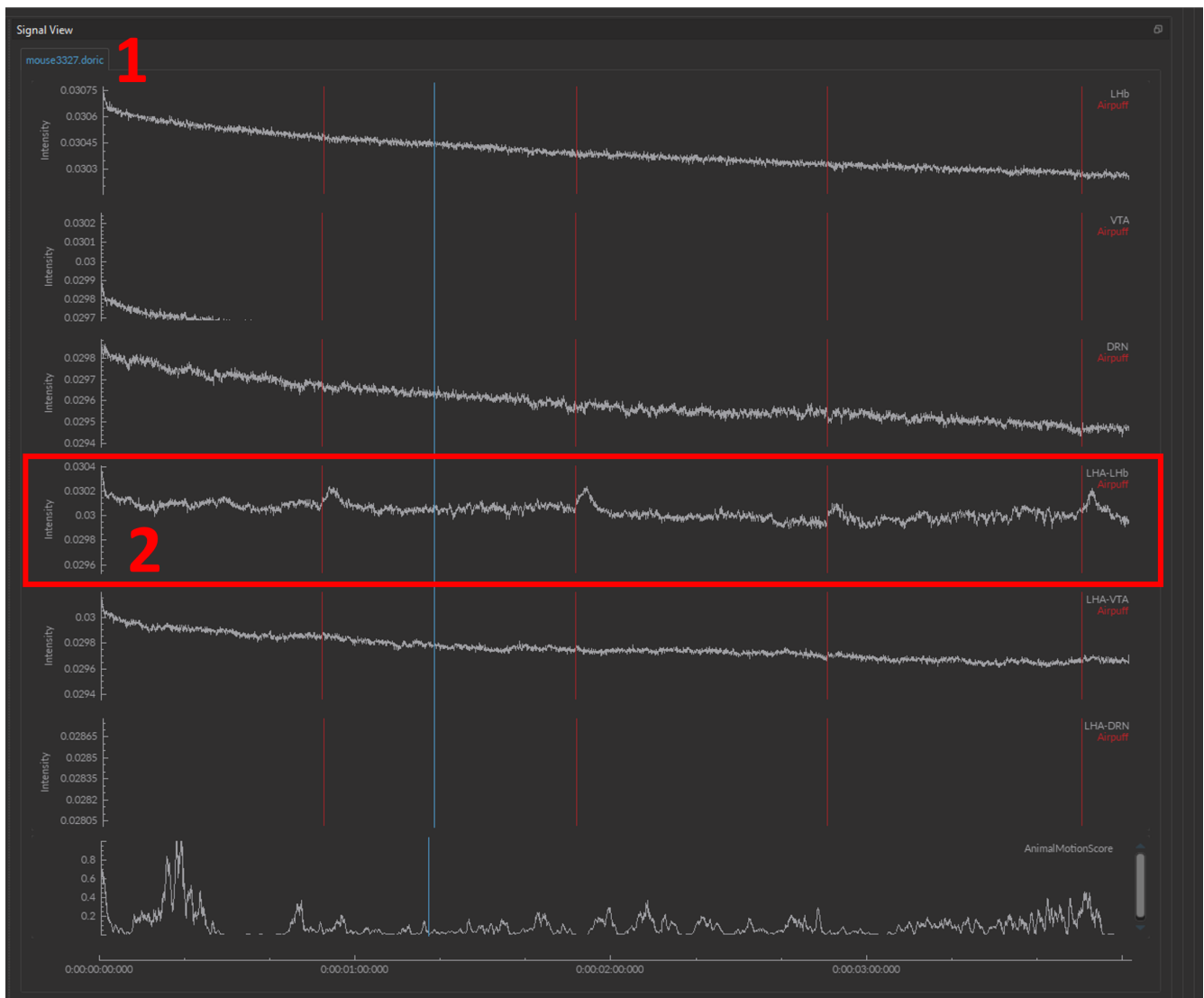

Figure 11.14: *Signal View*

1. The **Signal Tab** allows multiple signal files to be simultaneously loaded into the module. Each file will have its own tab, labeled with the filename. Only one tab can be viewed at a time. To switch between loaded videos, select the tab of choice.

2. The **Graph** includes the following elements:
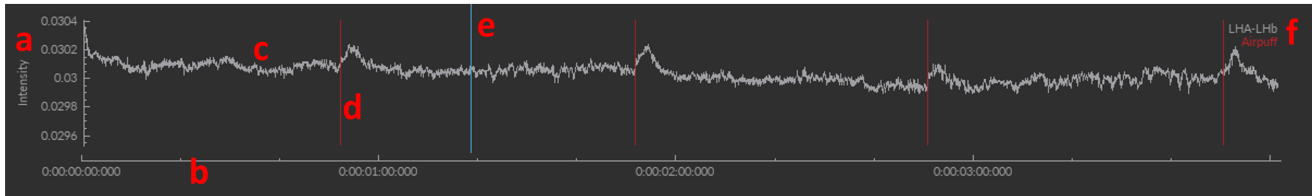
Figure 11.15: *Signal View, graph*

a) The **Y-axis** (Fig. 13.44, a) for loaded signal, represents the intensity of the pixel, which is unitless. For the **Animal Speed**, the unit is in pixel/second and for the **Animal Motion Score** is plotted with Arbitrary Units (AU).

b) The **X-axis** (Fig. 13.44, b) represents the time in d:hh:mm:ss:zzz.

c) The **Trace** (Fig. 13.44, c) is the curve of the signal, corresponding to fluctuations in pixel intensity, from which $\Delta F/F_0$ will be calculated.

d) The **Event indicators** (Fig. 13.44, d) are the overlayed **Keypress Events**, which can be imported using the **Load Events** button (Fig. 11.3, 3).

e) The **Current time indicator** cursor will move in response to the current video frame. **NOTE:** that if the timestamps of the behavior video and signal data do not match this alignment will be incorrect.

f) The **Legend** (Fig. 13.44, f) displays the color code of the graph traces. The trace name of the signal is always included and, when present, keypress event(s).

Image Analyzer

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 .doric formats, implements image processing functions and an export tool saves the fluorescence data in .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 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. To open Image Analyzer, select Analysis in the tab and choose Image Analyzer(Fig: 12.1).
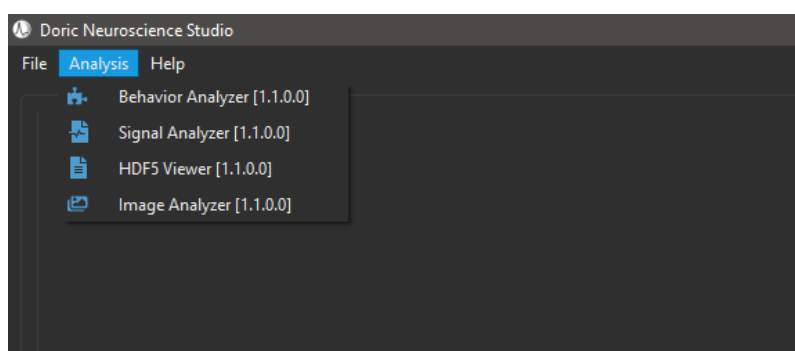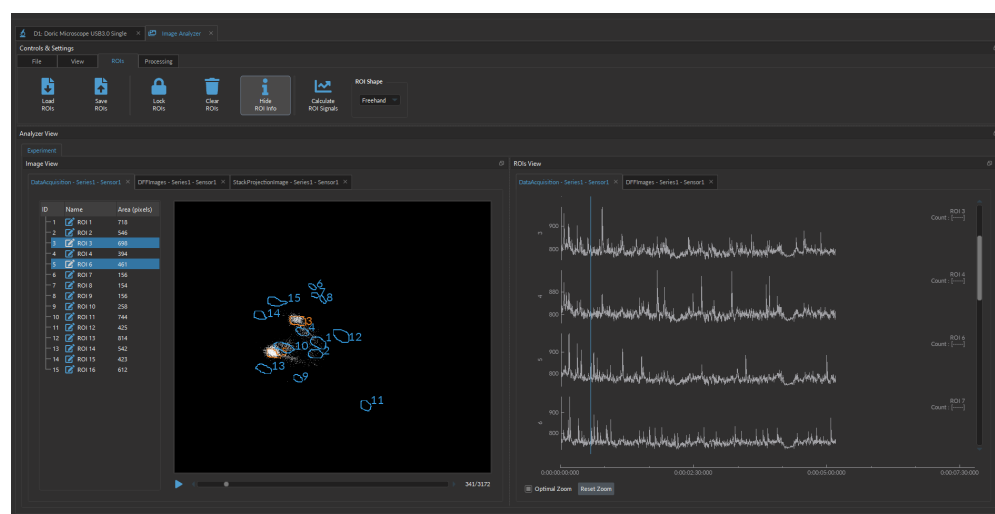

Figure 12.1: *Image Analyzer*

Figure 12.2: *Image Analysis Module Interface*

The Image Analyzer is composed of 2 main parts:

1. The **Controls & Settings** which regroups four tabs developed further in the document: **File** (section 12.1.1), **View** (section 12.1.2), **ROIs** (section 12.1.3), and **Processing** (section 12.1.4).

2. The **Analyzer View** 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, and displays the average intensity in each ROIs.

12.1   Controls & Settings

12.1.1   File
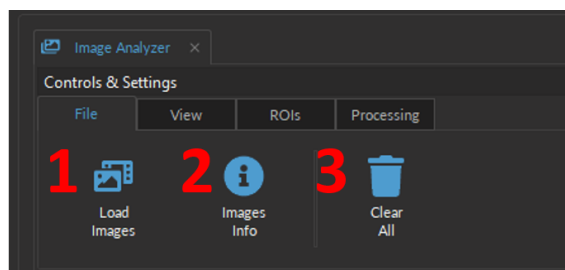
Figure 12.3: *File Tab*

The **File tab** (Fig. 12.3) is used to load data, obtain information about data and clear the **Analyser View**.

1. The **Load Images** button loads the images that must be a .doric containing images in a square, 16 bits format.

2. The **Images Info** displays a window with information about the images (Width x Height, Bits Count, Timestamp, Sensor ID, LED power, Exposure, and the Gain).

3. The **Clear All** button clears the **Analyser View** and close the analysis in progress.

12.1.2   View

The **View tab** (Fig. 12.4) is used to manipulate the appearance of an image without changing the base data.

Figure 12.4: *View Tab*

1. The **Reset Zoom** and **Zoom factor** functions adjusts the displayed size of the current image. **Fit in View** needs to be unselected to use these functions.

2. The **Fit In View** check box adjusts automatically the size of the current image to the **Image Viewer** window.

3. The **Contrast** function applies a different luminance response curve (gamma). See section 12.3.1 for details.

4. The **Min** function applies a lower threshold with the cut-off value defined by the slider. See section 12.3.2 for details.

5. The **Max** function applies an upper threshold with the cut-off value defined by the slider. See section 12.3.2 for details.

6. The **Autocontrast** function directly applies the equalizeHist function of the OpenCV library.

7. The **Reset** function returns the contrast and range values to their default.

8. The **Pseudocolor** function is a drop-down list for selecting alternate coloring schemes for the images presented.

9. The **Frame Display Time** function adjust the frame rate in **Play** mode.

10. The **Show/Hide Processing Info** can be selected to display, near the images in the **Analyzer View**, the list of the process operated on the images in the order in which they are applied.

12.1.3 ROI

The **ROI** tab (Fig. 12.5) is used to save/load data relating to regions of interest drawn on an image.
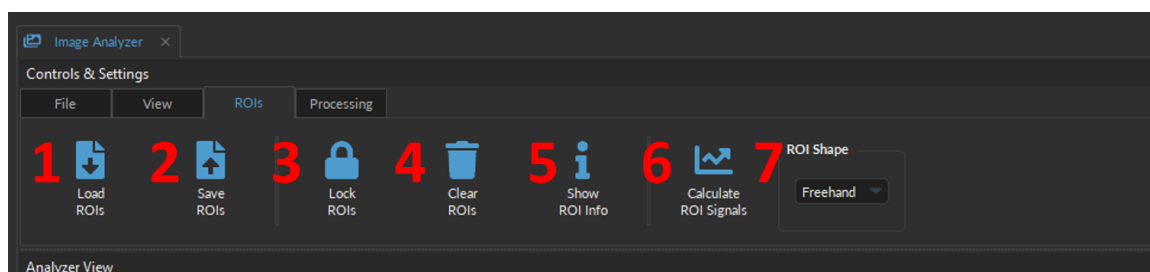
Figure 12.5: *ROI Tab*

1. The **Load ROIs** function loads .doric file containing informations about the saved ROIs.

2. The **Save ROIs** function saves the current ROIs information to a .doric file.

3. the **Lock/Unlock ROIs** can be selected to lock and unlock changes for ROIs. When it is active, you can not move or draw a ROI.

4. The **Clear ROIs** button clears all ROIs.

5. The **Show ROI Info** function display near to the images in the **Analyzer View** the **ID**, **Name**, and size (in pixels) of each ROIs.

6. The **Calculate ROI Signals** start the computing of ROI Signals depending of the ROI(s) drawn in the **Image Viewer**.

7. The **ROI shape** function is a drop-down list that allows the selection of the **ROI** shape. These include **Freehand, Circle, Rectangle** and **Square**.

12.1.4 Processing

The **Processing** tab (Fig. 12.6) is used to process the image data.

Figure 12.6: *Processing Tab*
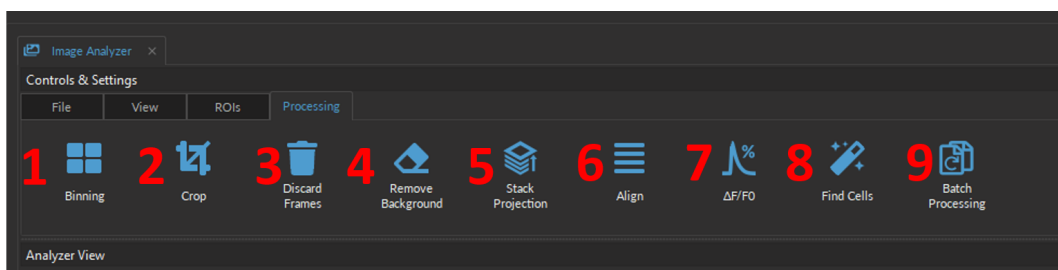
1. The **Binning** function combines a cluster of pixels in a single pixel to reduce the amount of data and facilitate the processing. Note: in 2x2 binning, an array of 4 pixels becomes a single larger pixel.

2. The **Crop** function allows to crop the current image in smaller dimensions to reduce the amount of data and facilitate the processing.

3. The **Discard Frame** function allows to remove user-defined frames in a data set. Note: The timestamps of the remaining frames stay the same when discarding frames.

4. The **Remove Background** function removes the average value of a selected ROI from all images in the stack. Note: it is not recommended to use the **Remove Background** function before the $\Delta F/F_0$ function.

5. The **Stack Projection** function projects all movie frames to a single frame using the method selected in the Settings dialog. See section 12.3.6 for details.

6. The **Align** function aligns the image stack to the user-defined key frame. See section 12.3.3 for computational details. Selecting this button will open the **Align Images** window (Fig. 12.7). 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.
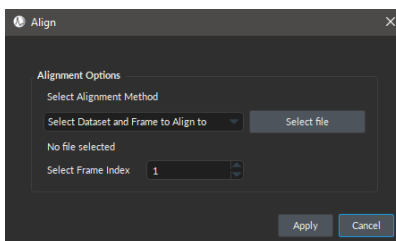

Figure 12.7: *Align Images Window*

- The **Align to the First Frame** method uses the first image in the set to align the rest.
- The **Select Frame to Align to** method allows the selection of a single image in the set to use for alignment of all other frames. Select the frame in the **Select Frame Index** display under **Select Alignment Method**.
- The **Select Dataset And Frame to Align to** method aligns the current set using data from a different image set.
- The **Select Alignment Shifts from Other 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.

7. The $\Delta F/F_0$ function calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 12.3.4 for details.

8. The **Find Cells** function detects the cells and creates the ROI automatically. See section 12.3.5 for details.

9. The **Batch Processing** function opens the **Batch Processing Window** (Fig. 12.8). This allows the processing of large datasets in sequential order, without needing to activate each individual function. The processing defined in the batch processing window is applied to all the data saved in the destination file.
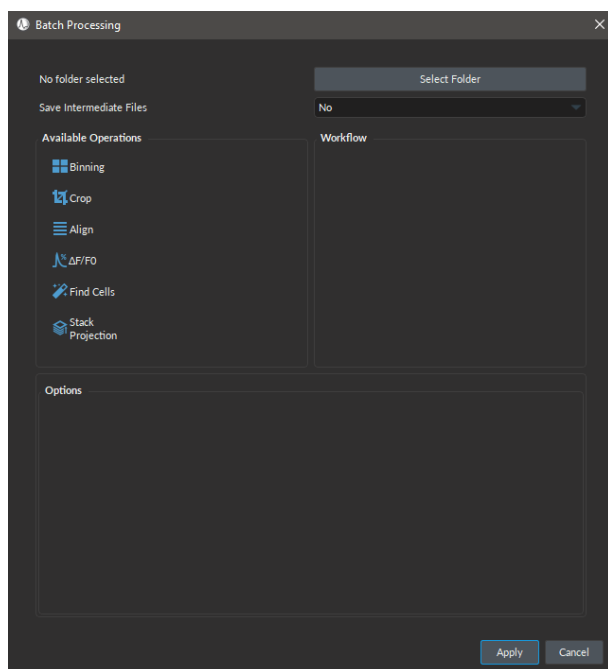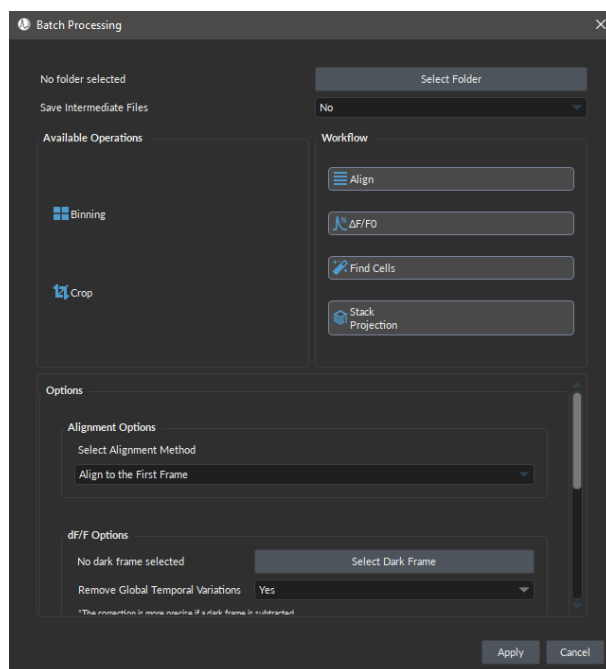
(a) *Batch processing window* (b) *Typical batch processing sequence*

Figure 12.8: *Batch Processing Window*

- The **Available Operations** 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 **Binning** function combines a cluster of pixels in a single pixel to reduce the amount of data and facilitate the processing.
  - The **Crop** function allows to crop the current image in smaller dimensions to reduce the amount of data and facilitate the processing.
  - The **Align** process aligns the image stack to the user-defined key frame. See section 12.3.3 for computational details.
  - The $\Delta\mathbf{F/F}_0$ process calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 12.3.4 for details.
  - The **Find Cells** process detects the cells and creates the ROI automatically. See section 12.3.5 for details.
  - The **Stack Projection** process projects all image frames to a single frame using the method selected in the Settings dialog. See section 12.3.6 for details.
- The **Workflow** box displays the order in which image processing actions will be taken. The parameters of the selected functions are adjusted in the **Options** box.
- The **Select a Folder** button allows the selection of a folder to save batch processing results.
- The **Save intermediate Files** option will save intermediary files in the image processing process alongside the completed files.
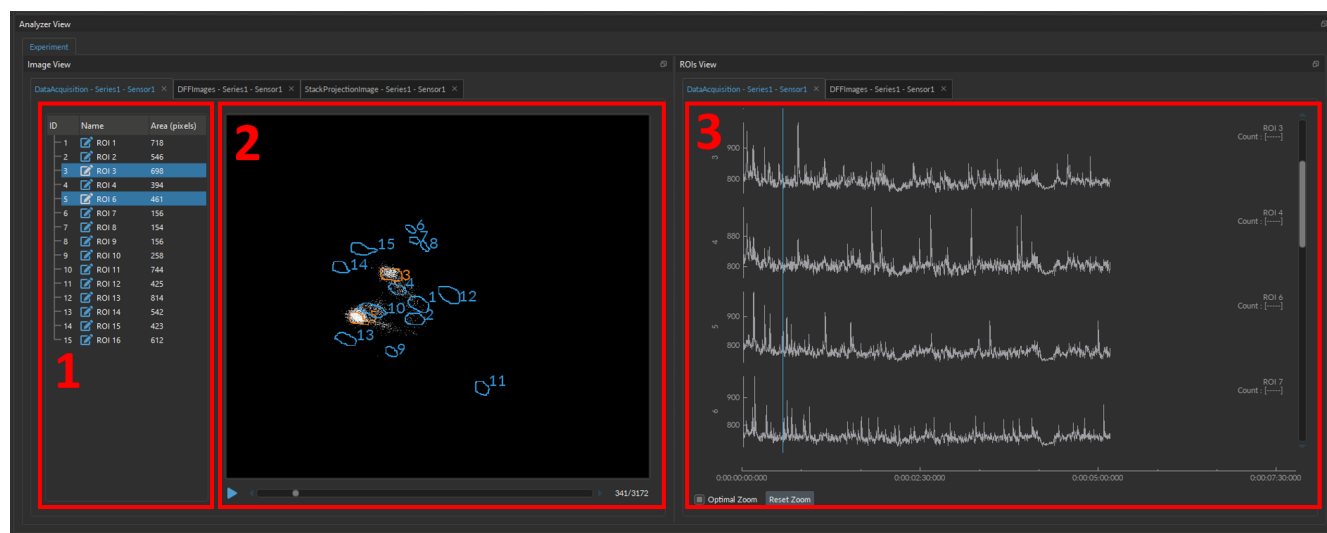
12.2   Analyzer View

Figure 12.9: *ROI View*

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 **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.

    a) The **ID** shows the order of the ROI (starting at 1).

    b) The **Name** of the ROI, by default ROI ROI_ID. It can be change by clicking twice on the name.

    c) The **Area** shows the area (in pixels) contained in the ROI.

2. 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. All selected ROI can be moved together directly in the *Image Viewer*.

3. The **ROIs View** panel shows the plot of average intensity as a function of the frame index. The Y-axis represents the average count of all the pixels of the ROI, or the variation to the baseline for ROI on normalised images. Each trace on a separate graph represent an ROI, allowing for precise intensity measurements (see Fig. 12.10)
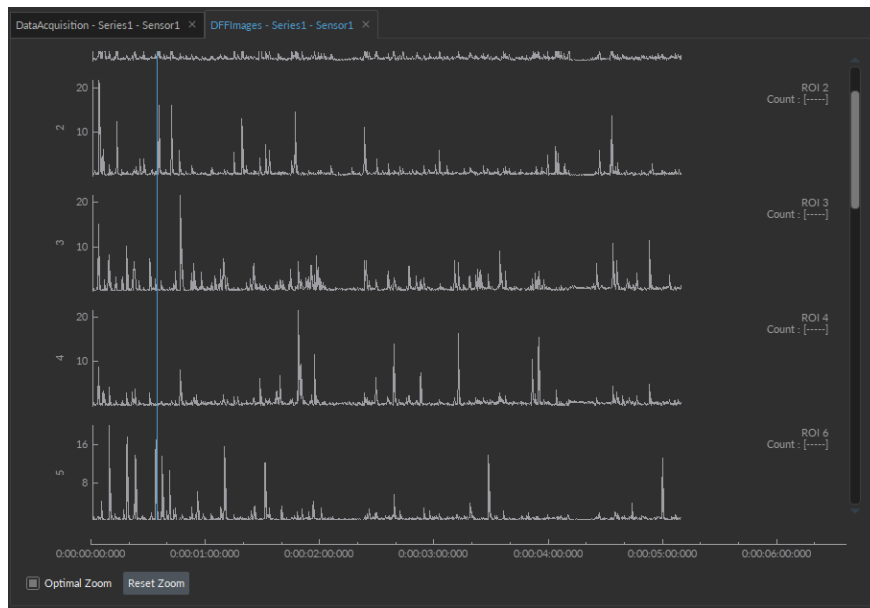
Figure 12.10: *ROI View Graph*

12.3   Algorithms

12.3.1   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.

12.3.2   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.

12.3.3   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.

12.3.4   ΔF/F₀

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. 12.11). Keep in mind that removing the average temporal profile can also remove global activity patterns.

Figure 12.11: ΔF/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 $R(t) = (F(t) - F_0)/F_0$.

12.3.5   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. 12.12. 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 filter the object found by the PCA/ICA.
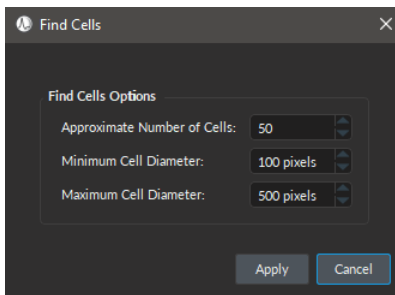

Figure 12.12: *Find Cells Settings*

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.

12.3.6   Stack Projection

This function can be used to help for ROI drawing. It calculates a temporal projection using the user-defined method (see Fig. 12.13).
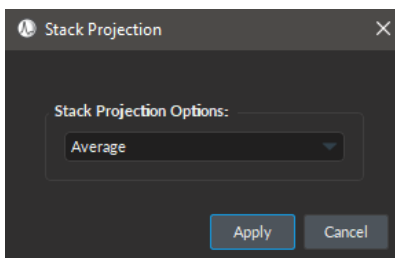

Figure 12.13: *Stack Projection Settings*

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

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

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

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

Bundle-imaging Fiber Photometry Driver (BFPD)

The Bundle-imaging Fiber Photometry Driver (BFPD) module controls the *Fiber Photometry BFPD*. This FPGA-based data acquisition unit synchronizes the output control and the input data of the acquisition. The photometry-oriented interface provides different functionalities for multi-channel experiments.
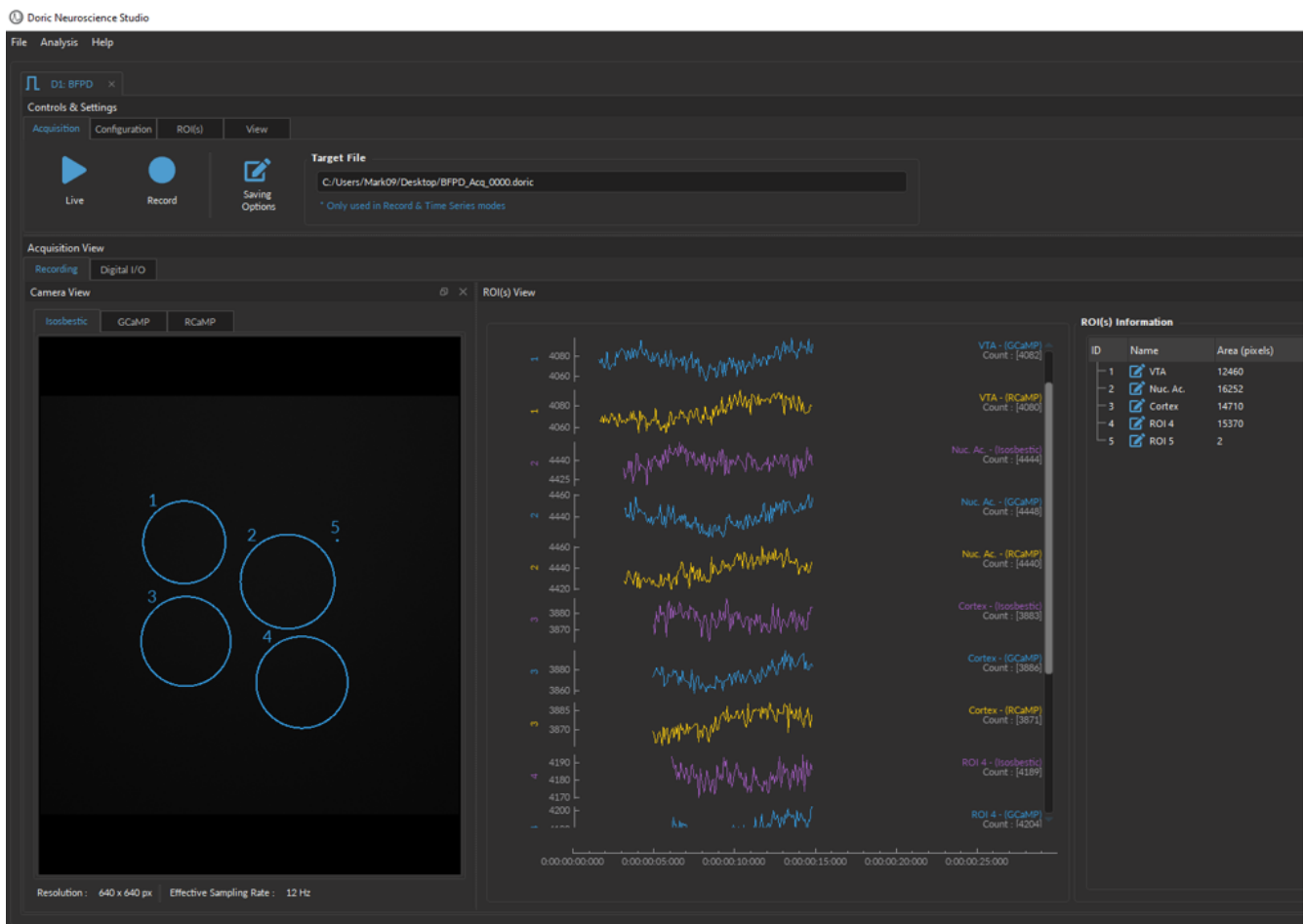

Figure 13.1: *BFPD interface*

13.1 Device Selection Window

Once *Doric Neuroscience Studio* (DNS) is opened, the *Device Selection* window should automatically pop up if the device is properly connected to the computer withe the USB cable (as in Fig. 13.2).

To add a device, **double click** on the device of choice in the *Available device(s)* sections (bottom half of window). If the device in question does not show up, double-check that the two ends of the USB cable are correctly connected to the USB ports. Then click *Refresh*. When properly connected to the system, the device will appear in the *Connected/Opened device(s)* section of the Window (see the green checkmark in Fig. 13.2).
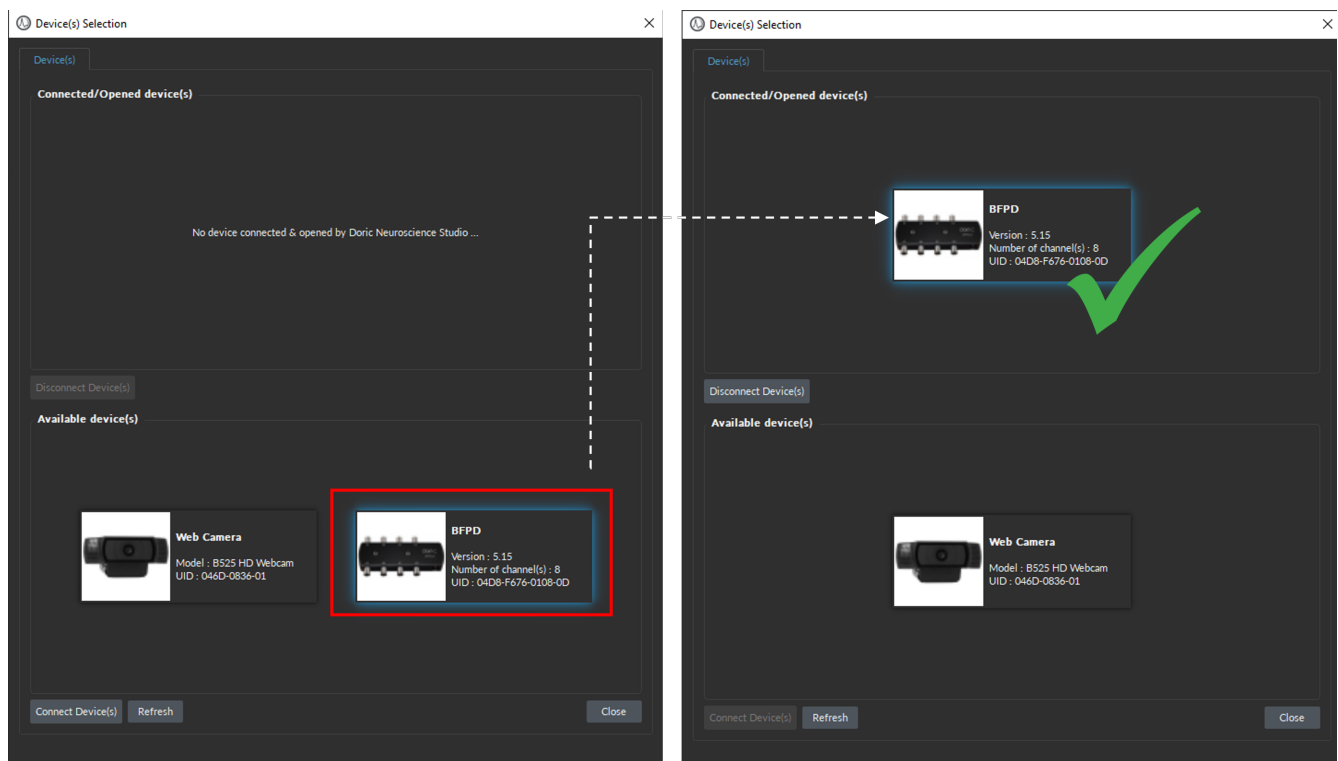

Figure 13.2: *Double click on the device of choice to connect it to DNS*

NOTE: If you have switched to DNS v6, older devices will require a firmware update to be recognized by the new version of the software. This update can be easily done using *Doric Maintenance Tools (DMT)* application and must be done one by one for each device. Further instructions can be found **HERE**.

Manually opening the *Device(s) Selection* window:
To manually open the *Device(s) Selection* window, select *File*, then *Device Selection* (as per Fig. 13.3) or use the hot key: *Ctrl+N*.
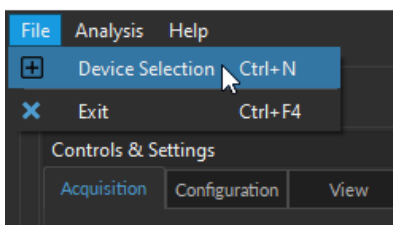

Figure 13.3: *Open Device Selection Window*

13.2 Overview

The **BFPD** interface is split into two sections (Fig. 13.4):

1. **Control and settings tabs** (Section 13.3) are used to manage different parameters and settings of the software (Acquisition, Configuration, ROI, and View).

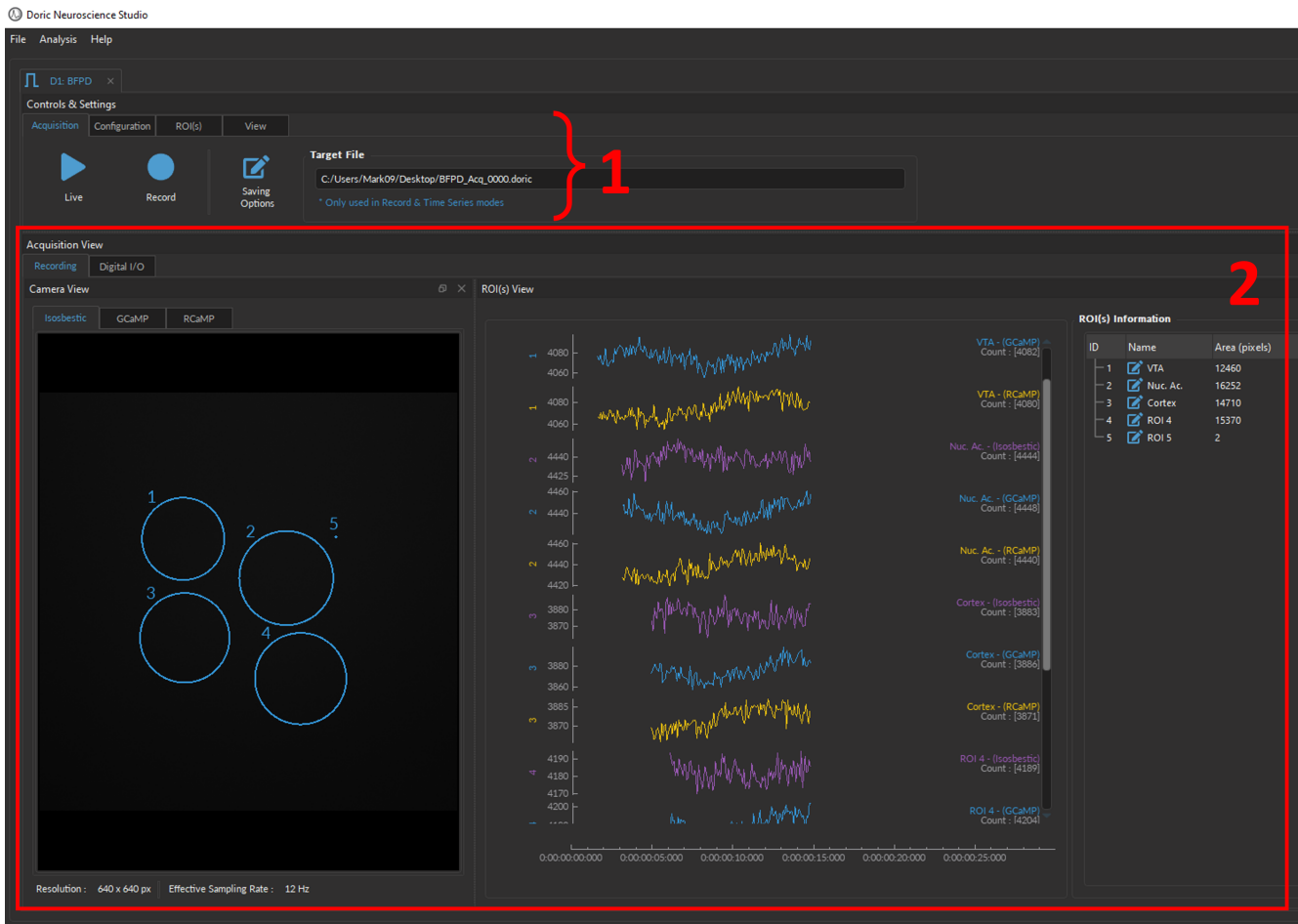2. **Acquisition view** (Section 13.5) displays the input and output traces for visualization.

Figure 13.4: *BFPD interface*

13.3   Control and Settings tabs

The **Control and settings tabs** (Fig. 13.4, 1) are used to manage the different parts of the software and are split into four separate tabs, each of which are detailed in the following sections:

- *Acquisition* tab - Section 13.3.1;

- *Configuration* tab - Section 13.3.2;

- *ROI(s)* tab - Section 13.3.3;

- *View* tab - Section 13.3.4.

13.3.1   Acquisition Tab

The **Acquisition** tab is used to start a live/recording session and set the saving parameters. The **Live** and **Record** buttons will not function if channels have yet to be set-up. See section 13.4.1 to configure channels for recording.
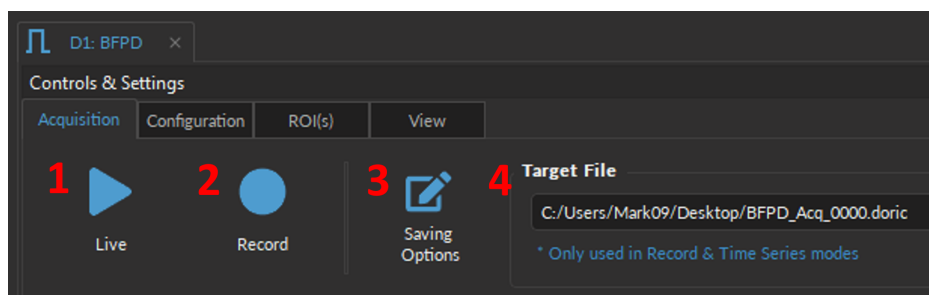
Figure 13.5: *Acquisition Tab*

1. The **Live** button (Fig. 13.5, 1) activates all prepared channels. This mode does not save data, keeping only the most recent 700 000 data points in memory. This mode is not recommended for long or critical measurement sequences. **Live** mode is useful to quickly test the recording software and to ensure that the parameters were properly set.

2. The **Record** button (Fig. 13.5, 2) activates all prepared channels while periodically saving recorded data to the computer. This mode is recommended for long measurement sequences.

3. The **Saving Options** (Fig. 13.5, 3) button opens the **Saving Parameters** window (Fig. 13.6). See section 13.3.1.1 for more details.

4. The **Target File** (Fig. 13.5, 4) displays the path and file name where the data will be stored once the **Record** button is selected. Select the **Saving Options** button to change the path and file name.
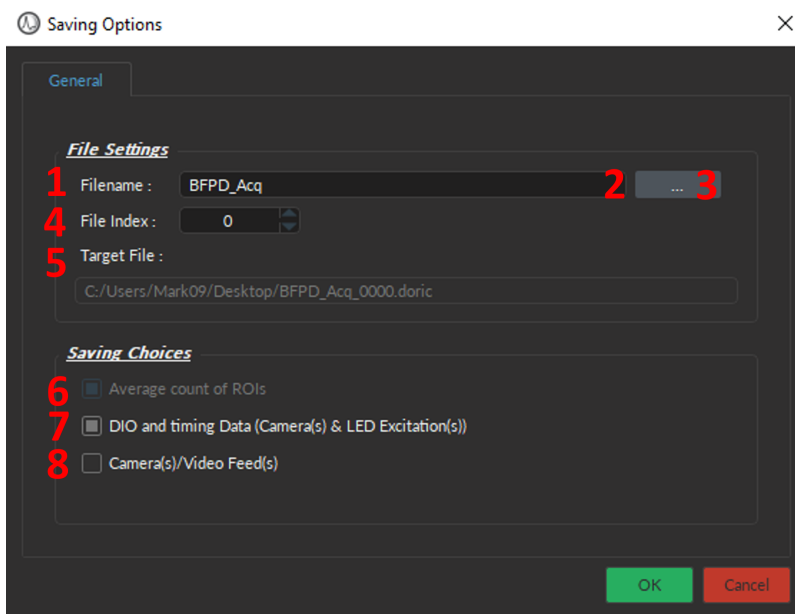

Figure 13.6: *Saving Menu Window*

13.3.1.1 Saving Parameters

The **Saving Parameter** window is used to define how and where the file is saved. This window is opened by selecting the **Saving Options** button in the Acquisition Tab (Fig. 13.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 13.6, 1).

2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 13.6, 2).

3. The **File format** (Fig. 13.6, 3) is **.doric**, an HDF5-based format that supports metadata (signal, video, images, tables, parameters, etc.). Version 6 of *Doric Neuroscience Studio* is no longer compatible with other file formats (.csv, .excel, or .tiff). We provide Matlab, Python, and Octave codes to read **.doric** files HERE (at the bottom of the web page). While not recommended, it is possible to export a *.doric* file into .csv format through the **Doric File Editor** module.

4. The **File Index** (Fig. 13.6, 4) box is used to define the current indexation number used for multiple files saved during the same measurement session. The suffix is incremented automatically when recording multiple files.

5. The **Target File** (Fig. 13.6, 5) displays the absolute path and filename where the data will be saved.

The **Saving Choices** allows users to select which type of data to save during the recording:

6. The **Average count of ROI(s)** checkbox (Fig. 13.6, 6) saves the mean pixels intensity of each defined region of interest. This setting cannot be disabled.

7. The **DIO and timing Data** checkbox (Fig. 13.6, 7), if enabled, will save the digital TTL outputs of the Camera(s) & LED Excitation(s). This value is by default enabled.

8. The **Camera(s) / Video Feed(s)** text-box (Fig. 13.6, 8), if enabled, will save the raw image stacks collected by the CMOS sensor(s). Note that selecting this option will generate large files.

13.3.2   Configuration Tab

The **Configuration** tab is used to set the channels and the global settings (such as sampling rate and Master trigger options), as well as save and load the preset channel configurations.
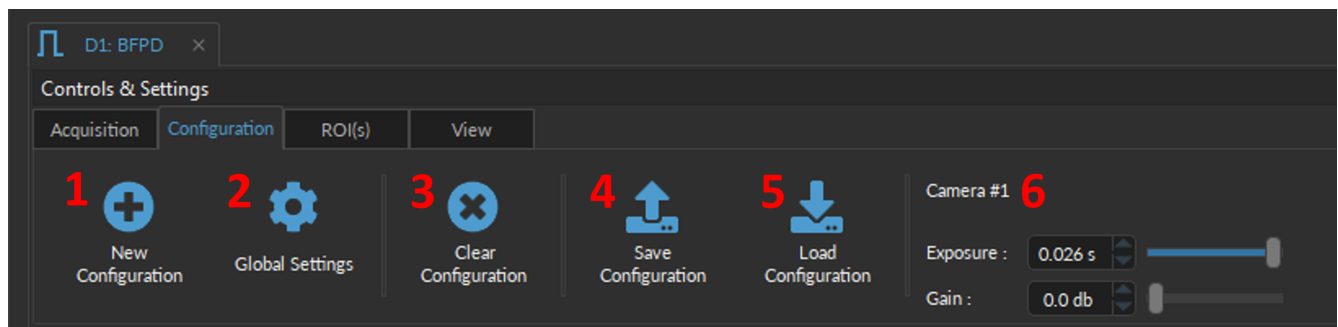

Figure 13.7: *Configuration Tab*

1. The **New Configuration** button (Fig. 13.7, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 13.4. Table 13.1 describes different types of channels available, their use cases and their individual sections.

2. The **Global Settings** (Fig. 13.7, 2) opens the **Global Options** window in Fig. 13.8, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 13.3.2.1 for more details.

3. The **Clear configuration** button (Fig. 13.7, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.

4. The **Save configuration** button (Fig. 13.7, 4) allows a BFPD configuration to be saved in the **.doric** format. This file preserves the current channel configuration/parameters, the Acquisition View window organization, and any custom trace colors and names.

5. The **Load configuration** button (Fig. 13.7, 5) imports a pre-configured **.doric** file into the module.

6. The **Camera #** options (Fig. 13.7, 6) are used to adjust the CMOS sensor. When the *BFMC* in use has more than one sensor, multiple **Camera** boxes will be displayed, one for each sensor, each of which includes:

   - The **Exposure (s)** textbox specifies the length of time that the sensor collects light from the sample. There are trade-offs between exposure time, image brightness, and phototoxicity.

- The **Gain (dB)** text-box corresponds to the electronic amplification of the signal after collection by the sensor, in logarithmic decibels (dB). Note that increasing the gain will simultaneously increase both the signal and noise. We recommend keeping the gain low unless signals are very weak.

Tips:
Prior to the start of the experiment, the camera exposure time should be maximized, while the gain should be set to 0 dB. If the signal is too strong or the camera is saturated, reduce the excitation power before reducing the exposure to minimize fluorophore bleaching. If the detected signal is too weak, the gain should be increased. However, increasing the gain will also amplify electronic noise and reduce signal noise ratio.

13.3.2.1 Global Settings

Through the **Global Settings**, user can set the acquisition **Sampling Rate** and specify the **Master Trigger Options** that will start recordings.
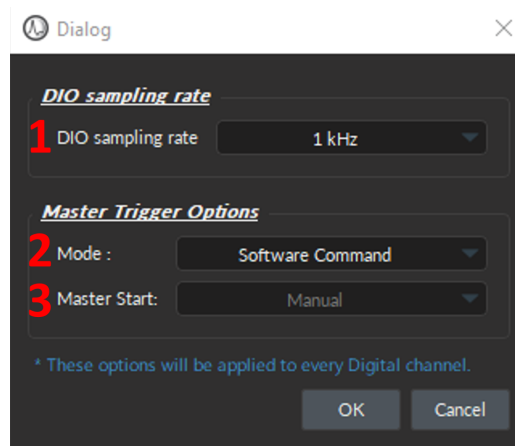

Figure 13.8: *Global Options Window*

1. The **DIO sampling rate** (Fig. 13.8, 1) is the number of data points collected per second, measured in Hz or kHz. By default, the sampling rate is set to 1kHz, but can range between (100Hz to 10kHz). Note that this value ONLY refers to the **CAM** and **EXC** digital outputs which use TTL pulses to synchronize the **BFMC** device. The **effective sampling rate** of each individual **CAM# EXC#** channel is displayed at the bottom of the **Camera View** (Fig. 13.41, no. 4).

2. The **Mode** (Fig. 13.8, 2) of the **Master Trigger Options** sets the origin (internal, external or time-series) of the trigger that will start the recording session and synchronize all the external and internal devices. Four options are available for different use cases:

   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 13.5, 2). The **Master Start** is, by definition, always **Manual**.
   - *Triggered* - The recording session starts when a trigger signal is received (from the **Master Start**, either manual or from an external digital source), and continues even if the trigger signal stops. Thus, the **Triggered** mode only controls the START of the recording session (and NOT the endpoint).
   - *Timeseries* - This mode allows users to record pre-defined series over longer periods of time (that can span several days) (Fig 13.9a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 13.9b) pops up. See section 13.3.2.2 for more details.

3. The **Master Start** (Fig. 13.8, 3) defines the source that will automatically start the recording. This source can either be:

   - *Manuel* - the user ultimately starts the recording session by clicking **Record** within *Doric Neuroscience Studio*;
   - *Digital I/O Channel (1-4)* - The specified channel will automatically begin the recording session when it receives a digital trigger pulse from an external device. ***However, this mode still requires that the **Record** button is selected BEFORE the TTL trigger signal is received.***

13.3.2.2 Time Series

The **Time Series** mode enables users to perform long-term recordings with a long delay. For example, 1 minute of recording every hour for 12 hours.

The **Time Series** Window (Fig 13.9b) can be opened by clicking on the **Record** button (Fig. 13.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 13.8, 2). Every **Time series** sequence is automatically saved to the same *.doric* file defined in **Saving Options** (Section 13.3.1.1).

(a) *Time Series Acquisition Timing Diagram*

(b) *Time Series Window*
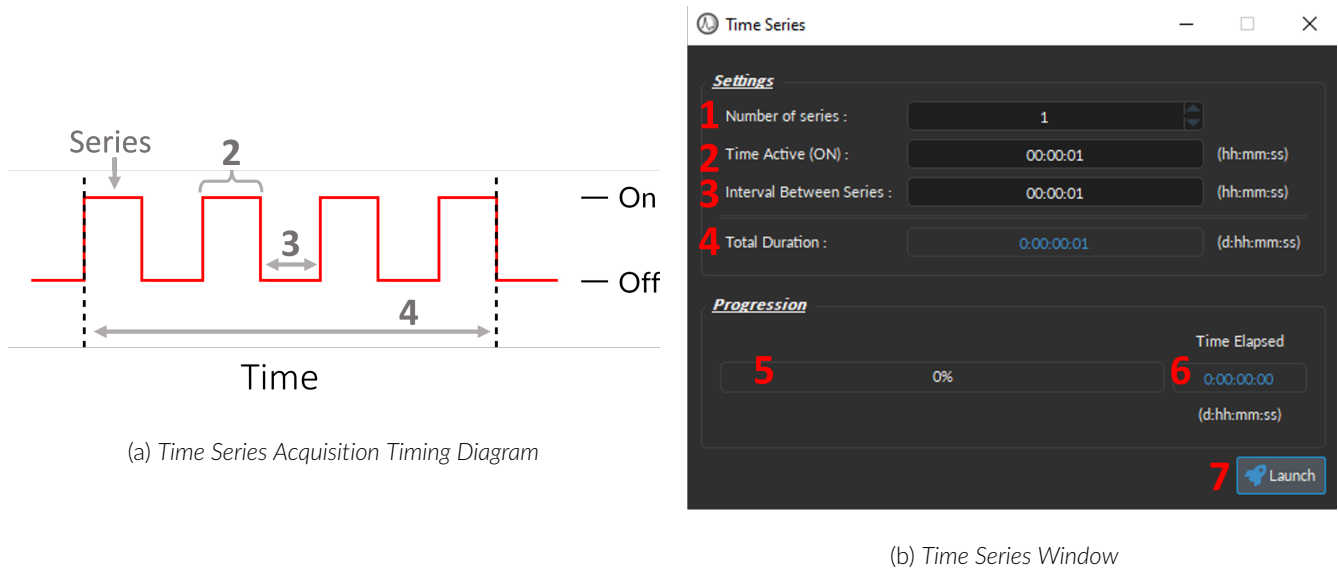
Figure 13.9: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 13.9b) sets the following parameters:

1. The **Number of series** (Fig. 13.9b, 1) defines the amount of times the series is repeated.

2. The **Time Active (ON)** (Fig. 13.9b, 2) defines the duration of the series.

3. The **Interval Between Series** (Fig. 13.9b, 3) defines the amount of time between each series, if the **Number of series** is greater than 1.

4. The **Total Duration** (Fig. 13.9b, 4) displays the total amount of time that the timeseries recording will take place.

5. The **Progression bar** (Fig. 13.9b, 5) indicates the progression of the timeseries (in %).

6. The **Time Elapsed** (Fig. 13.9b, 6) counter indicates the amount of time that has already passed in d:hh:mm:ss.

7. The **Launch** (Fig. 13.9b, 7) 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.

13.3.3 ROI(s) Tab

The **ROI(s) Tab** (Fig. 13.10) contains parameters to save, load, clear, or edit bundle fiber photometry ROI(s).
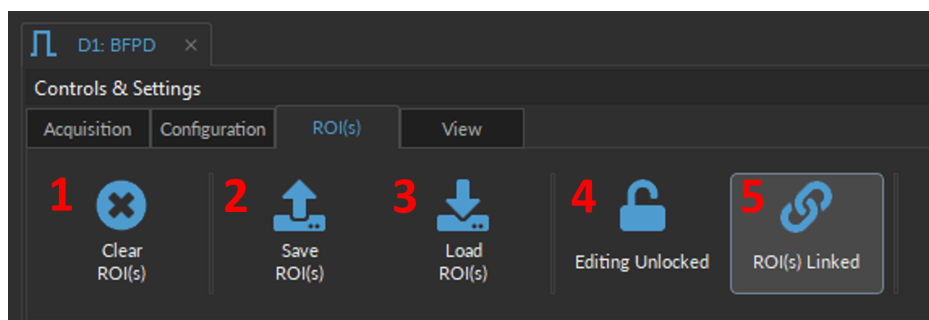
Figure 13.10: *ROI(s) Tab*

The **ROI(s)** parameters are as follows:

1. The **Clear ROI(s)** button (Fig. 13.10, 1) deletes all drawn regions of interest (ROI) within the **Camera View**. Note that unless the ROI(s) were previously saved, these ROI(s) cannot be recuperated.

2. The **Save ROI(s)** button (Fig. 13.10, 2) saves the region of interests drawn in the **Camera View** in a *.doric* file, so that the identical ROI can be re-imported into the module at a later time. At least one ROI must be drawn for this feature to work.

3. The **Load ROI(s)** button (Fig. 13.10, 3) imports a previously saved .doric file. Note that this ROI(s) configuration can be edited, but must be re-saved in order for changes to be conserved.

4. The **Editing Unlocked** button (Fig. 13.10, 4) when enabled prevents new ROI(s) from being drawn, but does not prevent moving or reshaping a selected ROI (see section 13.5.2).

5. The **ROI(s) Linked** button (Fig. 13.10, 5) automatically redraws identical ROI(s) in the other CAM# excitation tabs (Fig. 13.41, 1) within the **Camera View**. **Unlinking** previously linked ROI(s) deletes the ROI(s) from the **Camera View**. Note that it is preferred to uncheck **ROI(s) Linked** between cameras in order to move and resize ROI on each camera window independently to properly select the optical fibers.

   TIP 1: We suggest drawing one ROI for each optical fiber, plus one outside to monitor the background.

13.3.4   View Tab

The **View Tab** (Fig. 13.11) is used to modify the presentation of graphs in the **Acquisition view**.
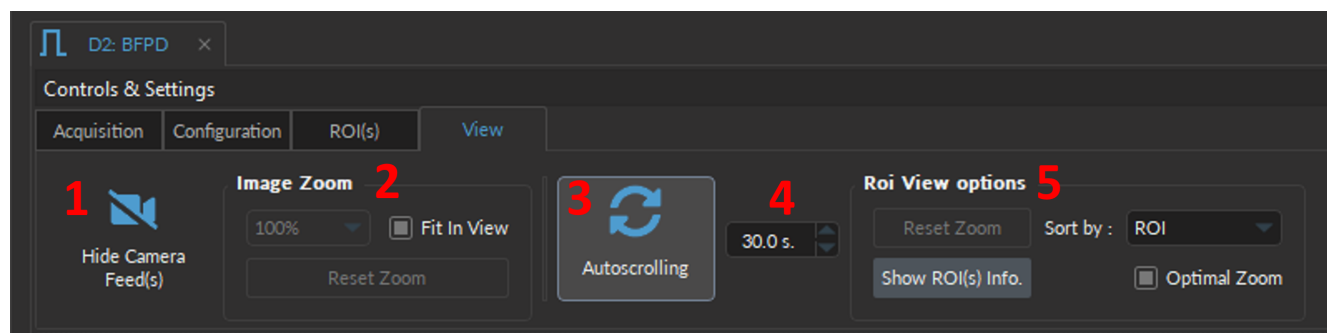

Figure 13.11: *View Tab*

The **View** parameters are as follows:

1. The **Hide Camera Feed(s)** button (Fig. 13.11, 1) will remove the **Camera View** from the **Acquisition View**, automatically enlarging the **ROIs View**. Disabling the same button (renamed **Show Camera Feed(s)**) makes the **Camera View** reappear.

2. The **Image Zoom** (Fig. 13.11, 2) includes the following:

- The **Zoom %** specifies the zoom factor for the image display, which ranges between 10%-500%.

- The **Fit In View** checkbox automatically adjusts the image to fit the entire **Camera View** window.

- The **Rest Zoom** button returns the zoom factor to 100%.

3. The **Autoscrolling** button (Fig. 13.11, 3), when selected, automatically sets the graphs to scroll as new data appears.

4. the **Autoscrolling range** (Fig. 13.11, 4) sets the graph zoom to the value of choice, specified in the text-box.

5. The **ROI View Options** (Fig. 13.11, 5) includes the following:

   - The **Reset Zoom** button readjusts the graph zoom to the default value.

   - The **Soft by:** drop-down lists allows users to organize the order of the traces within the **ROI(s) View** by either their *ROI* (Fig. 13.12a) or by their *Excitation* type (Fig. 13.12b).

(a) *Sort by ROI* (b) *Sort by EXC*
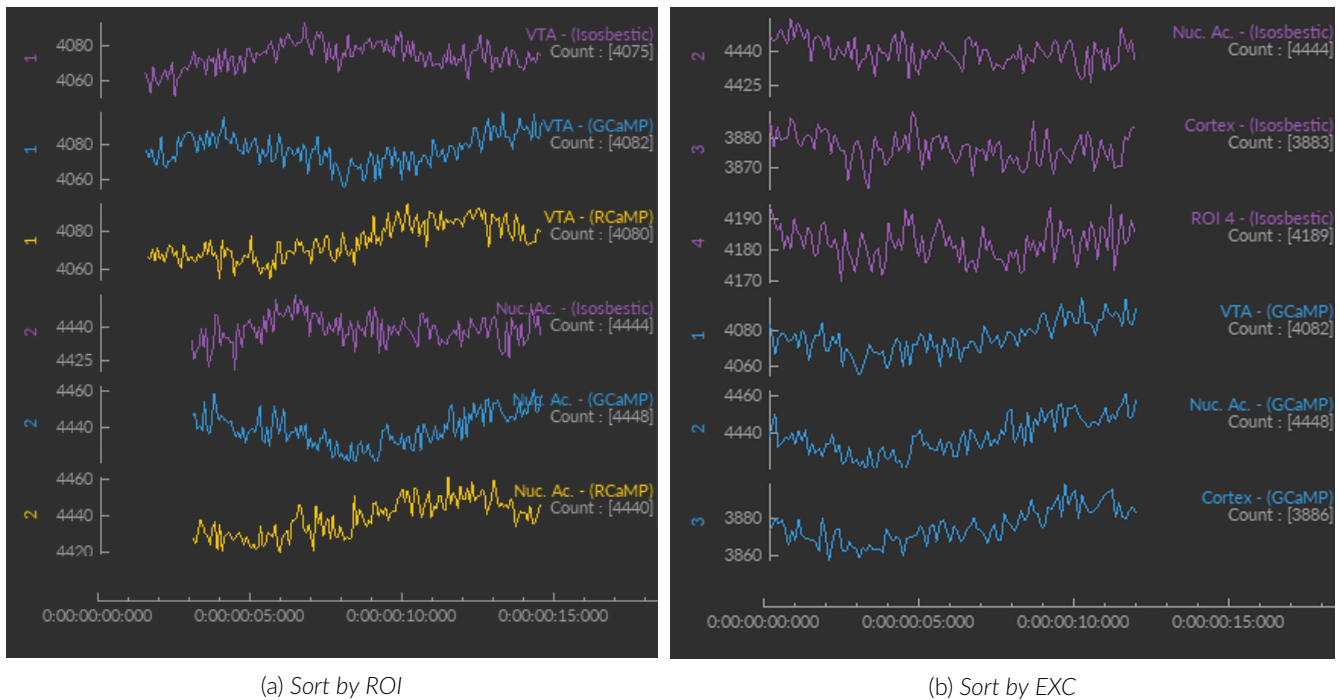
Figure 13.12: *Sort ROI(s) View options*

- The **Show ROI(s) Info.** button opens or closes the **ROI(s) Information** Tab in Fig. 13.43, 2.

- The **Optimal Zoom** check-box automatically adjusts the graph range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

13.4   BFPD Configurations

13.4.1   New Configuration:

To create a new channel, regardless of the input and/or output type, select the **New Configuration** button, which can be found under the **Configuration** tab (Fig. 13.7, 1). This will open the **Channel(s) Configuration** window (Fig. 13.13). To generate a new **Channel** using the **Channel(s) configuration** window (Fig. 13.13):

1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 13.13, 1). Table 13.1 describes the use case of each type.

2. Clicking on the icon will display the **Channel Type**-specific options on the right side of the window. Each **Channel Type** has a number of parameters that can be configured to fit the needs of the experiment(s). Details of the parameters and their options will be covered in the following sections. See Table 13.1 for hyperlinks to the relevant sections.

3. Select the **Add/Apply** button (Fig. 13.13, 3) to generate the defined channel or to update an already configured channel. It does not automatically close the *Channel Configuration* window. This allows the user to conveniently set up all required channels one after the other.

4. Select the **Close** button to shut the window once all channels are configured.
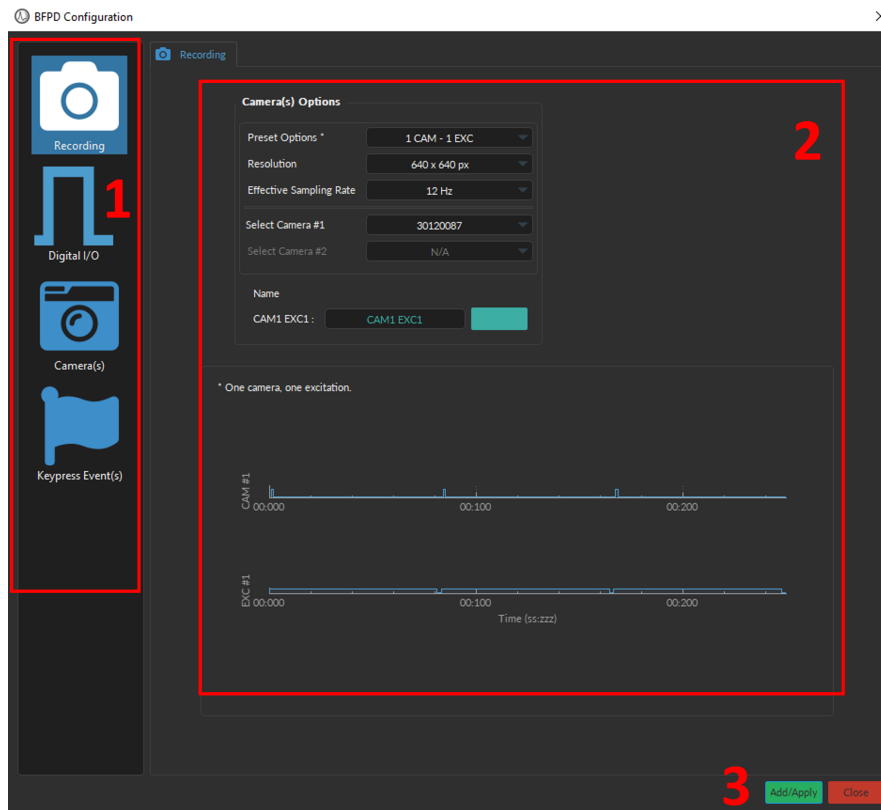

Figure 13.13: *Channel(s) configuration window*

13.4.2 Channels Types

Different input and output types can be configured for the experiment by creating a new Channel in the Configuration tab or editing an existing one (Fig 13.7). Table 13.1 details the types of inputs and output the BFPD and the software can handle and gives quick access to their sections.

Table 13.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	Recording	To collect the fluorescence signal of *BFMC* ROI(s)	13.4.3
	Digital I/O	For input and output of TTL signals	13.4.4
	Camera(s)	To collect images for behaviour experiments	13.4.5
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	13.4.6

13.4.3 Recording Channels

The **Recording** channel type allows users to select preset options especially designed for *Bundle Fiber Photometry*. These preset options will automatically create both the required inputs and outputs, including:

- The **Digital Output**, such as Camera (**CAM**) and LED Excitation (**EXC**) triggers required to drive data acquisition;

- The raw **Image stacks** inputs from the CMOS sensor inputs to collect the fluorescent signal;

- The **ROI(s) signal** input is calculated from the average pixel intensity of the user-defined regions of interest.
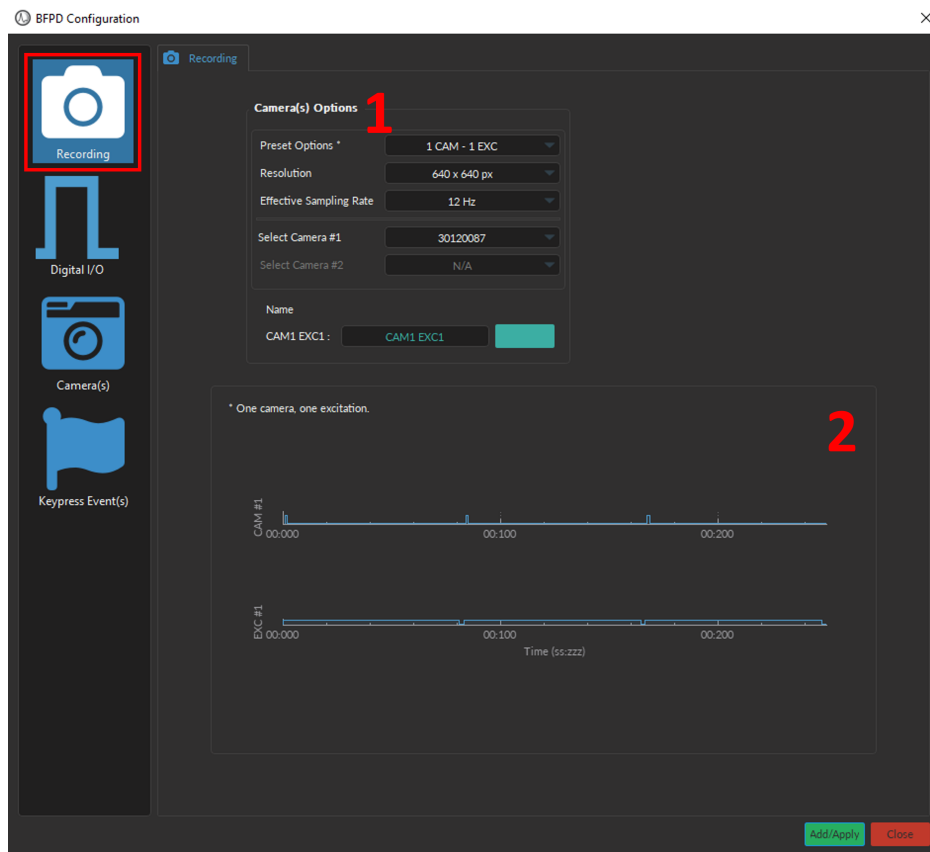
Figure 13.14: *Channel(s) configuration window, Recording*

REMINDER:
For the *BFPD* to appropriately **drive the LED excitations**, the LED Driver must be set to the **ExTTL** mode.

The **Recording** channel is divided into two sections (Fig. 13.14):

1. The **Camera Options** (Fig. 13.15) defines which preset option to use and allows users to select the proper camera and camera parameters for the recording, such as:
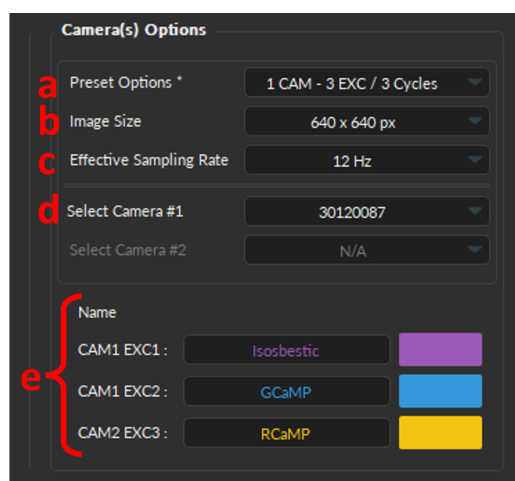
Figure 13.15: *Recording Channel - Camera Options*

a) The **Preset Options** drop-down (Fig. 13.15, a) contains six pre-configured options which are listed in Table 13.2, along with their use cases.

Table 13.2: *Preset option explanations*

Preset Option	CAM#	# of LED(s)	Cycles[1]	Application
1 Cam - 1 Exc	1	1	1	For a single fluorophore, without isosbestic point (such as RCaMP).
1 Cam - 2 Exc/2 Cycles	1	2	2	For a single fluorophore, with its isosbestic point (such as GCaMP).
1 Cam - 3 Exc/3 Cycles	1	3	3	For select custom *BFMC*.
2 Cam - 2 Exc/2 Cycles	2	2	2	For two fluorophores, and without isosbestic point.
2 Cam - 3 Exc/2 Cycles	2	3	2	For two fluorophores, and one isosbestic point (such as GCaMP and RCaMP), where both isosbestic and red fluorophore will be simultaneously sampled. *This preset option is ideal when a higher **Effective Sampling Rate** is required.*
2 Cam - 3 Exc/3 Cycles	2	3	3	For two fluorophores, and one isosbestic point (such as GCaMP and RCaMP), where none of the excitations overlap in time. *This preset option is ideal if biological cross-talk is a primary concern, but reduces the **Effective Sampling Rate**.*

b) The **Image Size** drop-down (Fig. 13.15, b) sets the resolution (pixel x pixel) of the Image stack from which ROI(s) will be computed. There are six options, among which the lowest available *Image size* is 256 x 256, and the maximum is 1024 x 1024. Note that if the save **Image Stacks** option is enabled, using a large resolution will result in larger *.doric* data files.

c) The **Effective Sampling Rate** drop-down (Fig. 13.15, c) sets the true frequency (in Hz) of each **EXC** since, when more than one excitation is used, the excitations are interleaved with one another, reducing the sampling rate by half (for 2 EXC) or by a third (for 3 EXC). Thus, the following **Effective Sampling Rate** are available according to the number of excitations:

---

[1]Series of events that occur during one measurement

- *1 EXC*: 3 Hz - 60 Hz
- *2 EXC*: 3 Hz - 30 Hz
- *3 EXC*: 3 Hz - 12 Hz

d) The **Select Camera #** drop-down (Fig. 13.15, d) sets which CMOS camera sensor will be labeled as **CAM1** and **CAM2** (if in use), by selecting the *Camera ID*.

e) The **Name CAM# EXC#** (Fig. 13.15, e) allows users to label the specific **Camera excitations** with more intuitive name. This setting also lets users select the trace color for the data collected during the corresponding excitations.

2. The **Sequence Preview** (Fig. 13.16) displays the TTL output pulses for the **CAM** and **EXC** that will be used during the recording.

Figure 13.16: *Recording Channel - Preview digital Camera and Excitations outputs*

f) The **CAM#** displays a preview of the TTL trigger that will drive the camera. One frame will be collected at the onset of each TTL pulse.

g) The **EXC#** displays a preview of the digital output signal that drives when the specific LED excitation.

13.4.4 Digital I/O Channels

Each **Digital I/O** channel can be configured as an output or an input to create TTL (On/Off) pulse sequences. **Digital Outputs** can provide triggers to external devices (such as light sources) required for the experiment while remaining synchronized with to recording system. In addition, **Digital Inputs** can record a copy of the trigger of an externally driven device used during the experiment (such as the timing of displayed stimuli or a measured behavior).

The *Channel(s) Configuration* window for the **Digital I/O** Channel is divided into three sections (Fig. 13.17): (1) the **Channel Options** (Section 13.4.4.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 13.4.4.2).
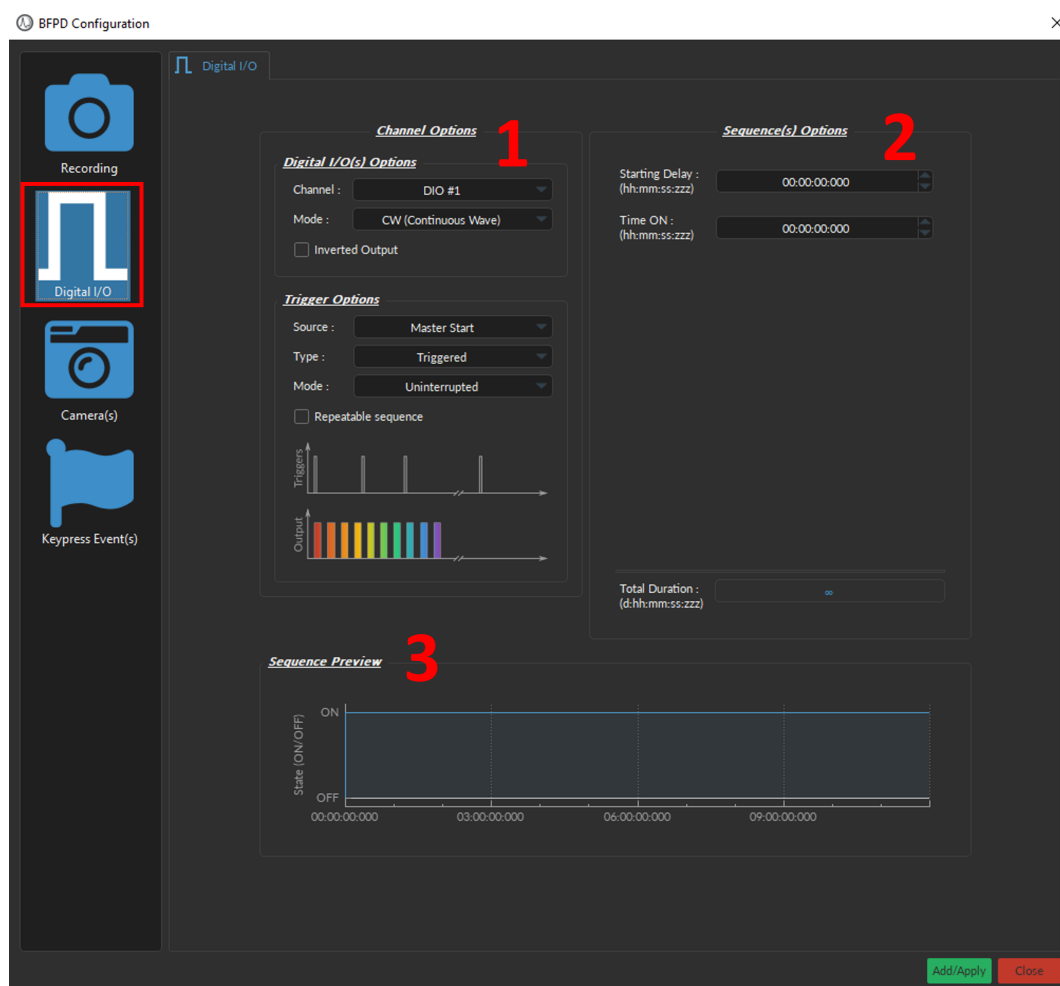
Figure 13.17: *Channel(s) configuration window, Digital I/O - CW mode*

13.4.4.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.
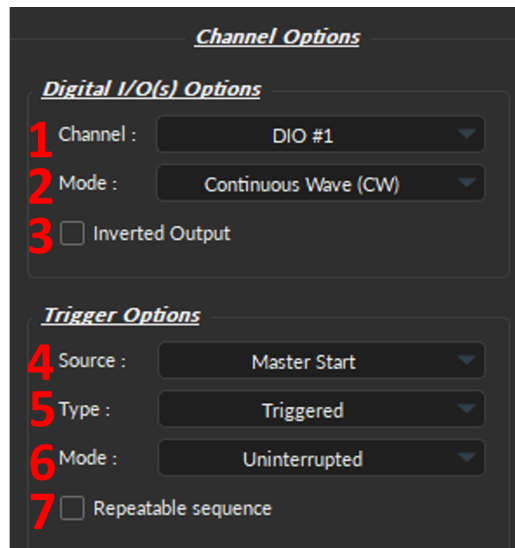
Figure 13.18: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel** (Fig 13.18, 1) identifies the channels available to create a Digital I/O. The channel can be changed by selecting a new one from the drop-down list. Each numbered channel on the physical BFPD corresponds to the same number of the digital channel within the software.

2. The **Mode** (Fig 13.18, 2) identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). Three modes are available:

   - The **Continuous wave (CW)** Mode (Fig. 13.19a);
   - The **Square (TTL)** Mode (Fig. 13.19b);
   - The **Input** mode receives a signal that are either 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the BFPD (See Section 13.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.
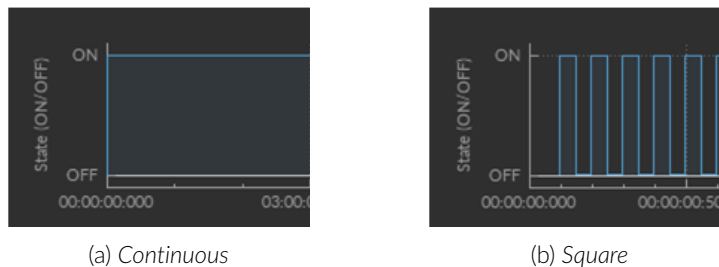

(a) *Continuous* (b) *Square*

Figure 13.19: *Channel Options - Output Modes*

3. The **Inverted Output** checkbox (Fig 13.18, 3), when enabled, will convert every 0 to 1 and 1 to 0, such as in Fig. 13.20.
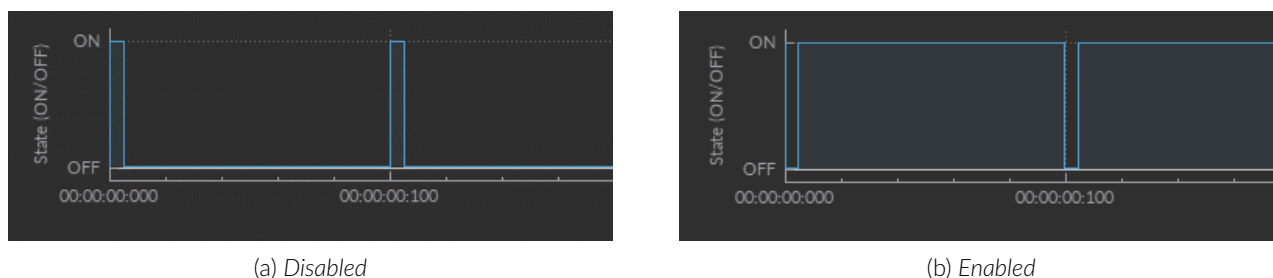
(a) *Disabled*

(b) *Enabled*

Figure 13.20: *Inverted Output*

Trigger Options:

4. The **Source** trigger option (Fig 13.18, 4) specifies the element that will set off the digital output. Two options are available:

   - The **Master Start** - will activate the output when the user selects the **Record** or **Live** button.
   - The **Digital I/O** channel - will activate the output when the console receives a TTL pulse from the selected DIO channel. Note that users must still first select the **Record** or **Live** button, setting it in a *listening* mode, which will wait until it receives the proper digital input.

5. The **Type** (Fig 13.18, 5) defines how the trigger activates a sequence. This includes input sequences, which can be triggered/gated by an outside source.

   - In **Triggered** mode (Fig. 13.21a), 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 (Fig. 13.21b), the sequence will start once the voltage reach a high TTL signal (4 V or more) on the input modulation BNC. When the TTL signal reaches a low TTL signal (0.4 V or less), the sequence stops and waits for another high TTL signal to continue. This mode can cut pulses, once the high signal returns. ***ONLY AVAILABLE FOR SQUARE (TTL) MODE***
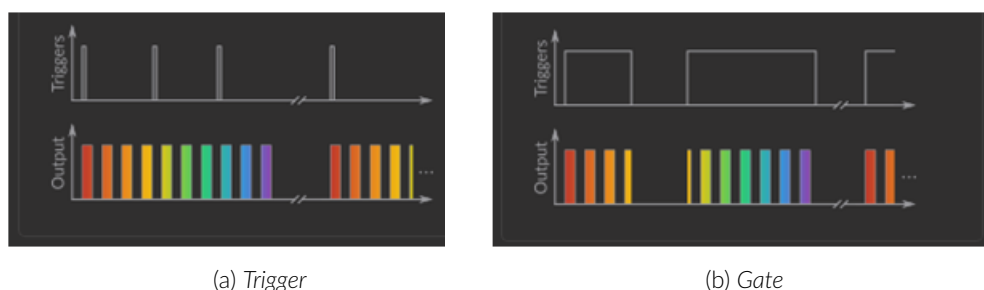

(a) *Trigger*

(b) *Gate*

Figure 13.21: *Trigger Options Modes*

6. The **Mode** (Fig 13.18, 6) defines how the sequence will run if a second TTL pulse is received before the sequence ends. This includes input sequences, which can be triggered/gated by an outside source. Four options are available:

   - The **Uninterrupted** mode - Ignores the additional TTL input until the sequence ran its course. If the TTL signal is received after the end of the sequence, it will trigger a new one.
   - The **Paused** mode - A second TTL pulse will stop the sequence at that time point. A third TTL pulse will continue the sequence, resuming the sequence from the moment it was paused.
   - The **Continued** mode - A second TTL pulse will stop the sequence at that time point. A third TTL pulse will start the sequence, resuming the sequence as if it was never paused.

- The **Restart** mode - A second TTL pulse will stop the sequence at that time point. A third TTL pulse will trigger the start of a new sequence.

7. The **Repeatable sequence** checkbox (Fig 13.18, 7), when enabled, will run the sequence when additional TTL pulses are received (Fig. 13.22).
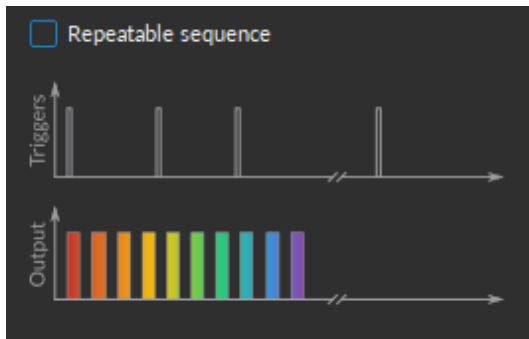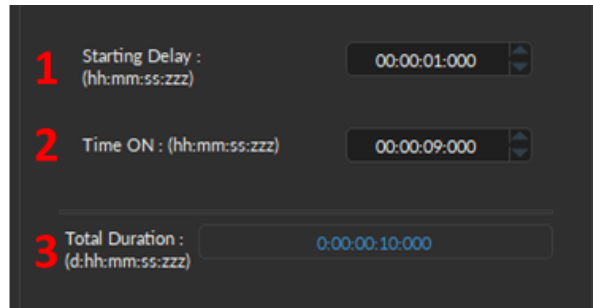

(a) *Disabled*
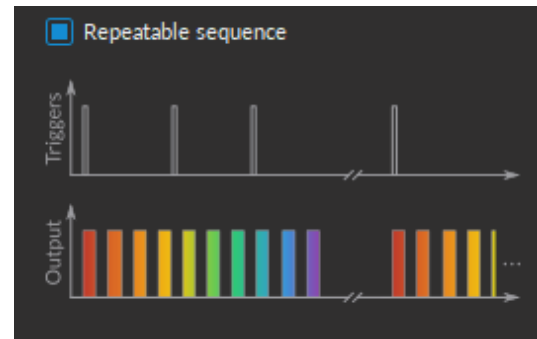
(b) *Enabled*

Figure 13.22: *Repeatable sequence*
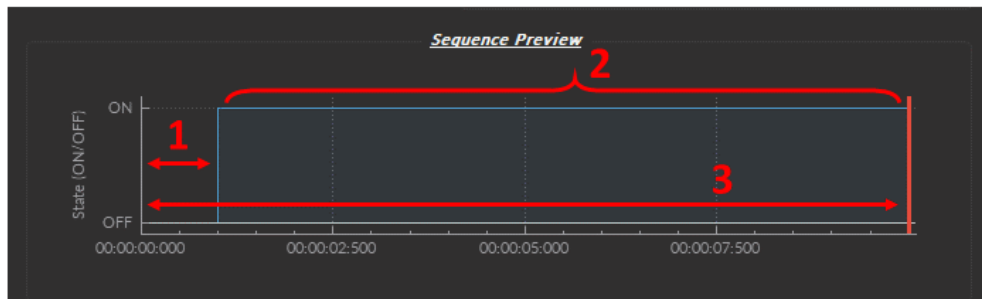
13.4.4.2   Sequence Options & Preview

The **Sequence options** section (Fig. 13.23a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 13.23b) displays the corresponding shape and timing of the sequence. 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**.

The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 13.18), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 13.19a) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

  1. The **Starting Delay** (Fig 13.23, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

  2. The **Time ON** (Fig 13.23, 2) 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** (Fig 13.23, 3) 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**.

(a) *Sequence Options*

(b) *Sequence Preview*

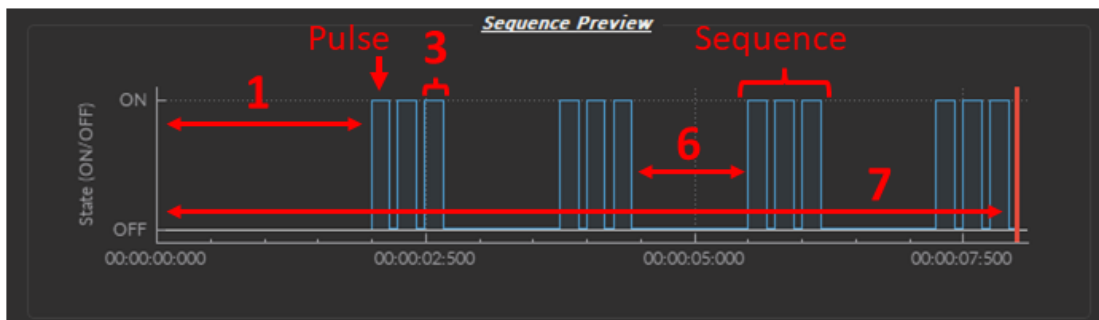Figure 13.23: *Channel(s) configuration window, Digital I/O - CW Mode*

- The **Square** channel mode (Fig. 13.19b) allows the creation of a square TTL pulse sequence. The elements included in the Sequence Option box are as follows (Fig. 13.23, 1-3):

  1. The **Starting Delay** (Fig 13.24, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

  2. The **Frequency** (Fig. 13.24, 2) 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).

3. The **Time ON** (Fig. 13.24, 3) 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.

4. The **Pulse(s) per sequence** (Fig. 13.24, 4) sets the number of pulses within a single sequence. If it is set to 0, the number of pulses will be infinite.

5. The **Number of sequence(s)** (Fig. 13.24, 5) sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** (Fig. 13.24, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).

7. The **Total Duration** (Fig 13.24, 7) 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**.

(a) *Sequence Options*

(b) *Sequence Preview*

Figure 13.24: *Channel(s) configuration window, Digital I/O - Square Mode*

13.4.5 Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for its measurement.
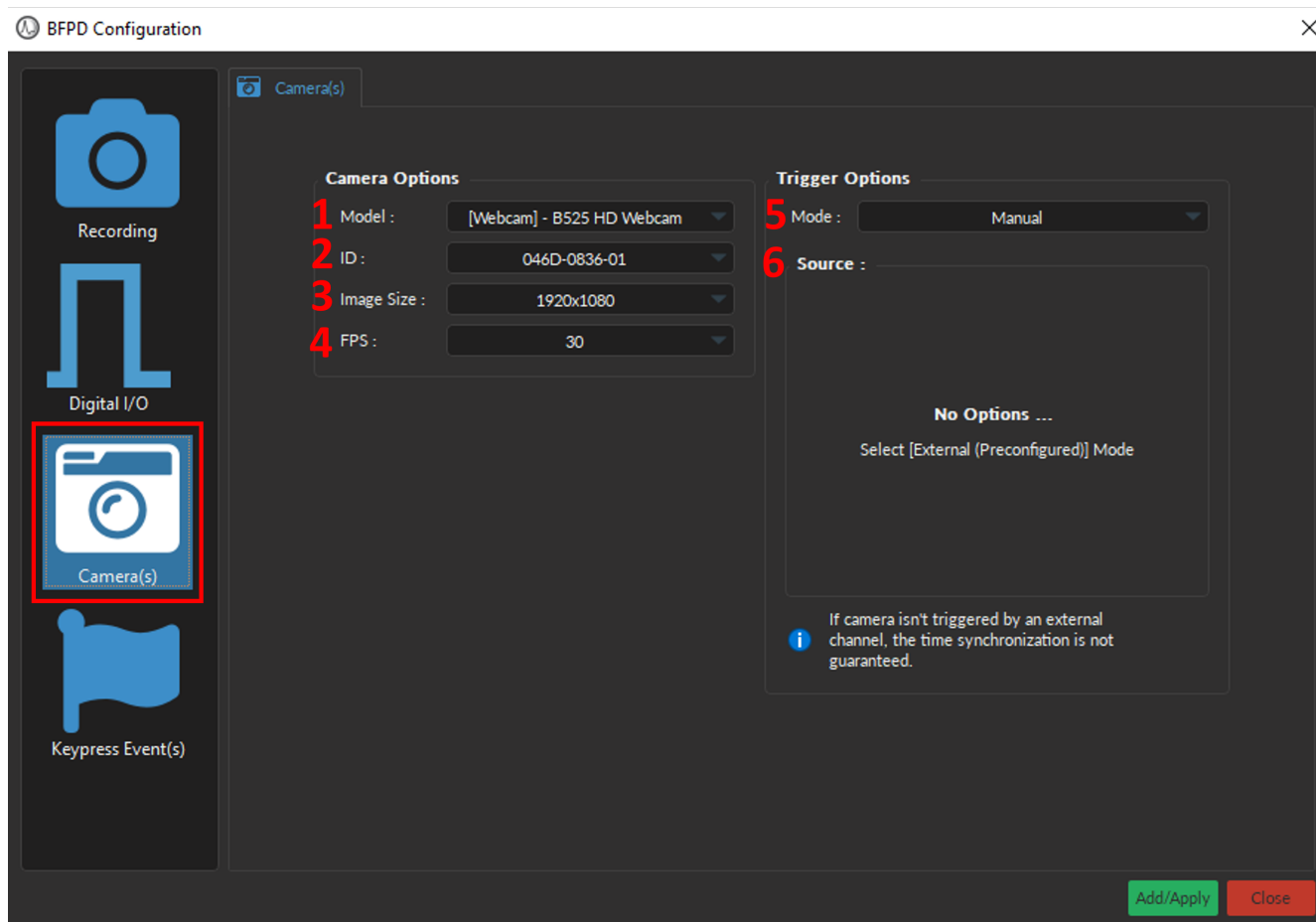

Figure 13.25: *Channel(s) configuration window, Camera*

WARNING:
A camera cannot be used for <u>BOTH</u> **BFPD** and **Camera** modules.
When creating a Camera Channel, if *No available camera detected...*, <u>disconnect</u> the camera in the **Device Selection** window to close the extra module.

Camera Options:

1. The **Model** (Fig. 13.25, 1) allows you to select the camera of choice based on the type of camera.

2. **ID** drop-down list (Fig. 13.25, 2) is used to select a camera based on its unique ID. The ID is particularly useful if multiple cameras of the same model are required for the experiment.

3. The **Image Size** (Fig. 13.25, 3) is used to set the resolution of the image. The large the number of pixels used for width x height, the better the resolution. Currently, image size can ranges between 160x120 to 1920x1080 pixels.

4. The **FPS** (Fig. 13.25, 4) is used to specify the frame rate of the camera (i.e. the number of images displayed per second). FPS can be any value between 5 to 30 for web cameras and up to 60 FPS for the *Doric Behavior Camera*.

Trigger Options:

5. The **Mode** (Fig. 13.25, 5) sets the type of trigger that will control the camera. Depending on the type of camera, at most three modes are available:

> **WARNING:**
> If the camera isn't triggered by an external channel,
> the **time synchronization is NOT guaranteed**.

- **Manual** - Selecting the *Live* or *Record* buttons located in the Acquisition Tab will the trigger the start of the camera recording. ***The time difference between the actual start time and when the first frame is received depends on the camera itself.*** Around a 1 second delay is pretty common for web cameras.

  The time delay (in ms) between the photometry and video data is recorded in the *DifferenceMasterStartToFirstImage* attribute, located in *.doric* file under the **Web Camera ID** folder (Fig. 13.26). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

Figure 13.26: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **External** - Will drive the camera using external TTL signal through the trigger cable (Frequency: 30 Hz (or camera FPS); Time ON: 5 ms). This signal can come from any external device connected to the opposite end of the trigger cable. If using *Doric Neuroscience Studio* to synchronize the recording, use *External (Preconfigured)* mode below instead. ***ONLY offered for the *Doric Behavior Camera.****

- **External (Preconfigured)** - This is the recommended mode to synchronize the camera with the rest of the Acquisition system. This mode automatically creates an additional Digital I/O channel configured to drive the camera at the proper frequency and Time ON. ***ONLY offered for the *Doric Behavior Camera.****

6. The **Source** (Fig. 13.25, 6 & Fig. 13.27) is only used for the **External (Preconfigured)** mode, and displays the **Digital I/O** channel with the preconfigured parameters that will be created at the same time as the **Camera Channel** (Fig. 13.27). For a detailed description of each Digital I/O parameter see Section 13.4.4. Briefly, key parameters include:

   a) The **Channel** (Fig. 13.27, a) corresponds to the physical Digital I/O channel number on the BFPD that is connected to the trigger cable of the *Doric Behavior Camera*.

   b) The **Mode** (Fig. 13.27, b) is by default set to the *Square (TTL)* which provides the external trigger signal to the camera. This parameter cannot be changed.

   c) The **Frequency** (Fig. 13.27, c) corresponds to the **FPS** set in the **Camera Options**. Changing the **FPS** will automatically change the **Frequency** in the **Sequence(s) Options**.

   d) The **Duty Cycle** (Fig. 13.27, d) is by default 50%. The frame will be taken at the start of each square pulse.

Figure 13.27: *Channel(s) configuration window, Camera - External (Preconfigured)*

13.4.6 KeyPress Event(s)

Keypress Event(s) are ideal when manually labeling or annotating events during experiments. Specifically, selecting any keyboard key during a recording will save the output synchronized to other measurements. Keypress events can be used to:

- Flag disruptions during the experiment, such as lights on, the door opening, construction noise, etc.

- Record experimentally relevant events/stimuli, such as air-puffs, licks, or any other behavior.

> **WARNING:**
> Keyboard event(s) timing are **accurate within 1 second** due to variations in Windows priority management and buffering of the signals.

To add a new **Keypress Event**, select the + sign at the botton of the window (Fig. 13.28, left). To remove a KeyPress, use - button (Fig. 13.28, right).

- **NOTE:** Selecting the + button (without clicking the *Add* button or the *Close* button of the *Channel Configuration* window) will **automatically** add the Keypress Event channel at the **bottom** of the Acquisition View window, below any pre-existing channels (Fig. 13.28).
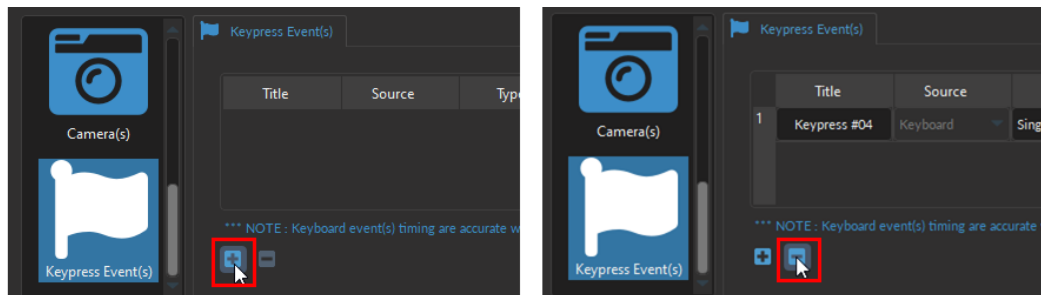

Figure 13.28: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 13.29) in the **Acquisition View**.
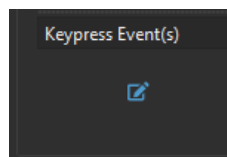

Figure 13.29: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 13.31:

1. The **Title** (Fig. 13.31, 1) allows you to give a name for the Keypress event.

2. The **Source** (Fig. 13.31, 2) is by default *Keyboard*.

3. Three **Types** of Keypress Event(s) (Fig. 13.31, 3) can be specified with the drop-down list:

    - **Single** - Records single event at the touch of a key (Fig. 13.30a).
    - **Toggled** - Records the start and end of an event using the same key. First press denotes the start of the event while a second press denotes the end of it (Fig. 13.30b).
    - **Timed** - Records an event for a predetermined duration of time (Fig. 13.30c). Every keypress is a new event, with the start of the event occuring when the key was depressed.
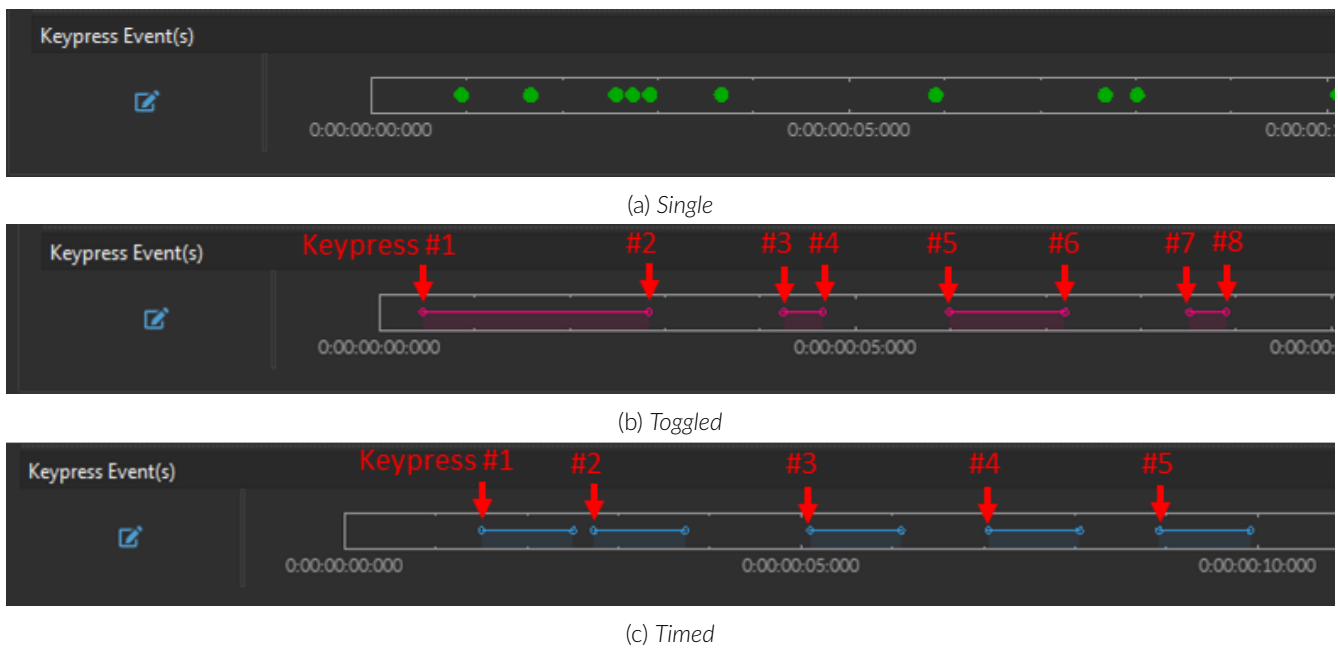
(a) Single

(b) Toggled

(c) Timed

Figure 13.30: *Three types of Keypress Event(s)*

4. The **Duration** (Fig. 13.31, 4) is only used for the **Timed** Keypress type to specify the predetermined amount of time a Keypress Event will span. The duration is set in hh:mm:ss:zzz.

5. Select the **Color** (Fig. 13.31, 5) field to open the **Select Color** window. Basic colors are provided, in addition to custom colors can be created and stored.

6. The **Shortcut Key(s)** (Fig. 13.31, 6) can be any keyboard key, including space bar, enter, backspace, any letters, number and special characters (*, !, ? etc.). To specify the key, click inside the *Shortcut Key(s)* cell, then press the keyboard key of choice. If a key was properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 13.31, column 6).

7. The **Information** column (Fig. 13.31, 7) provides space to make notes or write a short description of the Keypress Event.
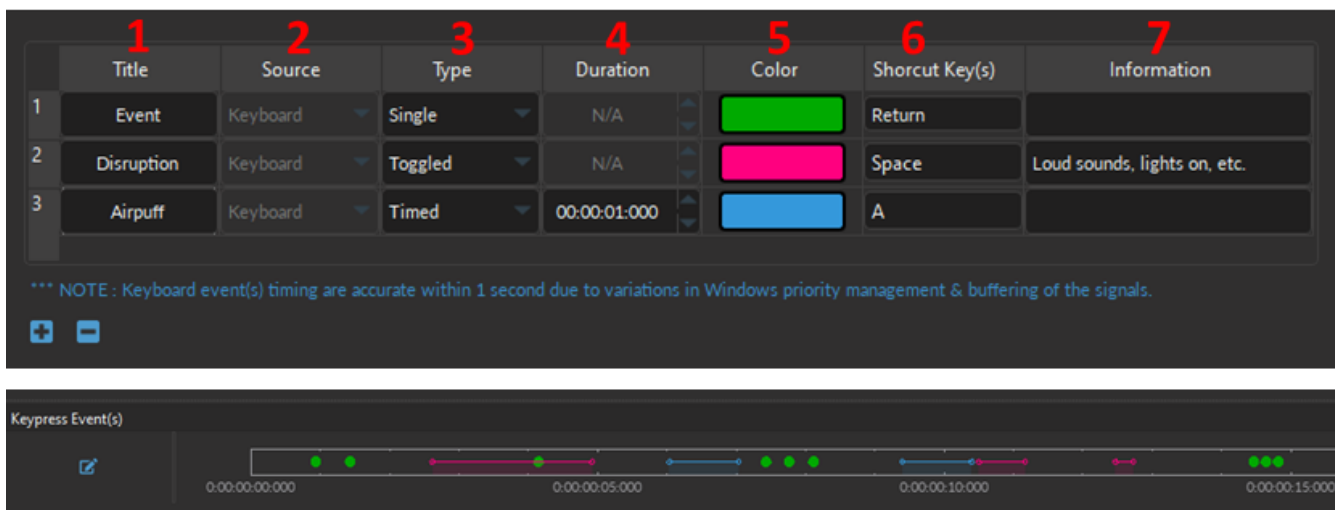

Figure 13.31: *Channel(s) configuration window, KeyPress Event(s)*

13.5   Acquisition View

The **Acquisition View** (Fig. 13.32) is split into three separate divisions, each of which visualizes different types of data in the following sections:

1. The **Digital I/O(s) View** (Fig. 13.32, 1) - Section 13.5.1;

2. The **Camera View** (Fig. 13.32, 2) - Section 13.5.2;

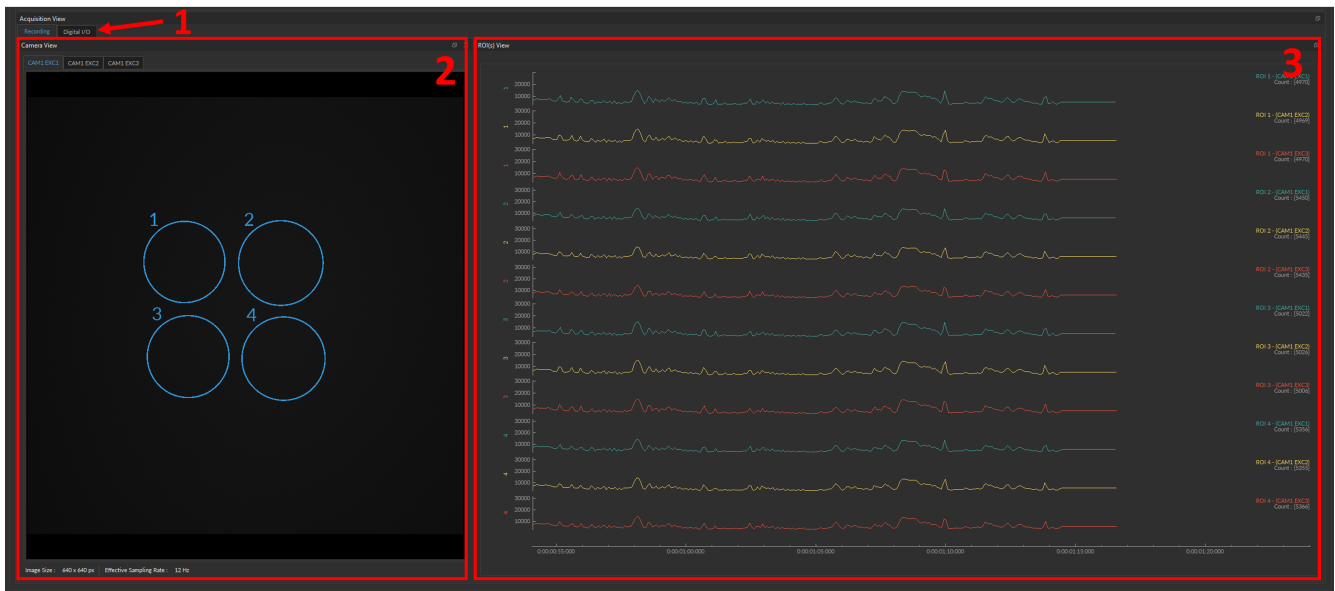3. The **ROI(s) View** (Fig. 13.32, 3) - Section 13.5.3.

Figure 13.32: *Acquisition View*

13.5.1   Digital I/O(s) View

The **Digital I/O(s) View** displays the active Digital channels, including **CAM** and **EXC** preset channels. Each Digital I/O channel includes: (1) a **Control Box** (Fig. 13.33, 1), and (2) a **Graph** (Fig. 13.33, 2).
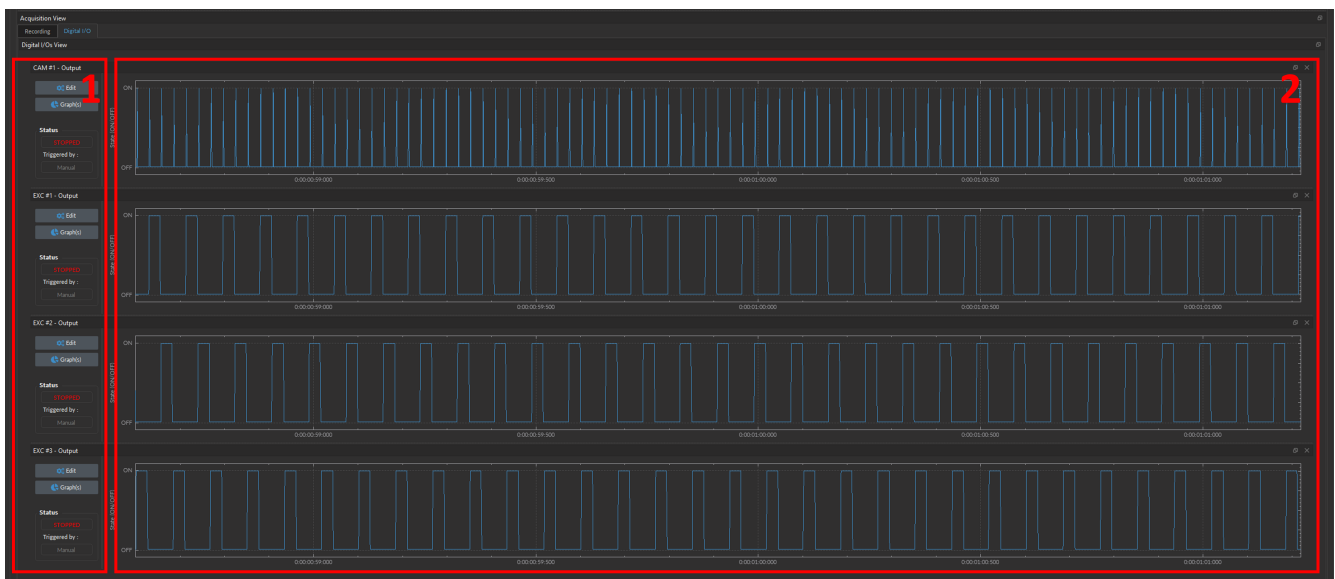
Figure 13.33: *Digital I/O(s) View*

13.5.1.1 DIO Control Box

The **Control box** of each channel allows users to track the status and edit the graph trace or the channel parameters.
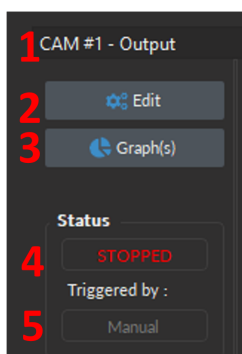

Figure 13.34: *Digital I/O View, Control box*

The following elements are contained within the **Control Box** of every Digitial channel (Fig. 13.34):

1. The **Channel name** (Fig. 13.34, 1) is located on the upper left of the **Control box**, identifying the type of channel and its number, corresponding to that on the *BFPD*.

2. The **Edit** button (Fig. 13.34, 2) opens the **Channel Configuration** window, where the preset digital outputs can be modified (Fig. 13.35). For details on individual parameters, see Section 13.4.4.
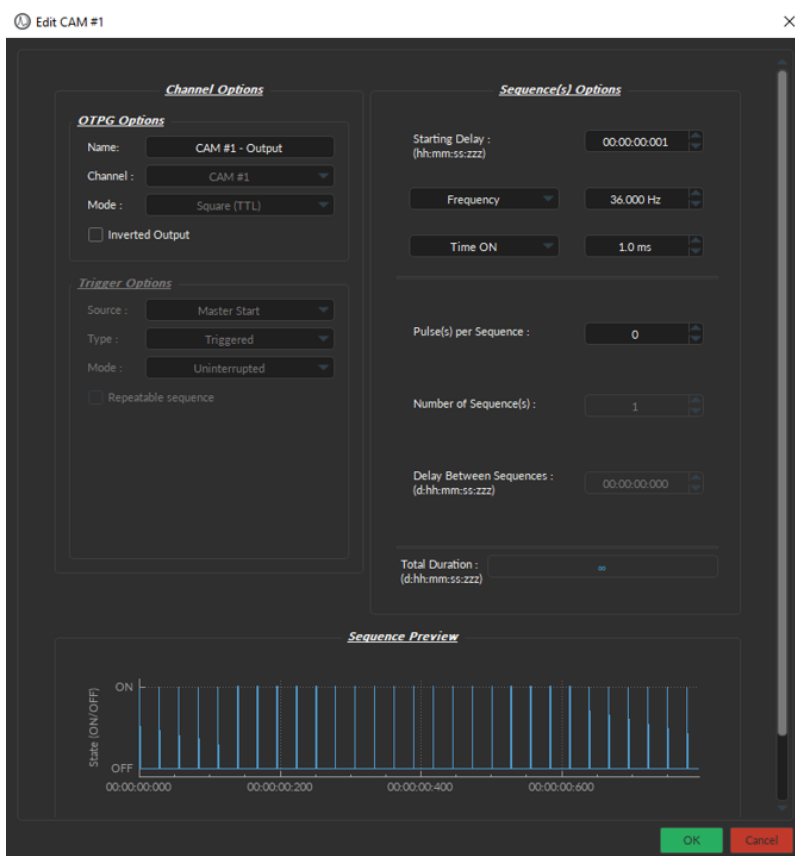

Figure 13.35: *Edit Preset CAM or EXC configuration*

3. The **Graph(s)** (Fig. 13.34, 3) button opens the **Graph Options** window (Fig. 13.36) corresponding to the channel whose graph will be modified. This window allows users to configure the visualization and naming parameters of each channel graph. If a channel has multiple traces, parameters to configure each trace individually will appear automatically on different rows. **Graph(s) Options** parameters are as follows:
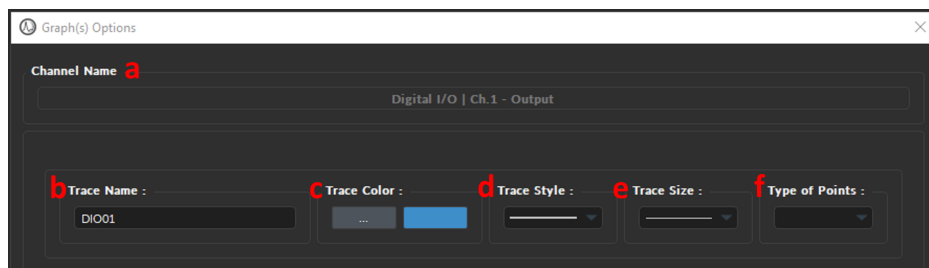

Figure 13.36: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 13.36, 1) is the default name assigned by the software, which includes the type of channel (Digital / Analog In or Out) and the location of said channel on the console (BNC connector 1-4).

b) The **Trace Name** text-box (Fig. 13.36, 2) allows users to specify a name for the trace, instead of the default name generated by the software.

c) The **Trace Color** button (**...**) (Fig. 13.36, 3) opens the **Color Select** window (Fig. 13.37), 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.

d) The **Trace style** drop-down list (Fig. 13.36, 4) 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.

e) The **Trace size** drop-down list (Fig. 13.36, 5) allows the selection of the trace size. Using a bigger **Trace size** than the default may result in slower display and performance degradation.

f) The **Type of points** drop-down list (Fig. 13.36, 6) selects the style data point used to demark instantaneous values on the graph. Using different point types than the default (none) may result in slower display and performance degradation.
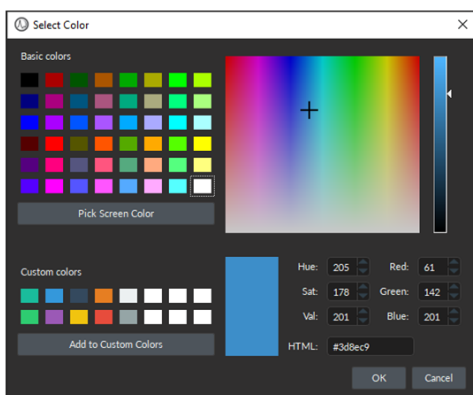

Figure 13.37: *Select Color Window*

4. The **Status** bar (Fig. 13.34, 4) displays acquisition status. **STOPPED** is displayed when the acquisition is inactive, **STARTED** when acquisition is active, and **WAITING...** when the **Master Trigger** is set to *Triggered* (see Section 13.3.2.1, no. 3).

5. The **Triggered by:** (Fig. 13.34, 5) text-box displays the source of the trigger for that channel, which can either be Manual (i.e. selecting the **Record**/**Live** button) or a specific channel that provides external trigger signal.

13.5.1.2   DIO Graph

The **Digital I/O** traces are displayed in the **Graph** box (Fig. 13.33, 2). Each channel graph includes the following components:
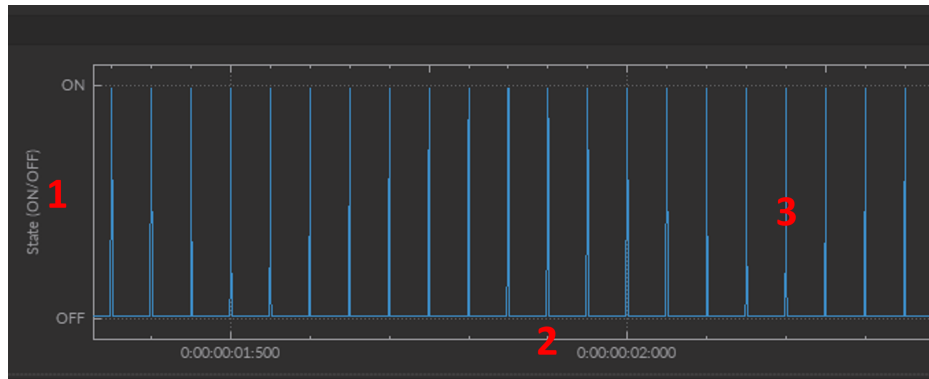

Figure 13.38: *Digital I/O(s) View - Graph*

1. The **Y-axis** (Fig. 13.38, 1) displays the Digital **State** of the channel, which can be either ON (1) or OFF (0).

2. The **X-axis** (Fig. 13.38, 2) displays the time in d:hh:mm:ss:zzz.

3. The **Trace** (Fig. 13.38, 3) can be edited by selecting the **Graph** button in Section 13.5.1.1, no. 3.

While Section 13.5.1.1, no. 3 allow users to control the trace display, there are other features of **Graph** view can be directly manipulated by selecting elements of the **Graph** itself, such as:

- *Axis Options* - Each **Graph** (Fig. 13.39) has both a **Voltage** or **State** as the vertical axis and **Time** as the horizontal axis. Double-clicking either axis will open an **Axis Options** window (Fig. 13.39) where the axis limits can be set, similar to the **Zooming Range** in the **View Tab**. Any changes done on a horizontal axis will change the axis limits for every channel.
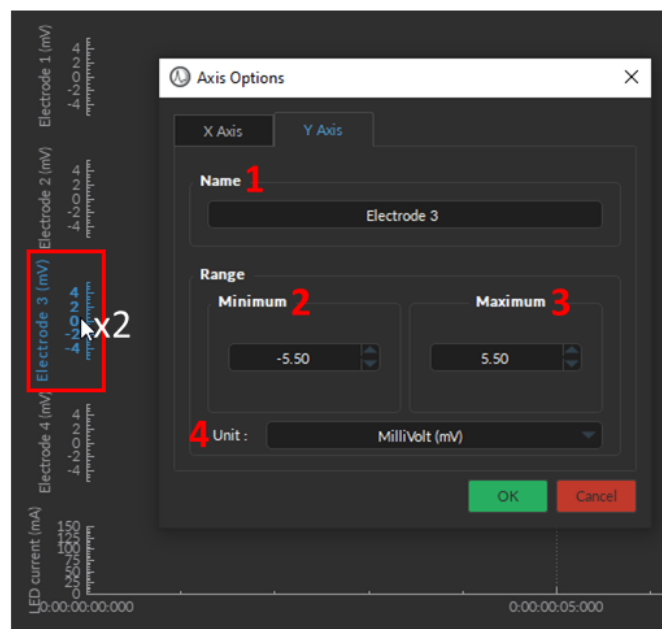

Figure 13.39: *Double click on any axis to open its Axis Options window*

- By clicking and **dragging the graph sideways or upwards**, one can scroll through nearby values on either axis, keeping the zoom range constant. Any changes done on a horizontal axis will change the axis limits for every channel.

- Using the **Mouse Scroll Wheel**, one can change the zoom range of the graph. Any changes done on a horizontal axis will change the axis limits for every channel.

- The **Instant values** box can be activated by double-clicking the **Input graph** box and selecting **Show instant values** (Fig. 13.40). 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**. To remove instantaneous value, double click on the dot.
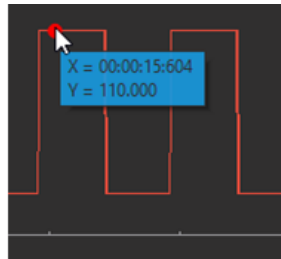

Figure 13.40: *Acquisition View - Instant values*

13.5.2   Camera View

The **Camera View** displays the live video feed from the CMOS **Sensor(s)**. This view contains the following components:
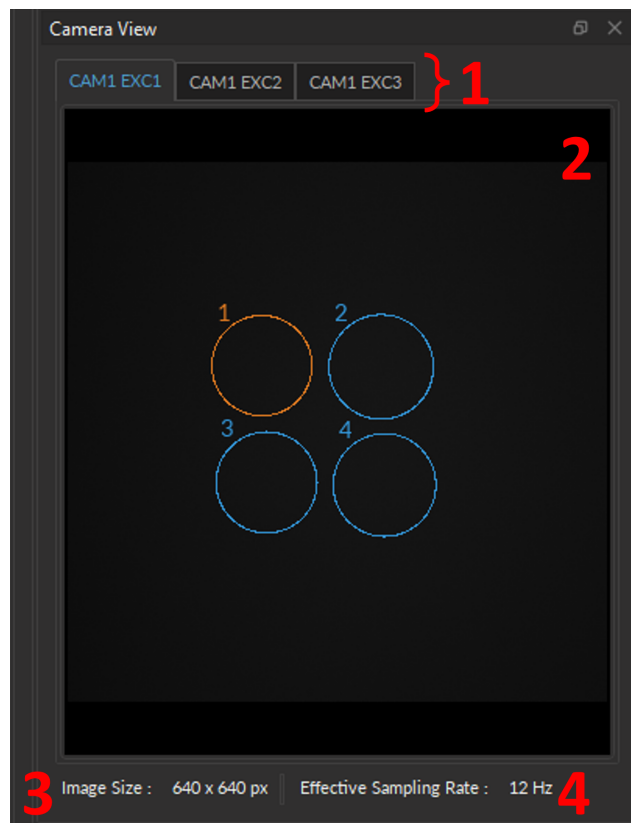

Figure 13.41: *Camera View*

1. The **CAM # EXC #** tab (Fig. 13.41, 1) - which displays each sensor's live video feed, where the ROI(s) can be drawn, edited, or deleted. Note that the tab will display the user-defined name if a name was assigned to each camera excitation when creating the channel in the **Configuration Window** (Fig. 13.15, e).

2. The **Sensor Feed** displays the live image of the CMOS sensor, where users can define ROIs that correspond to fibers within the bundle. The following mouse controls are available to draw, edit, or delete ROI(s) directly on the feed:

   - **Draw ROI** - click and drag the mouse over the area within the **Sensor View** that will be assigned as a ROI.
   - **Select ROI** - click either the edge or within the ROI will select it. Proper selection will become dotted and automatically highlight the corresponding ROI in the **ROI(s) Information** tab (Fig. 13.45).
   - **Delete individual ROI** - Select a ROI (as detailed above) and press the **Delete** key on the Keyboard. To delete all ROIs, see Section 13.3.3, no. 1.
   - **Displace ROI** - Select the ROI and hove above the center of the ROI until a *Move* icon (Fig. 13.42a) appears. Click and drag the ROI to its new desired location.
   - **Resize ROI** - Select the ROI and hove above the orange trace of the ROI until a *Resize* icon (Fig. 13.42b) appears. Click and drag the ROI to reduce or enlarge the shape. *Resize* option is not available for the *Freehand* shape.
   - **Select multiple ROIs** - Press *Ctrl* while selecting a second ROI, such that each selected ROI turns orange (Fig. 13.42c). This selection allows multi-ROI deletion or displacement.
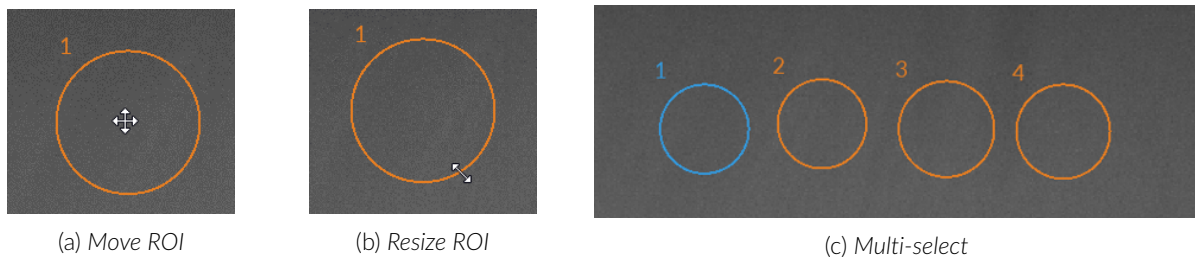

(a) *Move ROI*   (b) *Resize ROI*   (c) *Multi-select*

Figure 13.42: *Edit ROI(s)*

3. The **Image Size** (Fig. 13.41, 3) displays the image resolution, set in the **Configuration Window** (as in Fig. 13.15, 2).

4. The **Effective Sampling Rate** (Fig. 13.41, 4) the value set in Section 13.4.3, no. 1c.

13.5.3 ROI(s) View

The **ROI(s) View** displays the ROI traces calculated by averaging the pixel intensity value within each ROI. The following elements can be found in the **ROI(s) View**:
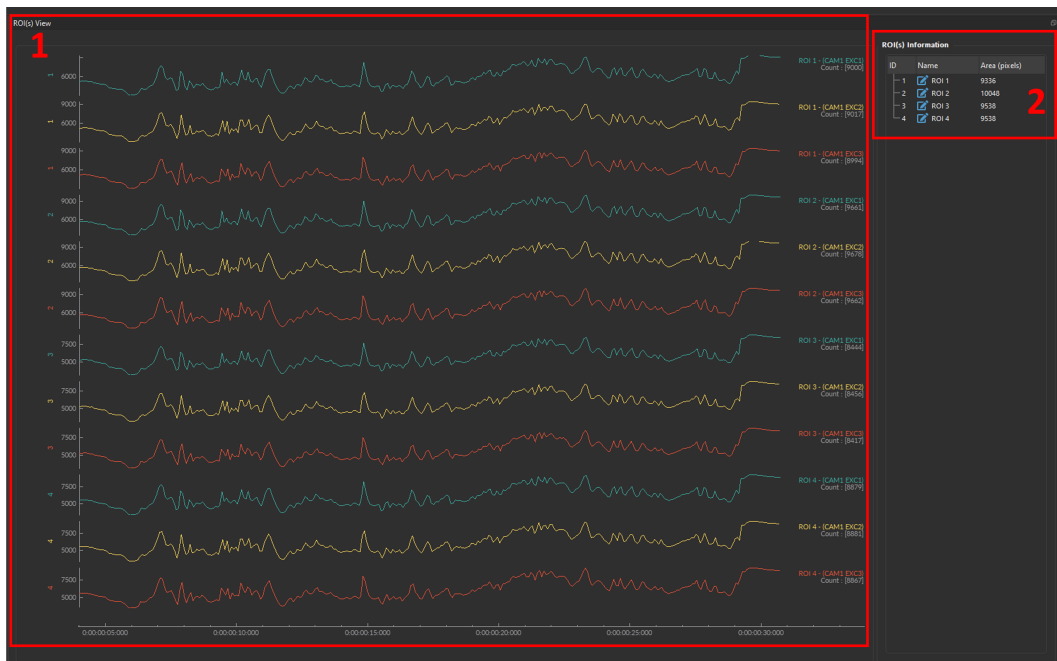

Figure 13.43: *ROI(s) View*

1. The **ROI(s) signal graph** (Fig. 13.43, 1) displays the raw signal trace for each ROI(s).
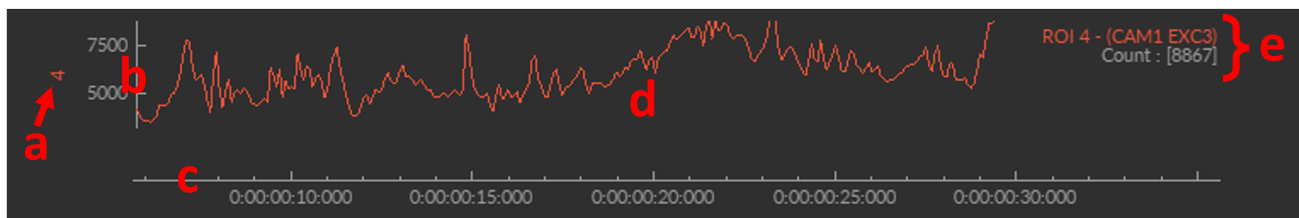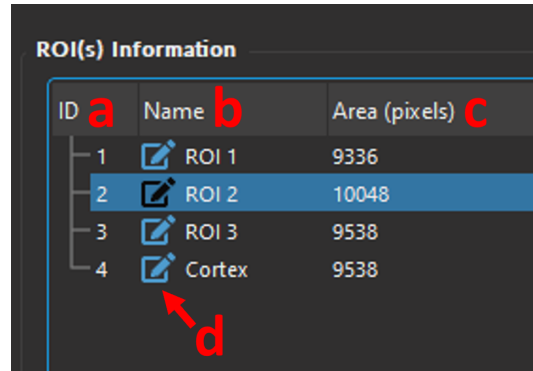

Figure 13.44: *ROI(s) View, Graph*

  a) The **ROI(s) ID** (Fig. 13.44, a) specifies which ROI the signal graph belongs to. The graphs are displayed in order of ROI created.

  b) The **y-axis** (Fig. 13.44, b) represents the mean signal intensity of the ROI, which is unit-less.

  c) The **x-axis** (Fig. 13.44, c) represents the time in d:hh:mm:ss:zzz.

  d) The **Trace** (Fig. 13.44, d) is the curve of the signal, corresponding to fluctuations in pixel intensity, from which $\Delta F/F_0$ will be calculated.

  e) The **Legend** (Fig. 13.44, e)

   - *ROI label* - displays the ROI **Name** (specified within the **Name** column of **ROI(s) Information** tab; Fig., 13.45, b), followed by the **Sensor Name** in parenthesis (which can be specified in Fig. 13.15, e).
   - *Counts* - displays the value of the last data point of the ROI trace (in average pixel intensity value).

2. **ROI(s) Information** Tab (Fig. 13.43, 2) displays a table with ROI basic data, including:

  a) *ID* (Fig. 13.45, a) displays the number associated with ROI.

b) *Name* (Fig. 13.45, b) displays the label associated with the ROI. Double-click on the text-box to rename the ROI.

c) *Area* (Fig. 13.45, c) displays the number of pixels that fill the perimeter of the ROI.

d) *Edit* button (Fig. 13.45, d) will highlight in orange the corresponding ROI in the **Camera View**. To edit or delete the selected ROI, see section 13.5.2, no. 2.

Figure 13.45: *ROI(s) View, ROI(s) Information tab*

14

Specifications

Table 14.1: *Doric Neuroscience Studio Hardware Requirements*

SPECIFICATIONS	VALUE	NOTES
Operating System	Windows 10, 11	64-bit
Memory (Minimum/Recommended)	8 GB/16 GB	
Processor Speed (Minimum/Recommended)	2 Ghz Quad-Core i5/ 3.46 Ghz Eight-core i7	
Hard Drive	2 GB of free space	SSD recommended

Table 14.2: *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	

Support

15.1 Contact us

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

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