doric

Doric Neuroscience Studio

User Manual

Version 6.1.5

Contents

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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 that allows control of the hardware and the acquisition of data.

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

- Control of our Programmable LED Drivers.
- Control of our Laser Diode Module Drivers.
- Control of our ⋆LISER™ Light Sources Drivers.
- Acquisition of the voltage of a chosen light source input BNC.
- Acquisition of data from our Fiber Photometry Console or Neuroscience Console 500.
- Acquisition of data from our Bundle Fiber Photometry Driver or Behavior and Bundle Photometry Console 300
- 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.
- Edit files acquired with Doric devices
- Analyse image data from the microscope.
- Analyse photometry data.
- Analyse electrophysiology data.
- Analyse behavioral tracking data.

Note: for more complex and specific data analysis, we now also offer a data analysis software, *danse*™, that covers the entire pipeline from raw data (including behavior) to the final plots. More information are available on *danse*™ webpage.

1.2 System requirements

Windows

For the recommended system requirements, please see Chapter **??**

2

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 **??** 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 ask and help to uninstall the previous version before installing 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**.

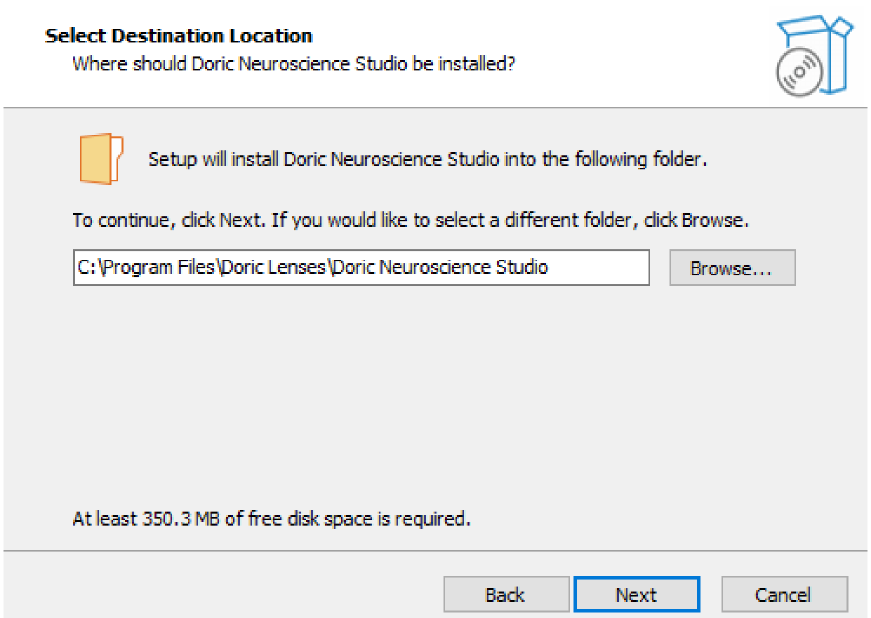


Figure 2.1: *Select Destination Location*

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 shortcut to launch the software on your desktop (Fig. 2.2).

- Select **Doric Maintenance Tool** to install the Doric service system to update device firmware, more update information will be provided further in this user manual. We highly recommend making this installation and keeping 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 install the drivers for Ethernet cameras (Fig. 2.2).

Click **Next** once the selection is done.

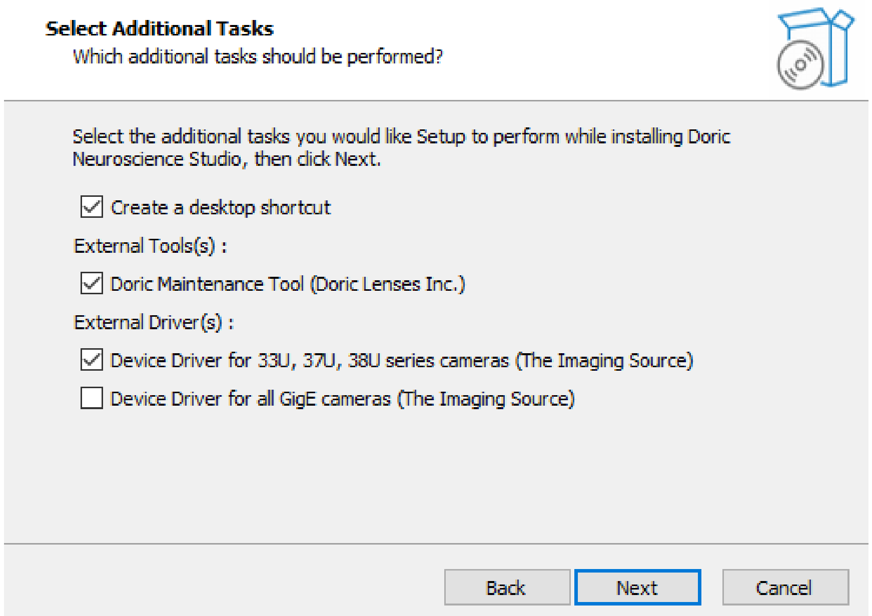


Figure 2.2: *Select complementary installations*

7. When ready, click **Install** to begin the process. This should take a few moments.

   <u>Note:</u> If you have selected the installation of **Doric Maintenance Tool** and/or the installation of another driver, their installation will start at the end of the installation of **Doric Neuroscience Studio**, before the next step. See the next sections for information about their installation.

   When the installation is done, the message in figure 2.3 will show up.

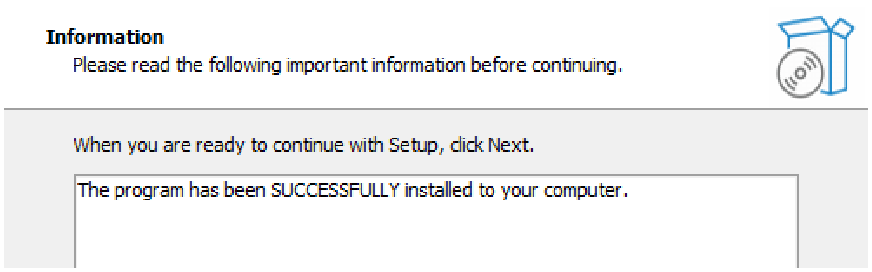


Figure 2.3: *Successful Installation of the Doric Neuroscience Studio*

8. Click **Finish** to exit the setup.
9. The software is ready for use.

2.1.2 Doric Maintenance Tool

If Doric Maintenance Tool has been selected to be installed during the installation of Doric Neuroscience Studio, the installation will 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 ask and help to uninstall the previous version before installing 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**.

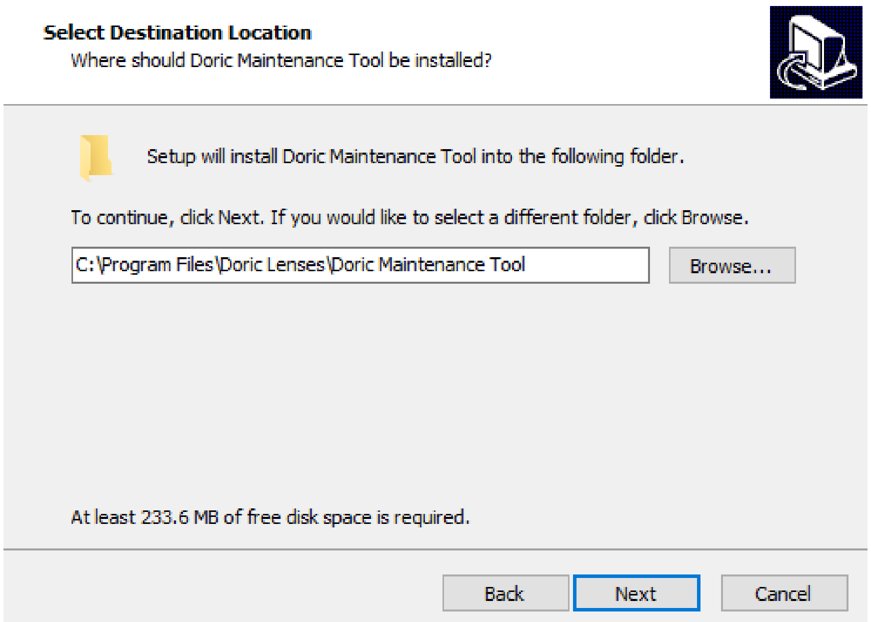


Figure 2.4: *Select Destination Location*

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.

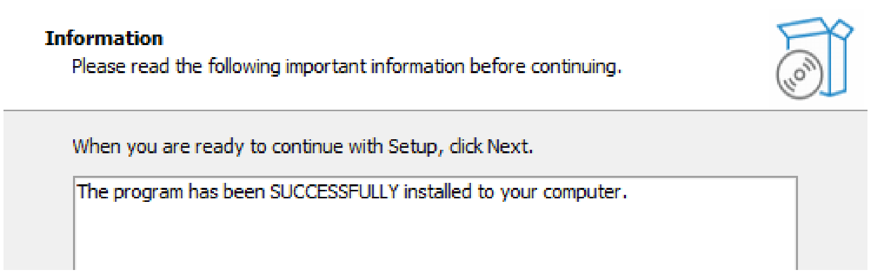


Figure 2.5: *Successful Installation of the Doric Neuroscience Studio*

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

2.1.3 USB Camera Driver

The installation of the Camera Driver is simpler than the other installations.

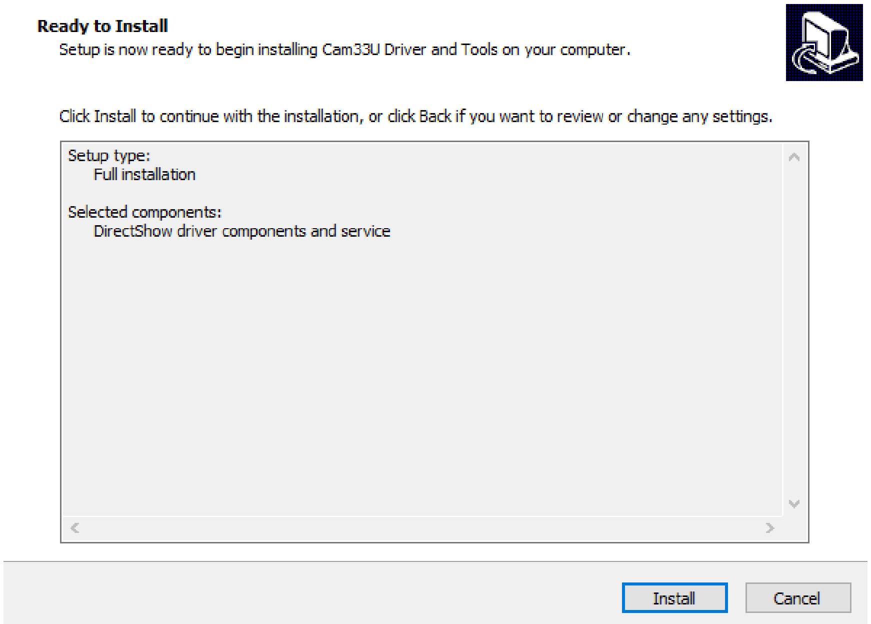


Figure 2.6: *Successful Installation of the Doric Neuroscience Studio*

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 2.7 will show up.

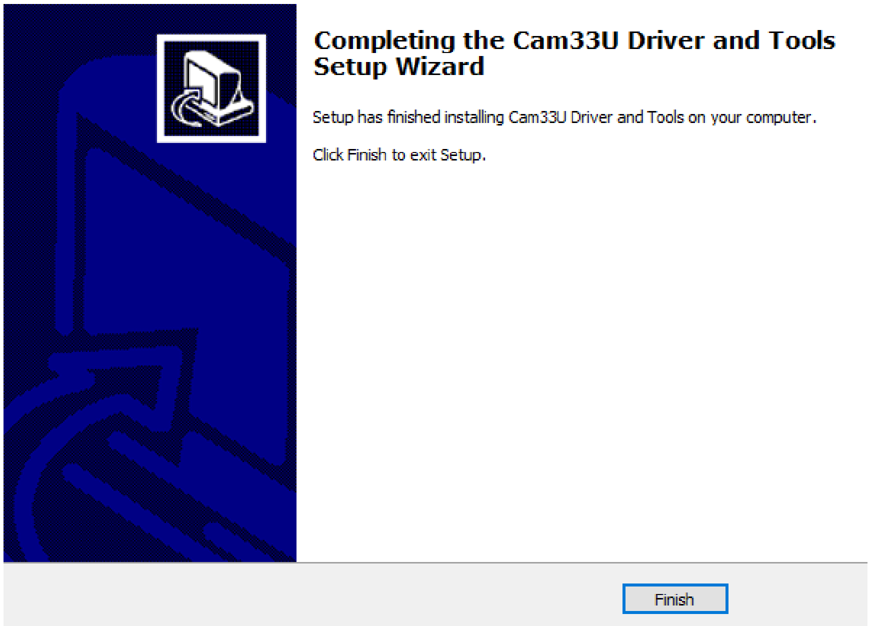


Figure 2.7: *Successful Installation of the Doric Neuroscience Studio*

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

2.1.4 Ethernet Camera Driver

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

1. Click **Next** in the Welcome window
2. In the Select Components windows, it is possible to select different options (Fig. 2.8):
   - Full installation: all the components will be installed.
   - Compact installation: to only install the driver.
   - Custom installation: to select what will be installed during the process.

   Note: At the same time as the driver it is possible to install 2 options (Fig. 2.8):

   - Kernel-Mode filter driver to enhance the performance. It is recommended to use it if possible.
   - IP configuration API files consist 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 the camera name, serial number, IP address, firmware version, and so on (for more information about this module, see here).

   When components have 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.

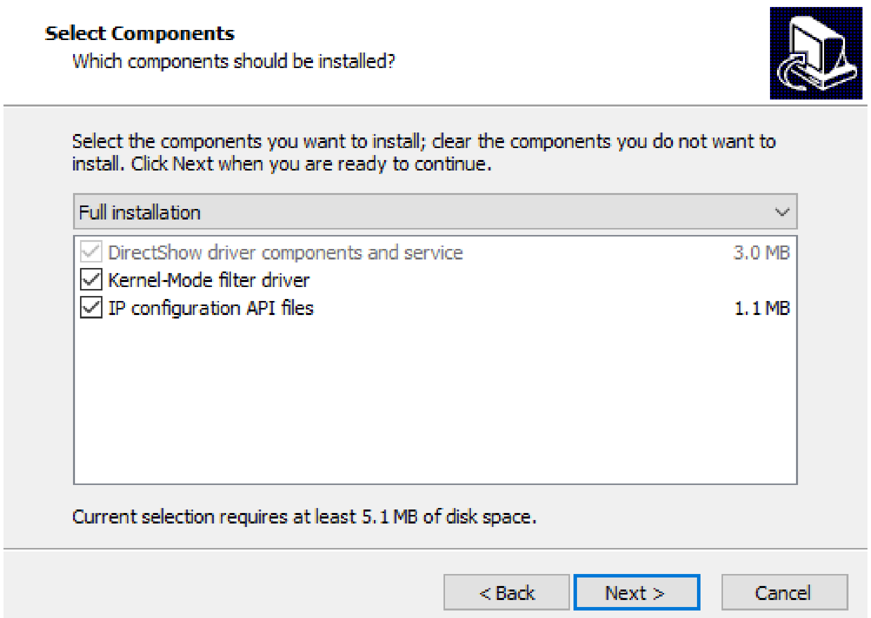


Figure 2.8: *Options selection in Ethernet Camera driver installation*

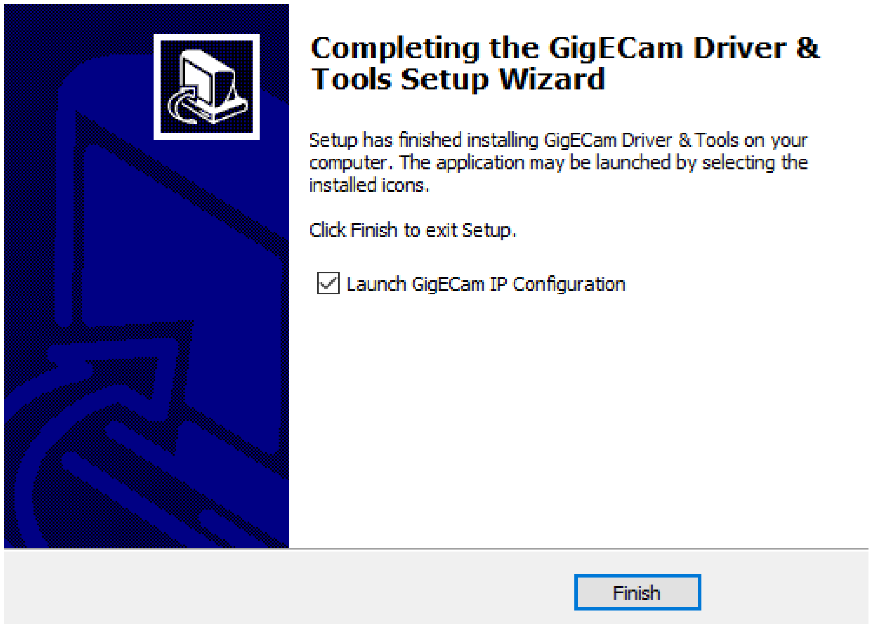
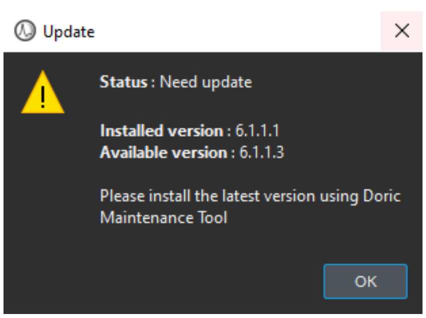


Figure 2.9: *Successful Installation of the Ethernet Camera Driver*

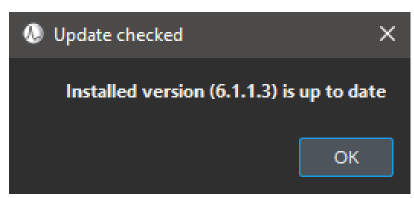
2.2 Updating Software & Firmware

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 require 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** will appear (Fig. 2.10a) asking to install the latest version with **Doric Maintenance Tool**. The procedure is described below. 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)**.

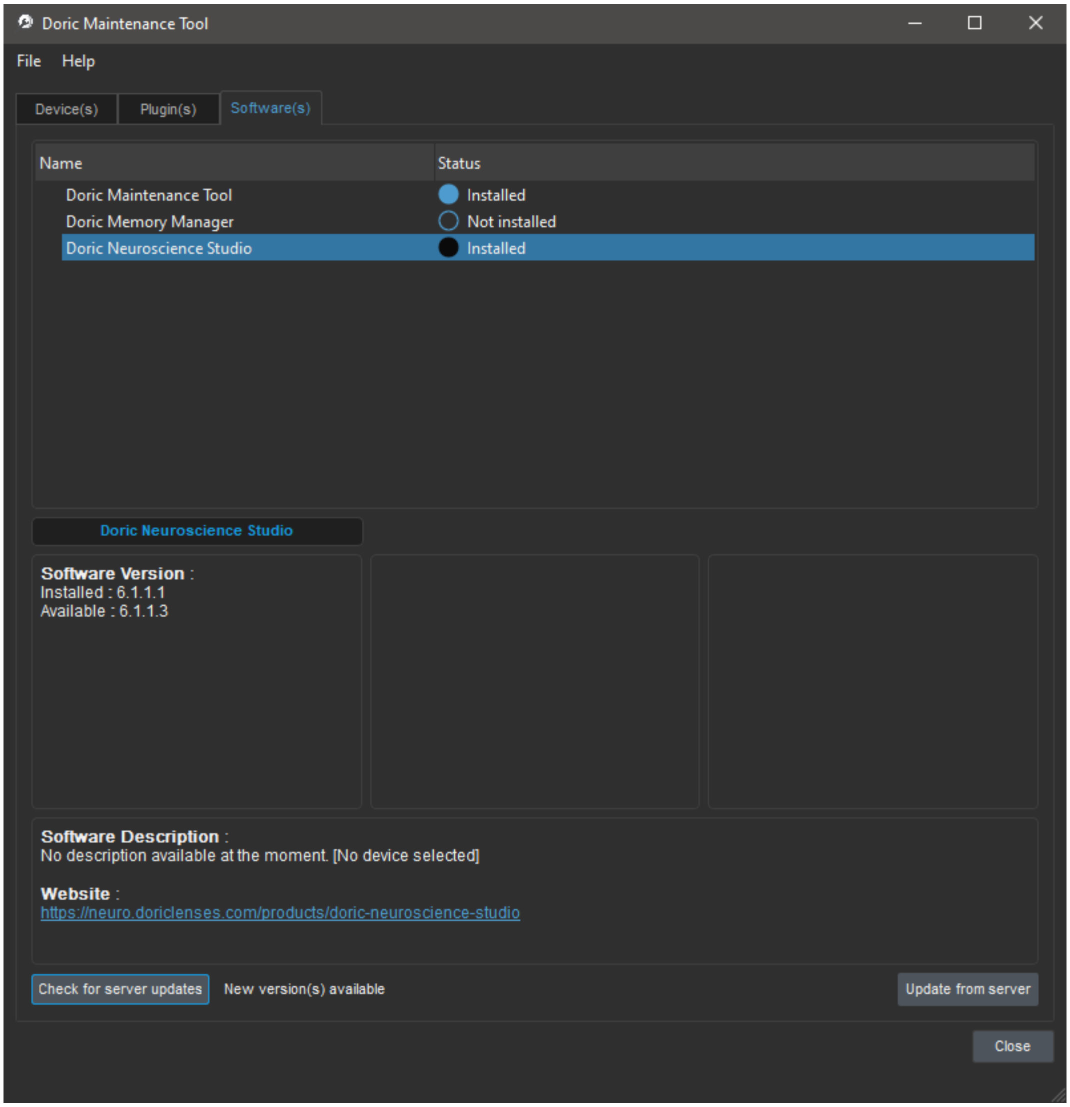


Figure 2.11: *Doric Maintenance Tool - Overview*

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 off 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. 2.12). **Click Yes**.

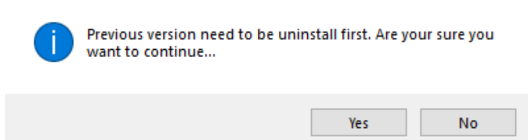


Figure 2.12: *Uninstall Window*

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

9. When the previous version is uninstalled, the installation of the new version needs to be done like the first installation (see section 2.1).

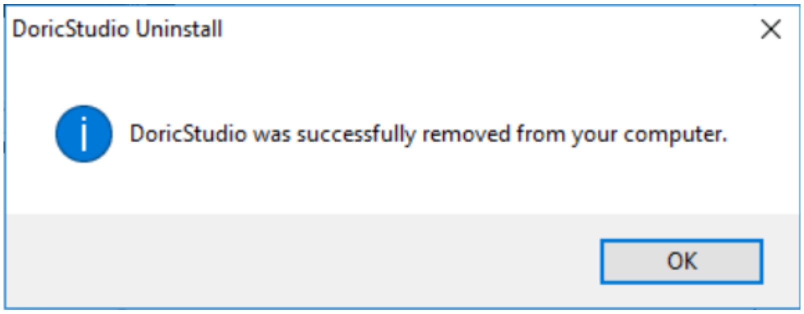


Figure 2.13: *Uninstall Completion*

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

<u>Note:</u> 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 installed at the same time as **Doric Neuroscience Studio**).
2. Turn On the device.

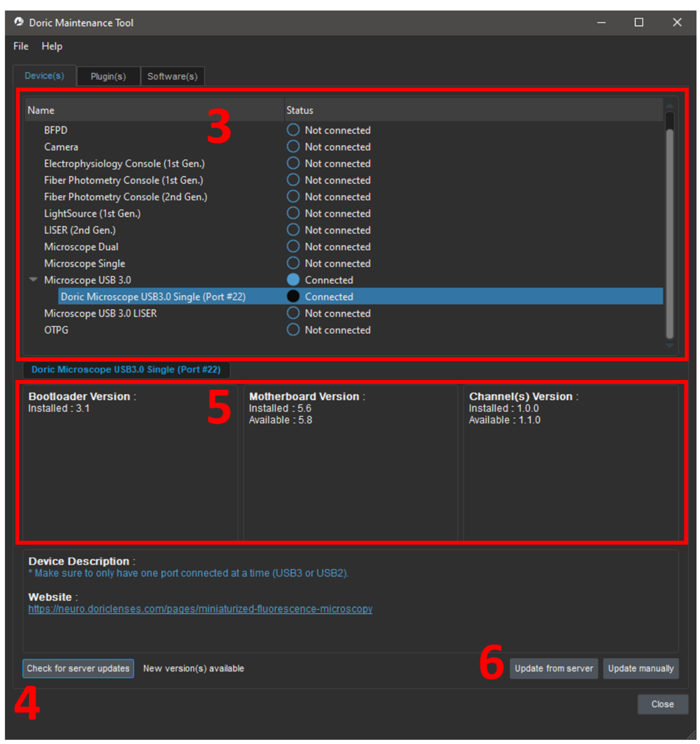


Figure 2.14: *Doric Maintenance Tool home page*

3. In the list, under **Name**, select the device to update (its status needs 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 cases, an update can be necessary without using the server version. In this case, a representative of Doric Lenses will send you the update file and you can use **Update manually** in replacement of **Update from server**) (Fig. 2.14).

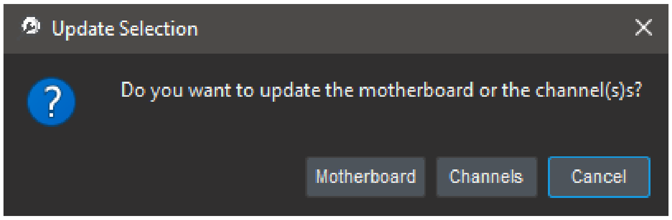


Figure 2.15: *Doric Maintenance Tool Update Selection*

7. A window asks 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 until 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**.

3

Software Organization

3.1 Device Selection

When *Doric Neuroscience Studio* (DNS) is first opened, a **Device Selection** window automatically appears (Fig. 3.1). Since each *Doric Lenses* device has a unique modular interface, users should select the *Doric Lenses* device that will be used during the recording session. Cameras are best left disconnected (as in Fig. 3.1) so that they can be integrated at a later time within the interfaces of other devices (like the *NC500*, *FPC*, or *BFPD*), for simultaneous neural activity and behavior visualization. Camera devices should only be connected when used on their own.

To connect Doric Neuroscience Studio to a device:

1. Select the device in **Available device(s)** (Multiple selections 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 appears in **Connected/Opened device(s)**.
3. Select **Close** to quit the device selection and access to Doric Neuroscience Studio menus.

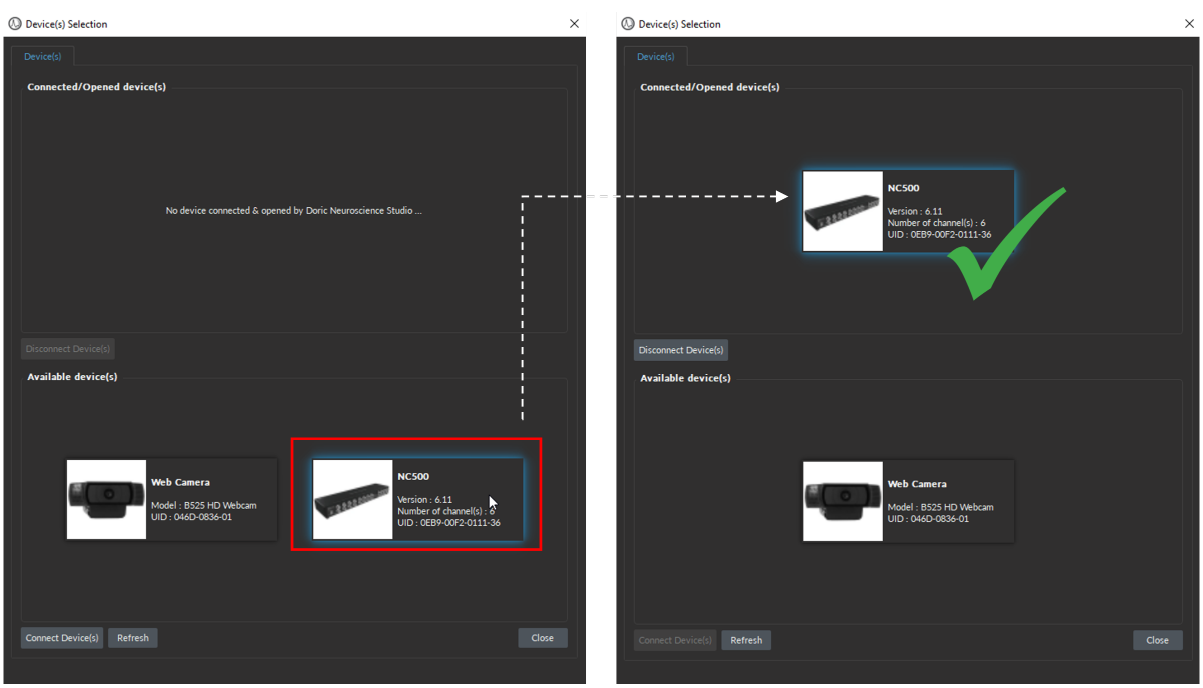
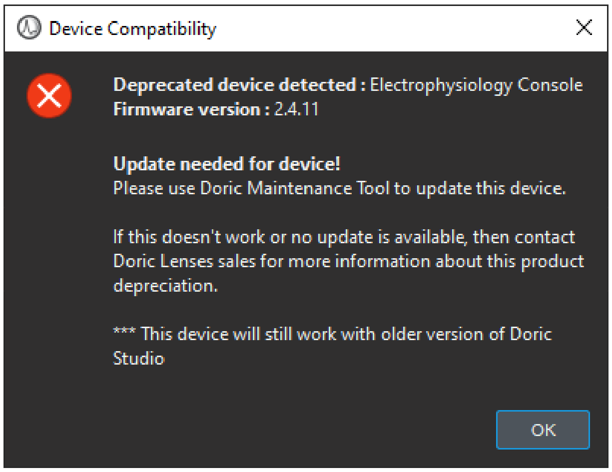


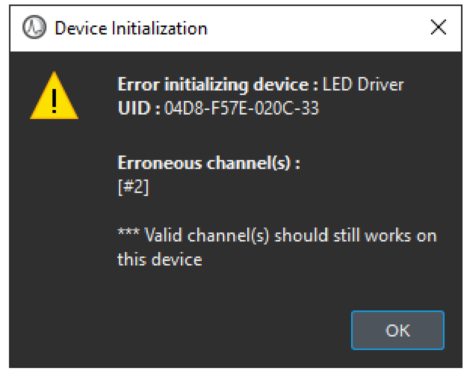
Figure 3.1: *Device Selection Window, connecting a device*

Notes:

- 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 to 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*

3.2 Menu

Doric Neuroscience Studio menu (Fig. 3.3) is split into four different drop-down options:

Figure 3.3: *Menu Options*

1. The **File** option (Fig. 3.4) is used to open the **Device selection** window to add the device-specific interface (see Fig. 3.1), **Save All Configurations**, **Load All Configurations** (more details are presented at Section 3.4) and also used to **Exit** the software.

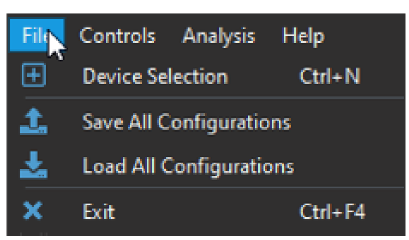


Figure 3.4: *File menu*

2. The **Controls** option (Fig. 3.5) is used to simultaneously start all the channels in multiple individual modules (such as *Acquisition Console* & *Light Source* modules).

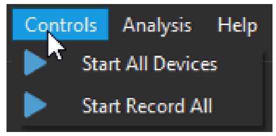


Figure 3.5: *Controls menu*

- **Start All Devices** sets every channels in all active modules to **Live** mode. This mode is useful to test the acquisition system, as no data will be saved.
- **Start Record All** sets every channel in all active modules to **Record** mode. Note that data are saved independently for each module. Select **Stop Record All** to end the recording session on all channels.

3. **Analysis** (Fig. 3.6) is used to open analysis modules, each of which are described in the following chapters:
   - **Behavior Analyzer** - Chapter 12
   - **Signal Analyzer** - Chapter 11
   - **Doric File Editor** - Chapter 4
   - **Image Analyzer** - Chapter 13

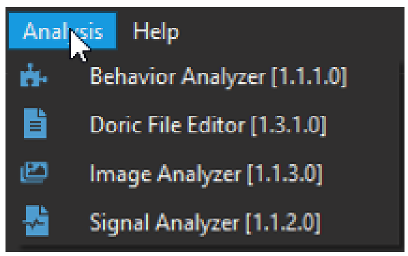


Figure 3.6: *Analysis menu*

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

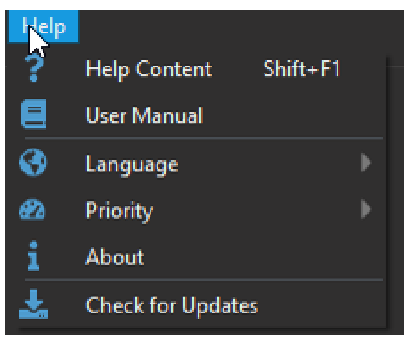


Figure 3.7: *Help menu*

- When the **Help Content** option 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.
- There are two **Language** options: French and English. Note that when changing the language, users must close and restart the software to update the user interface with the new language.
- The **Priority** options (Fig. 3.8) change the priority levels of *Doric Neuroscience Studio* within Windows, which can increase the reliability of the software. While the software is by default at **Normal** priority, a **High** priority will provide the greatest reliability.

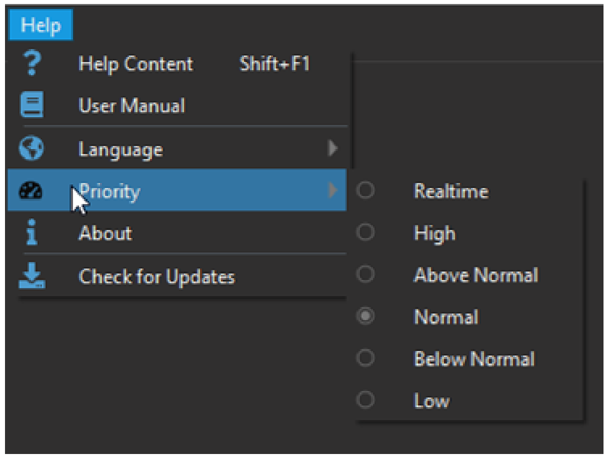
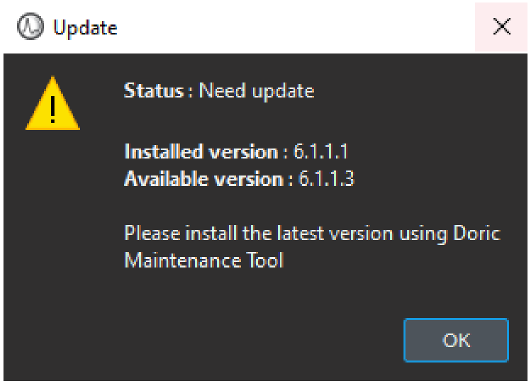


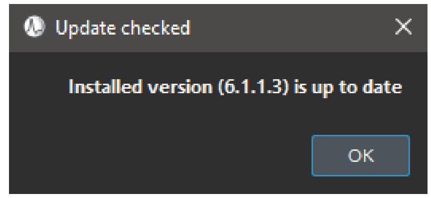
Figure 3.8: *Help, Priority*

- The **About** selection will open the **Software Info** window (Fig. 3.9), which shows the current version of the software.
- The **Check for updates** selection open the window (Fig. 3.10) of the same name. It can be used to check if the software can be updated. This function requires an Internet connection. To update the software, see Section 2.2.1.

Figure 3.9: *About: information on Doric Neuroscience Studio version*



(a) *Need update*



(b) *Up to date*

Figure 3.10: *Check for updates*

3.3 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.

3.4 Saving/Loading multiple configurations simultaneously

The latest versions of Doric Neuroscience Studio now offer the possibility to save the configurations of multiple Doric devices in an unique, Master configuration file, through the **Save All Configurations** option available in the **File menu** (Fig. 3.4). This Master configuration file can then be reopened for subsequent experimental sessions using the **Load All Configurations** option (Fig. 3.4).

Note: Doric devices are given a Unique Identifier (UID), an information that is also saved in the configuration file. A Master configuration file created with a particular set of devices can be reused with a different set of the same devices. However, while loading the Master configuration file, a dialog window will inform you that the configuration didn't find a match based on the devices' UIDs, and will offer to match the configuration of the device's UID saved in the file to the currently connected device (Fig. 3.11). For this purpose, simply tick the corresponding boxes on the Dialog box (Fig. 3.11). For older device models, a UID is automatically generated by the software upon every connection to the computer, and the same message type may appear. If this is the case, you can match the devices as previously described by altering the configuration.

In addition, Doric Neuroscience studio will automatically detect when a Master configuration file is being loaded while devices of a different type than the ones saved in the configuration file are connected, resulting in the error message shown on Figure 3.12.

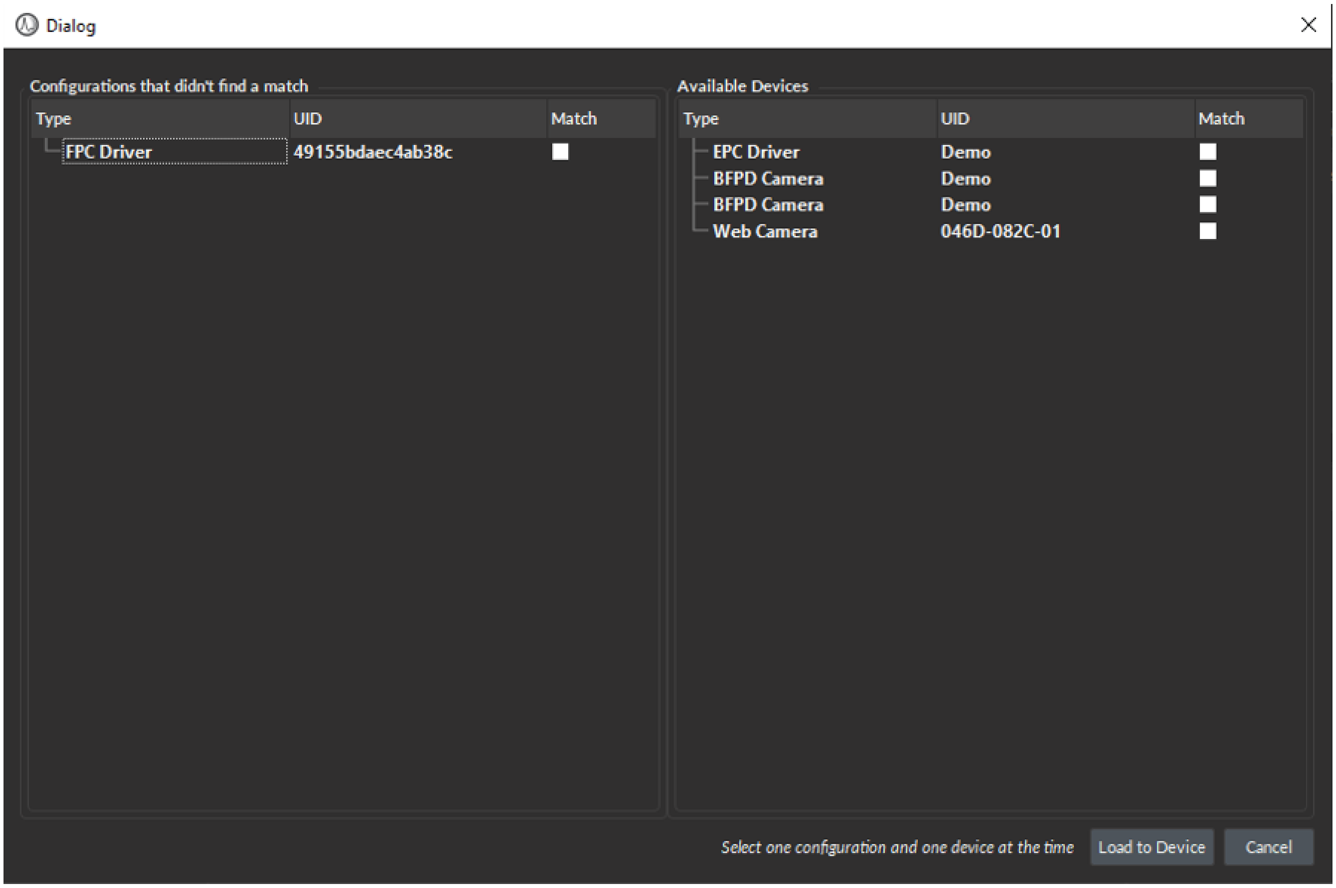


Figure 3.11: *Load All Configurations: didn't find a match*

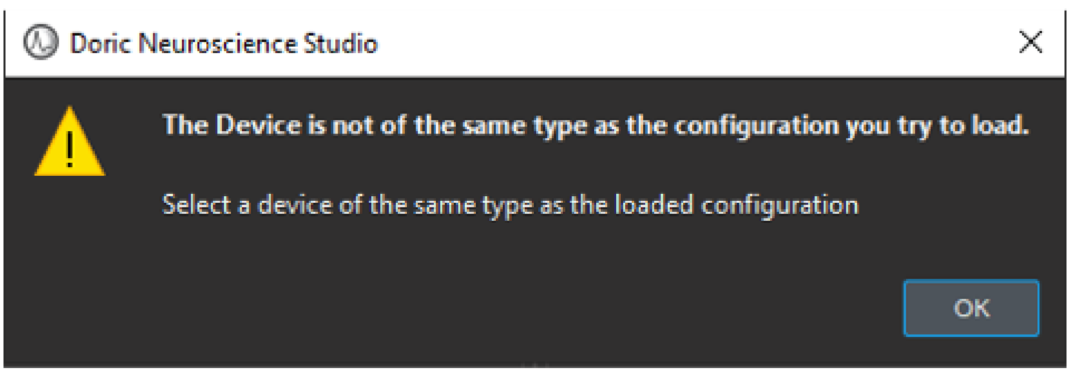


Figure 3.12: *Load All Configurations: device is not of the same type*

4

Doric File Editor

The **Doric File Editor** module (Fig. 4.2), allows users to view and manipulate *.doric* files. The *.doric* file, the format in which *Doric Neuroscience Studio* saves all data, is an HDF5-based file format.

This Hierarchical Data Format (HDF5) supports large, complex, and heterogeneous data. The HDF5 format is arranged in a nested structure, reminiscent of the organization of files in a computer directory. This type of structure supports metadata, including signals, keypress events, image stacks, and behavior videos. In addition, every configuration parameter used during recording is also stored within the same *.doric* file as the raw data.

Specifically, using this module users can:

- **View** the entire file structure, including configuration parameters, and recording variables used at the time of recording, facilitating experiment replication and/or troubleshooting; *Section 4.3*.
- **Check** the values of the raw data; *Section 4.5.1*.
- **Convert** old data into the *.doric* format (v6), compatible with *danse*^{TM}, Doric's neuroscience data analysis software; *Section 4.2.2*.
- **Import** external data and combine it into a *.doric* file; *Section 4.2.3*.
- **Export** data as *.csv* or *.tiff* file; *Section 4.2.4*.
- **Merge** multiple *.doric* files; *Section 4.2.1*.

4.1 Overview

To open the **Doric File Editor** module, select **Analysis**, then the **Doric File Editor** (Fig. 4.1). This will open the interface in Fig. 4.2.

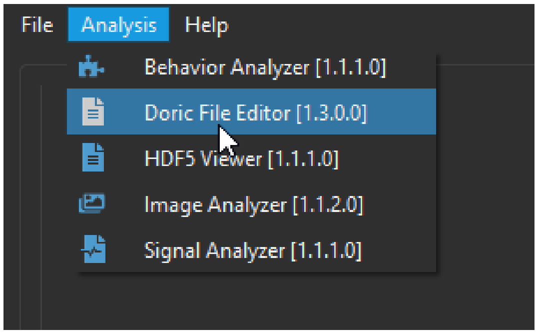


Figure 4.1: *Open Doric File Editor*

The **Doric File Editor** is organized in four sections (Fig. 4.2):

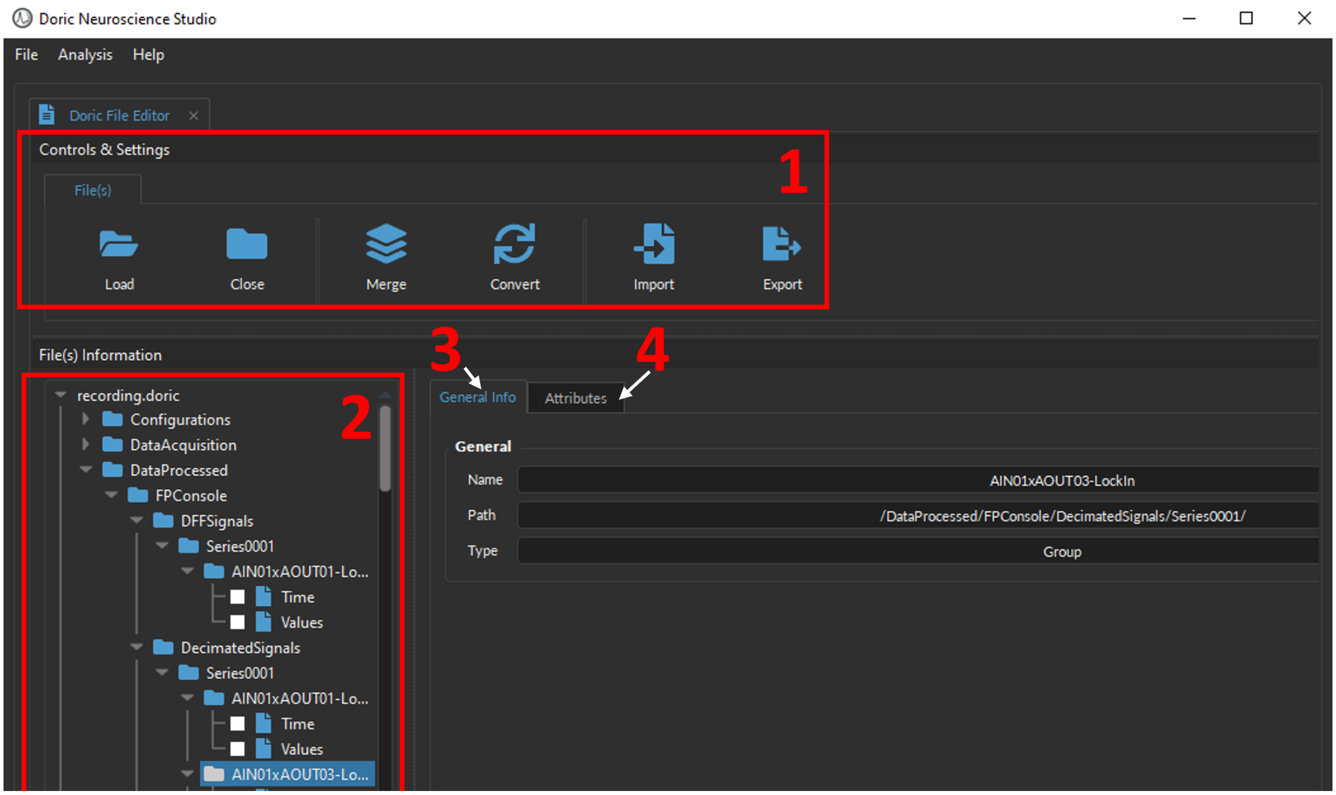


Figure 4.2: *Doric File Editor*

1. The **Control & Settings** (Fig. 4.2, 1) contains tools to manipulate *.doric* files. See section 4.2 for more details.
2. The **File Manager** (Fig. 4.2, 2) displays the nested structure stored within the file, from the name of the file itself and each of its branching folders. Section 4.3 details the specific HDF5-based organization within a single *.doric* file.
3. The **General Info** Tab (Fig. 4.2, 3) contains path and folder information and, for datasets, a **Data Viewer** function to quickly check the raw data. Sections 4.3.1 and 4.5.1 detail how the parameters are organized for **Configuration** and **DataAcquisition** folders, respectively.
4. The **Attributes** Tab (Fig. 4.2, 4) contains tables with parameters names and values. Sections 4.3.2 and 4.5.3 details how the attributes are organized for **Configuration** and **DataAcquisition** folders, respectively.

4.2 Control & Settings

The **Control & Settings** toolbar consists of a single **File(s)** tab (Fig.4.3), which contains the following functions:

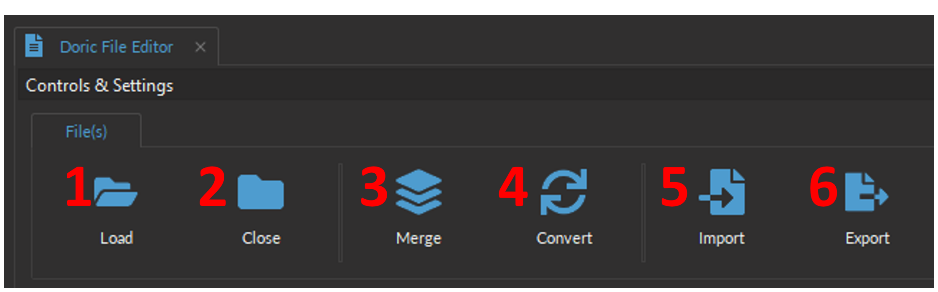


Figure 4.3: *Control & Settings*

1. The **Load File** button (Fig. 4.3, 1) opens a *File Explorer* window. Multiple files can be simultaneously loaded within the module and is in fact required when merging files. Note that only *.doric* files previously recorded on version 6 of the software are compatible with the **Doric File Editor**.

2. The **Close Current File** button (Fig. 4.3, 2) removes the selected file from the module (Fig. 4.2, 2). This button will only close one file at a time.

3. The **Merge** button (Fig. 4.3, 3) opens the **Merge** window (Fig. 4.4; see also Section 4.2.1), where users can specify how several *.doric* files will be merged (Fig. 4.24b).

4. The **Convert** button (Fig. 4.3, 4) converts .csv, .xlsx, .avi, .tiff or v5 .doric files to version 6 *.doric* files compatible with *Doric Neuroscience Studio* modules (**Signal Analyzer**, **Behavior Analyzer**, and **Image Analyzer**) and the *danse*^{TM} software. See Section 4.2.2.

5. The **Import** button (Fig. 4.3, 5) can add data from external files, including .csv or .xlsx data (for vector data) or a .avi, .tif, or .tiff files (for image data) and add it to one .doric file already loaded into the **File Manager**. See Section 4.2.3 for more details.

6. The **Export** button (Fig. 4.3, 6) outputs a *.doric* file as .csv (for vector data) or .tiff (for image data). See Section 4.2.4.

4.2.1 Merge

To merge several *.doric* files together, first load the files into the **Doric File Editor** (Section 4.2). Then select the **Merge Data** button (Fig. 4.3, 3) to open the **Merge** window (Fig. 4.4), where users must specify the following parameters:

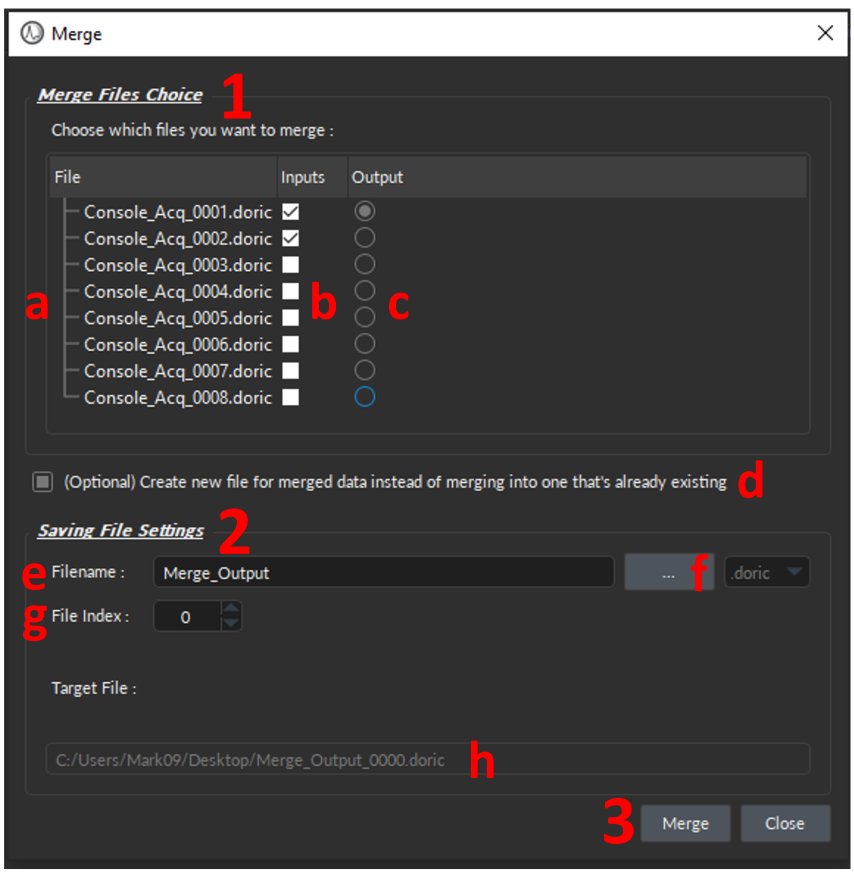


Figure 4.4: *Merge Window*

1. The **Merge Files Choice** (Fig. 4.4, 1)

a) The **File** column (Fig. 4.4, a) displays every *.doric* file loaded into the module. To add additional files, close the **Merge** window and select the **Load** button (Fig. 4.3).

b) The **Inputs** column (Fig. 4.4, b) is used to specify which of the previously loaded files to include during the merge.

c) The **Outputs** column (Fig. 4.4, c) is used to specify which file will be used as the output merged file. Thus, the files included in the merge should always have *identical outputs*.

d) If the **Checkbox** (Fig. 4.4, d) is enabled, a new file will be created for the merged data instead of merging it into a pre-existing file.

2. The **Saving File Settings** (Fig. 4.4, 2) are hidden unless the **Create new file** checkbox (see Section 4.2.1, no. 1d) is enabled. Here, users can specify:

e) The **Filname** text-box (Fig. 4.4, e) gives a new name to the merged file. By default, the filename will be *Merge_Output*.

f) The **[...]** button (Fig. 4.4, f) sets the new path of the merged file.

g) The **File Index** text-box (Fig. 4.4, g) allows users to specify the ending number of the file.

h) The **Target File** text-box (Fig. 4.4, h) displays the final path and filename where the merged file will be saved.

3. Selecting the **Merge** button (Fig. 4.4, 3) launches the function and combines the selected files according to the parameters set. Fig. 4.5 shows the final output of a merge file (if the checkbox was selected, Fig. 4.4, d). A merged file will have duplicate **Device** folders, matching the number of merged files (Fig. 4.24b).

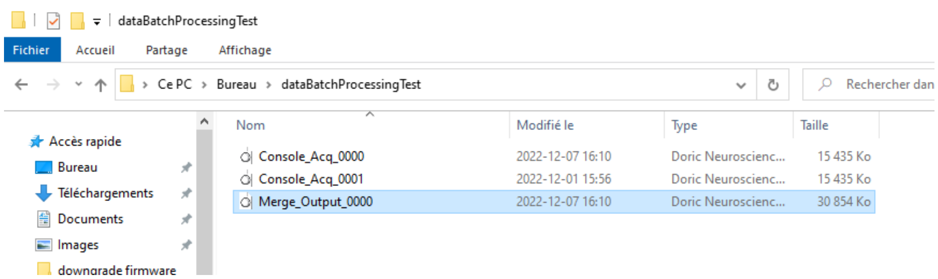


Figure 4.5: *Merge output File*

The file structure of the merged files follows the input file structure. However, in both the Configuration and Data Acquisition branches, the Device Type folders are duplicated, one for each of the merged files. The structure of the contents of each **Device** folder will be identical to the **Device** folder of each file included in the merge.

4.2.2 Convert

This function **Converts** all older *DNS* file formats into version 6 *.doric* file, matching the output of the newest version of *Doric Neuroscience Studio (DNS)* that is compatible with *danse*^{TM}. Select the appropriate tab from the **Convert** Window (Fig. 4.6, 1-3) to choose which type of file format to convert from.

Figure 4.6: *Three tabs allow conversion based on file type*

Each **Convert** tab (Fig. 4.6, 1-3) will be treated in the following section:

1. **Spreadsheet** (*.csv* / .xlsx) - Section 4.2.2.1

2. **Microscope Images** (*.avi* / *.tif* / *.tiff*) - Section 4.2.2.2
3. **Doric File (V5)** (*.doric* (v5)) - Section 4.2.2.3

4.2.2.1 Convert Spreadsheet

The **Spreadsheet** tab converts *.csv* or *.xlsx* data into v6 .doric files, using the following parameters:

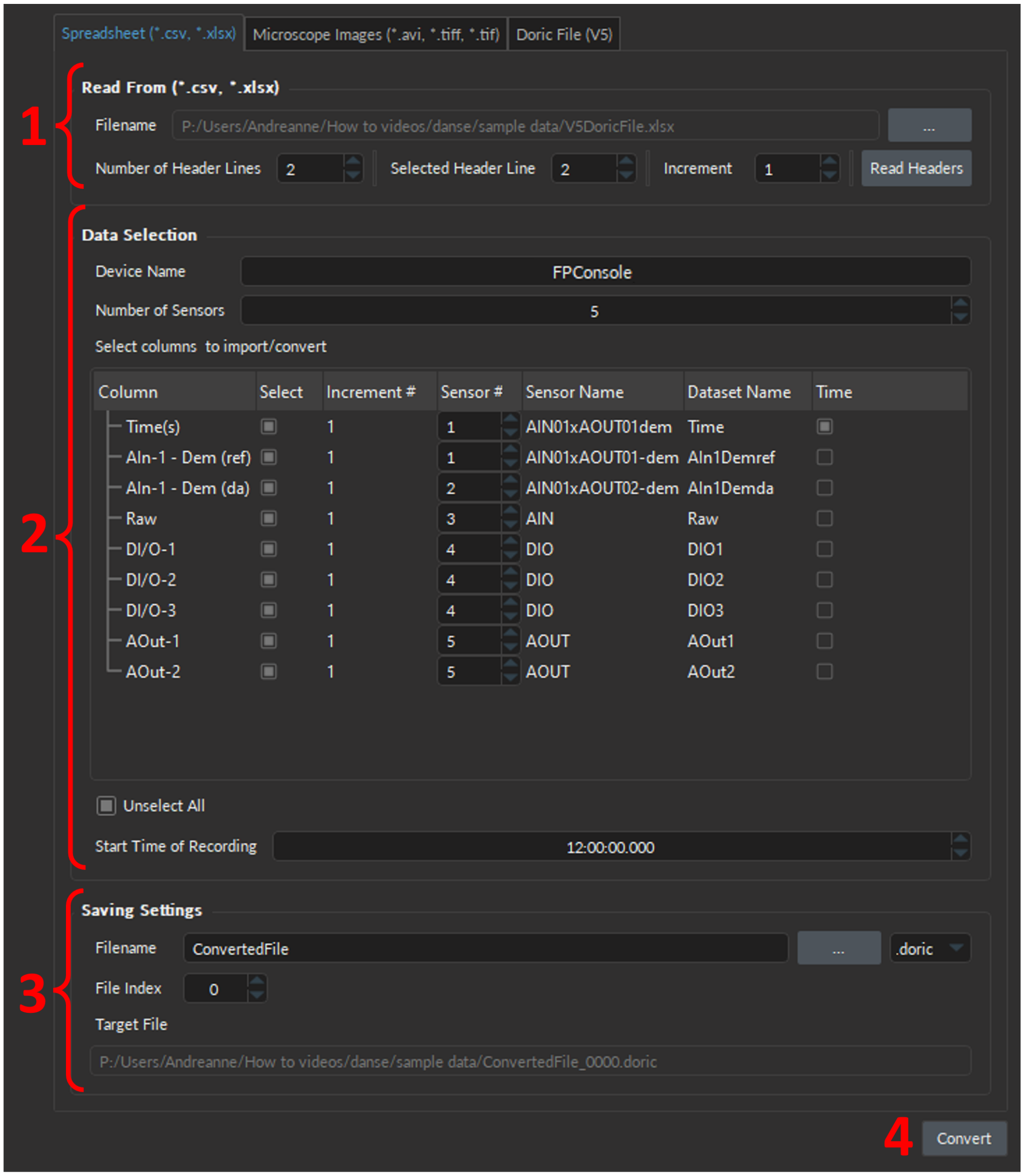


Figure 4.7: *Convert Window, Spreadsheet Tab*

1. The **Read From** section (Fig. 4.7, 1) is used to select the **Spreadsheet** that will be converted into a v6 .doric file.

   - The **Filename** displays the computer path and filename of the data that will be converted. The **Filename** is automatically generated once the file is selected.
   - Selecting the **[...]** button opens a File Explorer window to select the data file.
   - The **Number of Header Lines** specifies the total number of rows within the spreadsheet that contains heading information. For example, V5 Doric CSV file (Fig. 4.8, a) has two header rows above the raw data. Each header lines is attributed an index number (Fig. 4.8, b).

- The **Selected Header Line** specifies which header line (if there are multiple) will be read into the **Data Selection** table and then used when converting the file. The value selected in the text-box represents the index number of the **Selected Header Line**. For instance, a **Selected Header Line** of ***2*** reads the bottom header row in Fig. 4.8, b. Such that the name of the header is displayed in the first column of the **Data Selection** table (Fig. 4.7, 2).

	A	B	C	D	E	F	G
1	---	Analog In. | Ch.1	Analog In. | Ch.1	Analog In. | Ch.1	Analog Out. | Ch.1	Analog Out. | Ch.2	Analog Out. | Ch.4
2	Time(s)	AIn-1 - Dem (AOut-1)	AIn-1 - Dem (AOut-2)	AIn-1 - Raw	AOut-1	AOut-2	AOut-4
3	0.0833735	0.074755089	0.111966655	-0.059968871	1	1	0
4	0.0842035	0.074753995	0.111941634	0.031891842	1	1	0
5	0.0850335	0.07475949	0.111917428	0.103076266	1	1	0
6	0.0858635	0.074758808	0.111893109	0.013122959	1	1	0
7	0.0866935	0.074757691	0.111866474	0.038148137	1	1	0

Figure 4.8: *Spreadsheet Headers*

- The **Increment** parameter is used for very specific niche cases where data is not organized by column, and instead data from separate sensors/channels are alternated within a column, as in columns 2 and 3 of Fig. 4.9. The number of interleaving signals corresponds to the number of increments (Fig. 4.9).

  ****For V5 Doric spreadsheets (and all typical data), the value of the increment should ALWAYS be 1.****

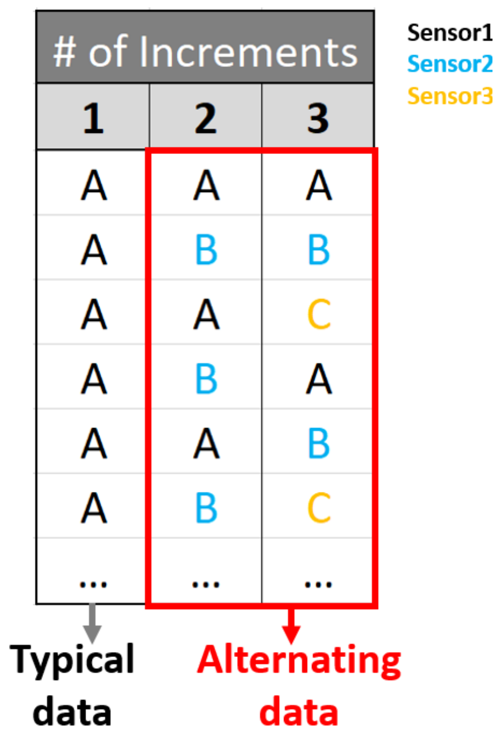


Figure 4.9: *Increment Schematic*

- The **Read Headers** button updates the **Data Selection** table (Fig. 4.7, 2) if the following parameters were modified: **Number of header lines**, **Selected Header Line**, **Increment**, and **Number of Sensors**.

2. The **Data Selection** section (Fig. 4.7, 2) contains the following parameters:

   - The **Device Name** specifies the type of device used to collect the data. For instance, if a Doric *Fiber Photometry Console* was used, you could put *FPConsole* as the **Device Name**, like for the spreadsheet example (Fig. 4.7).

- The **Number of Sensors** corresponds to the maximum number of channel or sensor types. For instance, continuing with the FPConsole example (Fig. 4.7 & 4.8) Analog In, Analog Out, and Digital In/Out are three separate types of channels. In addition, demodulated signals (when using the Lock-In mode) count as separate sensors. Thus, we would have a total of 5 sensors for this data file.
- The **Select Columns to import/convert** specifies which datasets to include during the conversion, specify the column that corresponds to the **Time** vector, which column corresponds to which sensor, and rename the **Sensors** and **Datasets**, if required.
- The **Select all** check box will enable all the columns for conversions. De-selecting the checkbox will disable them all and lose any changed names.
- The **Start Time of Recording** specifies the absolute time of the day as *hh:mm:ss:zzz* when the recording was initiated and can be used to align the data properly.

3. The **Saving Settings** section (Fig. 4.7, 3) specifies:

   - The **Filename** of the **converted** file.
   - Selecting the **[...]** button opens a File Explorer window to specify the folder where the newly converted file will be saved.
   - The **File Index** is used to define the current indexation number used for multiple files saved during the same measurement session. The suffix is incremented automatically when converting multiple files.
   - The **Target File** displays the absolute path and filename where the data will be saved. It is automatically generated based on the **Filename** and saving folder path.

4. The **Convert** button (Fig. 4.7, 4) initiates the conversion process. A **Process Complete** pop-up window (Fig. 4.10) will be displayed if the conversion was successful. To view the file in the **File Manager** you must first load it (See Section 4.2).

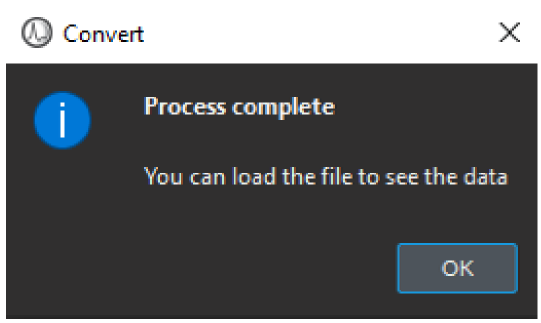


Figure 4.10: *Convert Spreadsheet, Process Complete Pop-up*

4.2.2.2 Convert Microscope Images

The **Microscope Images** tab uses the following parameters to convert data (.avi, .tif, or .tiff) into v6 .doric format:

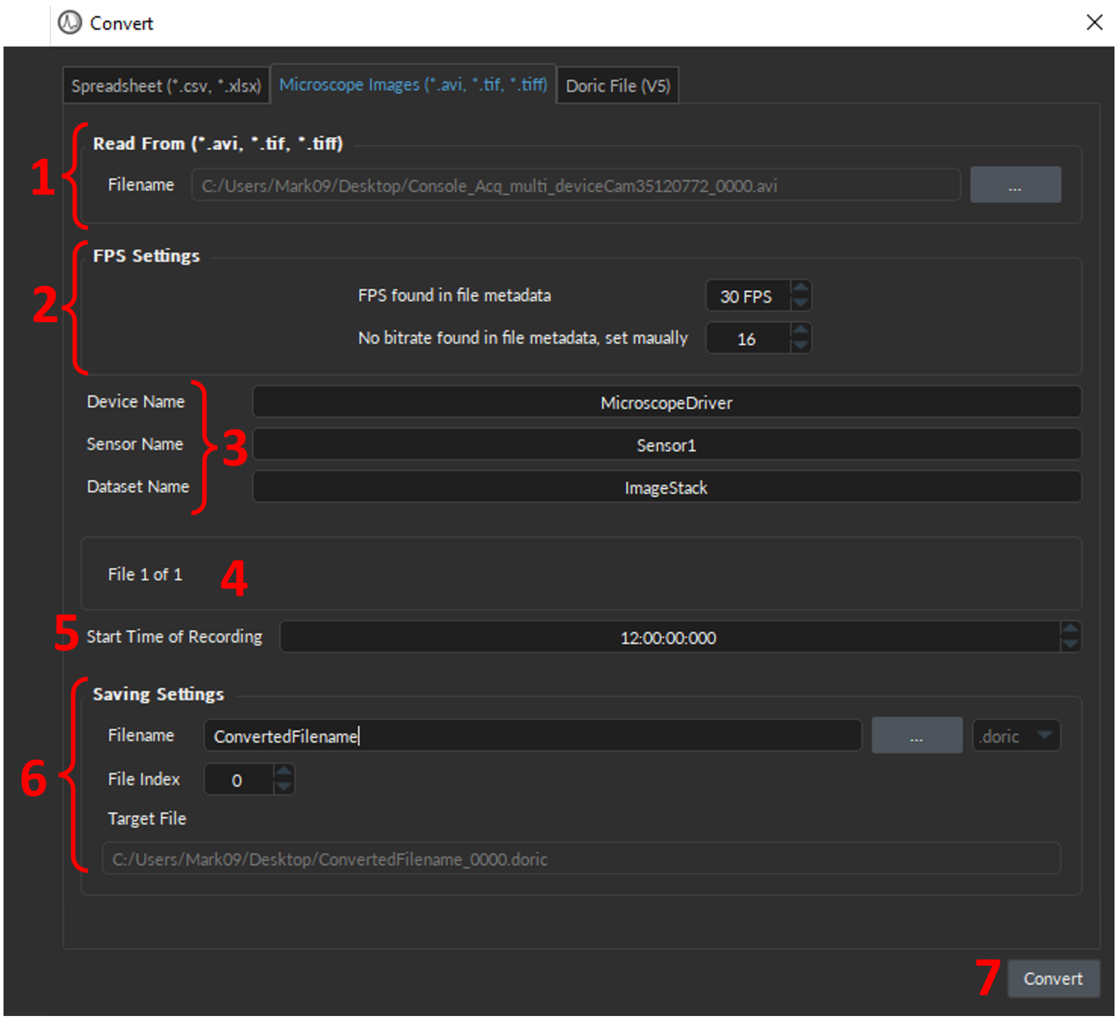


Figure 4.11: *Convert Window, Microscope Images Tab*

1. The **Read From** section (Fig. 4.11, 1) specifies the Microscope Image file that will be converted.
   a) The **Filename** displays the computer path and filename of the data file that will be converted. The **Filename** is automatically generated once the file is selected (Fig. 4.12, a).
   b) Selecting the **[...]** button opens a File Explorer window to select the data file.
2. The **FPS Settings** (Fig. 4.11, 2) contains the following parameters:
   c) The **FPS** automatically detects the **FPS** from the metadata of the file. However, users can specify or modify this value, if needed. If no **FPS** value is detected, *No FPS found in the file metadata, set manually* message will be displayed instead.
   d) The **Bitrate** automatically detects the **Bitrate** from the metadata of the file. However, users can specify or modify this value, if needed. If the **Bitrate** value isn't detected, a *No Bitrate found in the file metadata, set manually* message will be displayed instead. The **bitrate** can take any value between 8-16.
3. The **Structure Settings** (Fig. 4.11, 3) sets the name of the nested folders containing the converted data, recapitulating the file structure of v6 *.doric* files. The names of the following three layers (Fig. 4.12) must be specified:
   e) The **Device Name** specifies the device type used to collect the data, as in Fig. 4.12, e.

f) The **Sensor Name** specifies the type of sensor that was used to collect the data, such as red or green CMSO sensor, as in Fig. 4.12, f.

g) The **Dataset Name** specifies the type of data that was collected, as in Fig. 4.12, g.

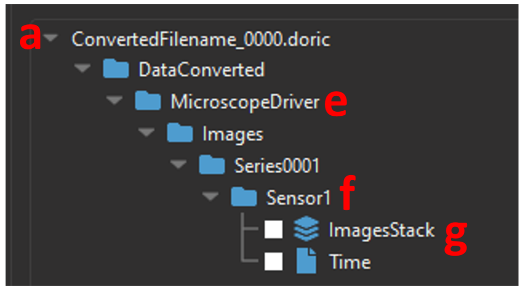


Figure 4.12: *Converted Microscope File Structure*

4. The **Loading bar** (Fig. 4.11, 4) appears when users selected the **Convert** button at the bottom of the window and indicates the progress of the file conversion (Fig. 4.13).

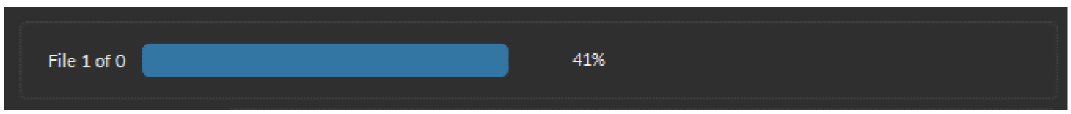


Figure 4.13: *Loading Bar*

5. The **Start Time of Recording** (Fig. 4.11, 5) specifies the absolute time of the day as hh:mm:ss:zzz when the recording was initiated and can be used to align the data properly.

6. The **Saving Settings** (Fig. 4.11, 6) specifies:

   h) The **Filename** of the **converted** file.

   i) Selecting the **[...]** button opens a File Explorer window to specify the folder where the newly converted file will be saved.

   j) The **File Index** is used to define the current indexation number used for multiple files saved during the same measurement session. The suffix is incremented automatically when converting multiple files.

   k) The **Target File** displays the absolute path and filename where the data will be saved. It is automatically generated based on the **Filename** and saving folder path.

7. The **Convert** button initiates the conversion process. A **Process Complete** pop-up window (Fig. 4.14) will be displayed if the conversion is successful. To view the file in the **File Manager** you must first load it (See Section 4.2).

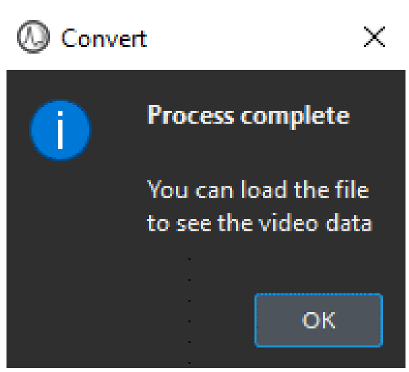


Figure 4.14: *Convert Microscope Images, Process Complete Pop-up*

4.2.2.3 Convert Doric File (V5)

Since significant improvements have been made to v6 *.doric* file format (compared to v5), there are several cases where converting the older format into the new one is useful or required, such as analyzing an experiment with data in both versions and/or using *danse*^{TM} data analysis software.

To **Convert** v5 .doric files to v6, the **Doric File (V5)** tab (Fig. 4.15) includes the following parameters:

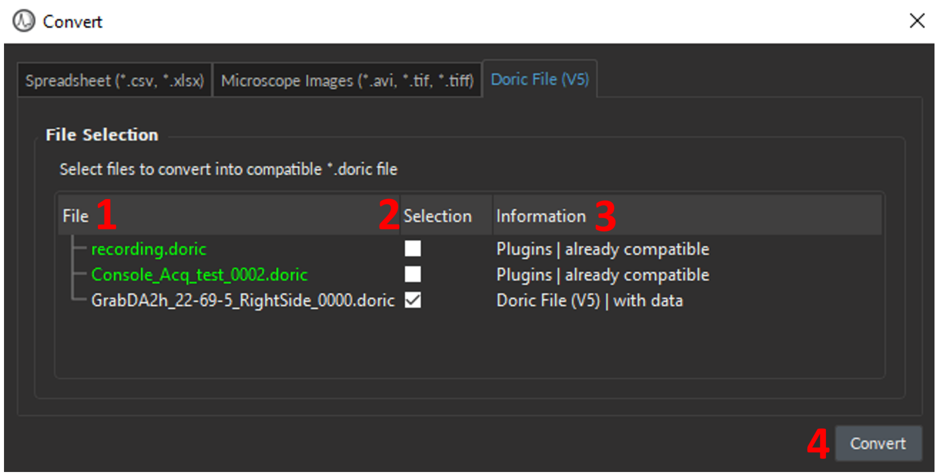


Figure 4.15: *Convert Window, V5 Doric File Tab*

1. The **File** column (Fig. 4.15, 1) lists all of the *.doric* files contained within the **File Manager** (Fig. **??**, 2). If the filename(s) are in green, then the folder is already in v6 *.doric* format and cannot be converted again. If the filename is in gray, it isn't in v6 format. However, if the filename is in red, then the data cannot be converted since it is most likely a V5 configuration file.

   NOTE: You can also slide a file into the **File Selection** table to add it to the list of **Files** within the column.

2. The **Selection** column (Fig. 4.15, 2) contains a checkbox where multiple files can be selected simultaneously for conversion. NOTE THAT Selecting a green file will give an error message.

3. The **Information** column (Fig. 4.15, 3) details whether a file was already converted. This section can even detect whether a V5 Doric File contains data or is a configuration file.

4. The **Convert** button (Fig. 4.15, 4) initiates the conversion process. Converted files will be saved in the same directory/folder as the original file, named as *FILENAME_converted_mmddyyyy*. A **Process Complete** pop-up window (Fig. 4.16) will be displayed if the conversion was successful. To view the file in the **File Manager** you must first load it (See Section 4.2).

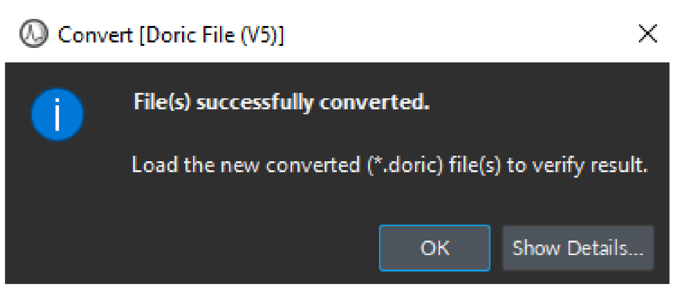


Figure 4.16: *Convert V5 Doric File, Process Complete Pop-up*

__REMINDER:__ *V5 Configuration files CANNOT be converted to V6, since new parameters have been added and old ones deleted in V6 Doric Neuroscience Studio. Contact support@doriclenses.com for assistance recreating configuration files.*

4.2.3 Import

The **Import** function embeds external data, either from a **Spreadsheet** or **Microscope Images** to an already generated *.doric* file. Note that this function cannot embed data into multiple files simultaneously, even if they are all pre-loaded into the **File Manager**.

The following parameters must be specified to embed data into the chosen file:

1. The **Import Into** section specify which *.doric* file will receive the embedded data. This process is common for both **Spreadsheets** and **Microscope Images** (Fig. 4.17), and is as following:

Figure 4.17: *Import, Select file to import into*

   a) The **File** column (Fig. 4.17, a) displays all the *.doric* files pre-loaded into the **File Manager**. If no files are displayed, close the **Import** window and use the **Load** button to select a data file.

   b) The **Import Into** column (Fig. 4.17, b) contains the checkboxes that select which file embeds the external data. Note that a single checkbox can be enabled at a time.

2. The **Spreadsheet** specific parameters in Fig. 4.18 are identical to the parameters details in Section 4.2.2.1.

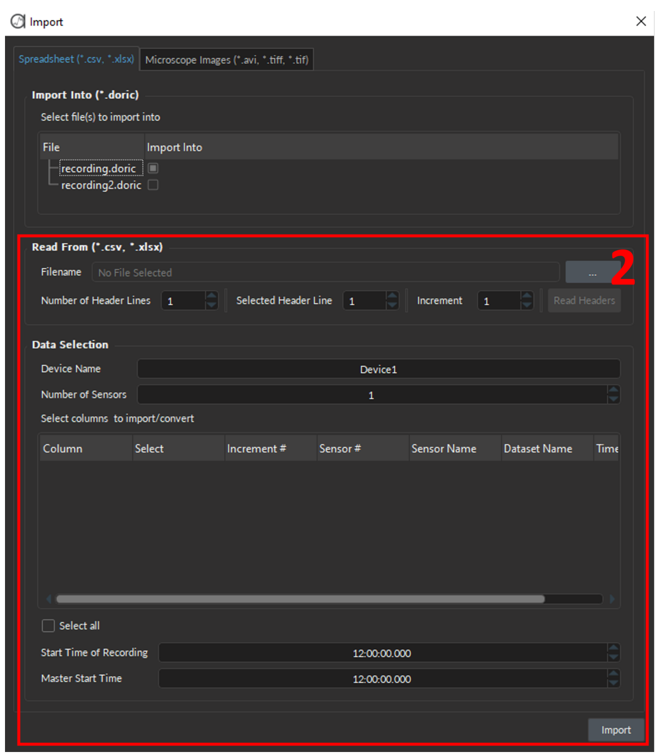


Figure 4.18: *Import, Spreadsheet-specific parameters*

3. The **Microscope Image** specific parameters in Fig. 4.19 are identical to the parameters details in Section 4.2.2.2.

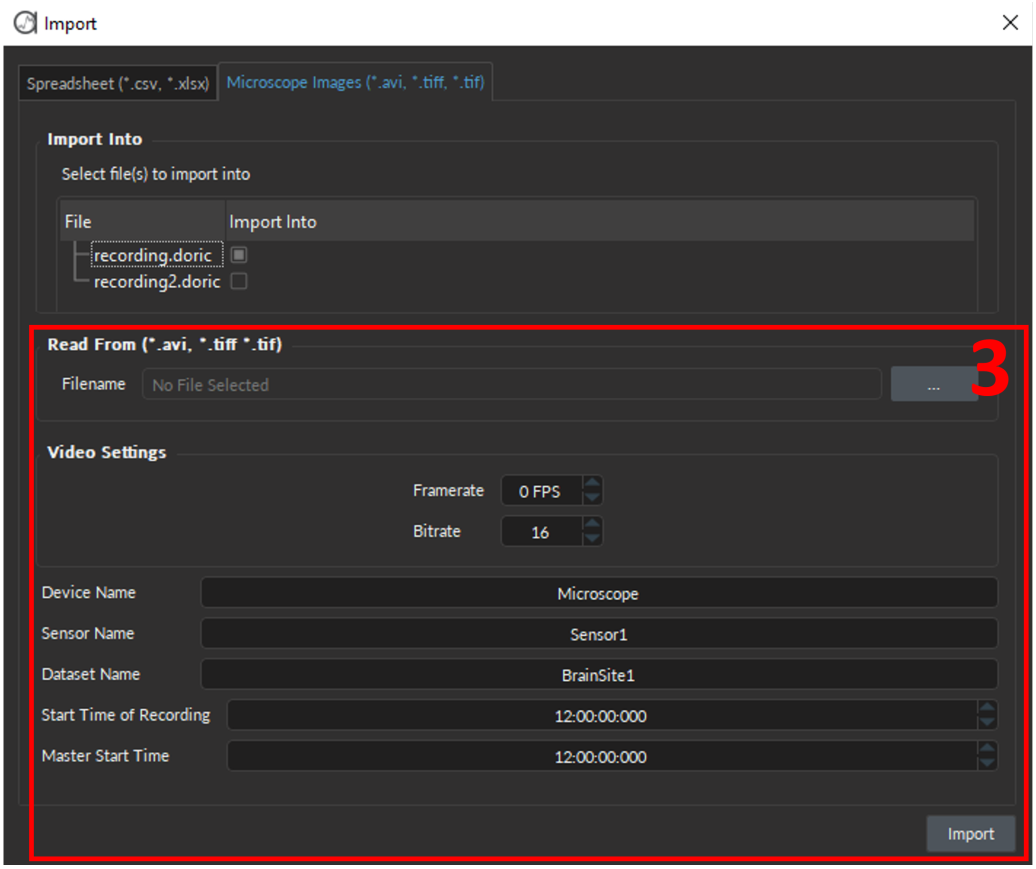


Figure 4.19: *Import, Microscope-specific parameters*

4.2.4 Export

To export v6 *.doric* files to *.csv* or to *.tiff* (for microscope images), first load the files into the **File Manager**, then select the **Export** buttons (Fig. 4.3, 6). This button opens the **Export** window (Fig. 4.21).

Note that only the **Sensor / Channel Type** folders (such as folders marked with *blue arrow* in Fig. 4.21) in the **Data Acquisition** folder will be converted into .csv (vector) or .tiff (images) format.

The following parameters can be specified when exporting data from the **Export** window (Fig. 4.21):

1. The **Loaded Files** section (Fig. 4.21, 1) contains a list of *.doric* files already loaded into the **File Manager** from which the contents will be displayed within the **Data Selection** section.

2. The **Saving Settings** section (Fig. 4.21, 2) specifies the following parameters:

    - The **Filename** text-box names the exported file.
    - The **[...]** button opens a File Explorer window where users can specify the path where the exported data will be saved.
    - The **File Extension** of the exported data file is automatically detected based on the signal type (vector: *.csv*, Fig. 4.21a; image: *.tiff*, Fig. 4.21b) of the **Sensor / Channel Type** folder in question.
    - The **File Index** text-box specifies the # attached at the end of the filename. By default, this value will be 0.
    - The **Target File** displays the location on the computer where the newly exported file will be saved.

3. The **Decimation Settings** section (Fig. 4.21, 3) includes the following:

    - The **Enable Decimation** check-box turns on the decimation setting. This parameter is used to reduce the sampling rate and thus, also reduces the file size of the exported file.

- The **Decimation Factor** text-box provides a way to reduce the file sizes. This method conserves one point over a number of data points equal to the **Decimation Factor**.[1]

4. The **Data Selection** section (Fig. 4.21, 4) is used to select the **Sensor/Channel** that is to be exported. Note that a single **Sensor/Channel** can be exported at once and should always include a **Time** vector and at least one **Dataset** vector (Fig. 4.20). If multiple **Dataset** are contained within a single **Sensor/Channel** folder, users can select which **Dataset** to include in the exported file (Fig. 4.20).

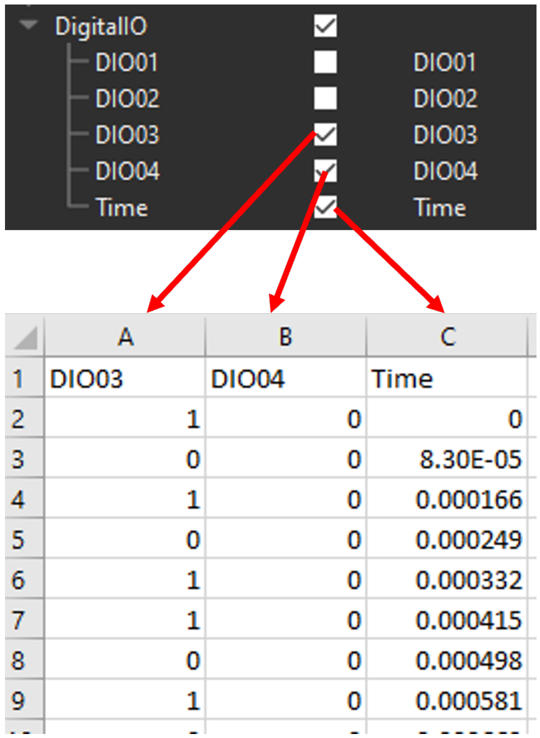
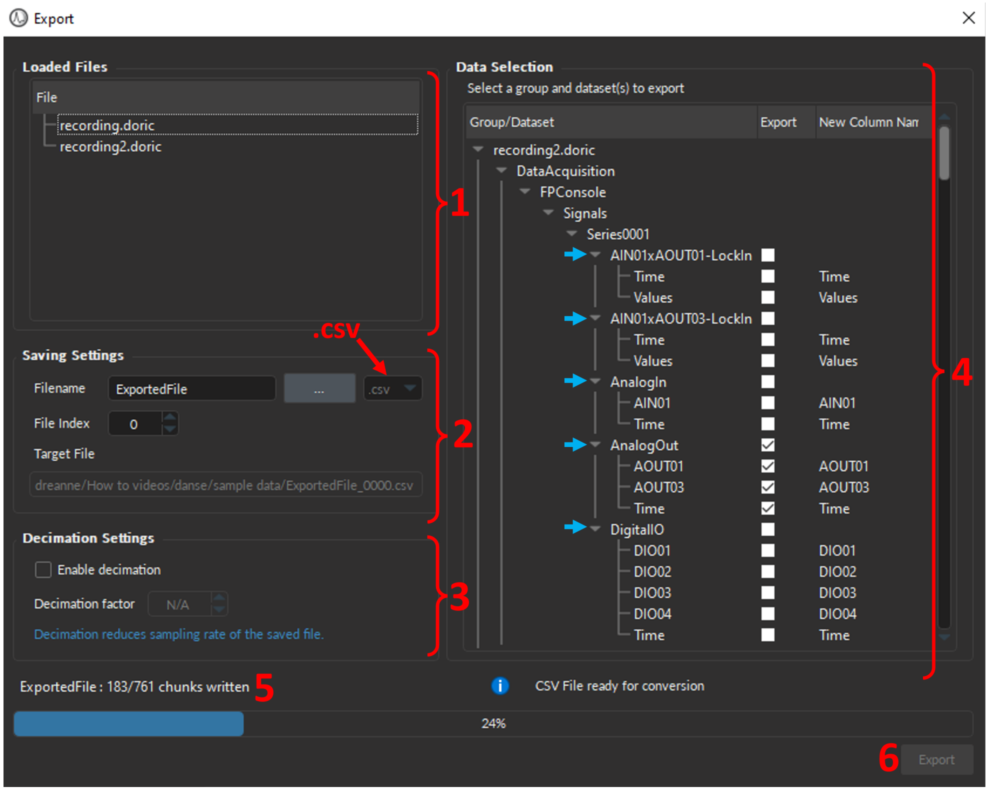


Figure 4.20: *Export, Dataset selection example*

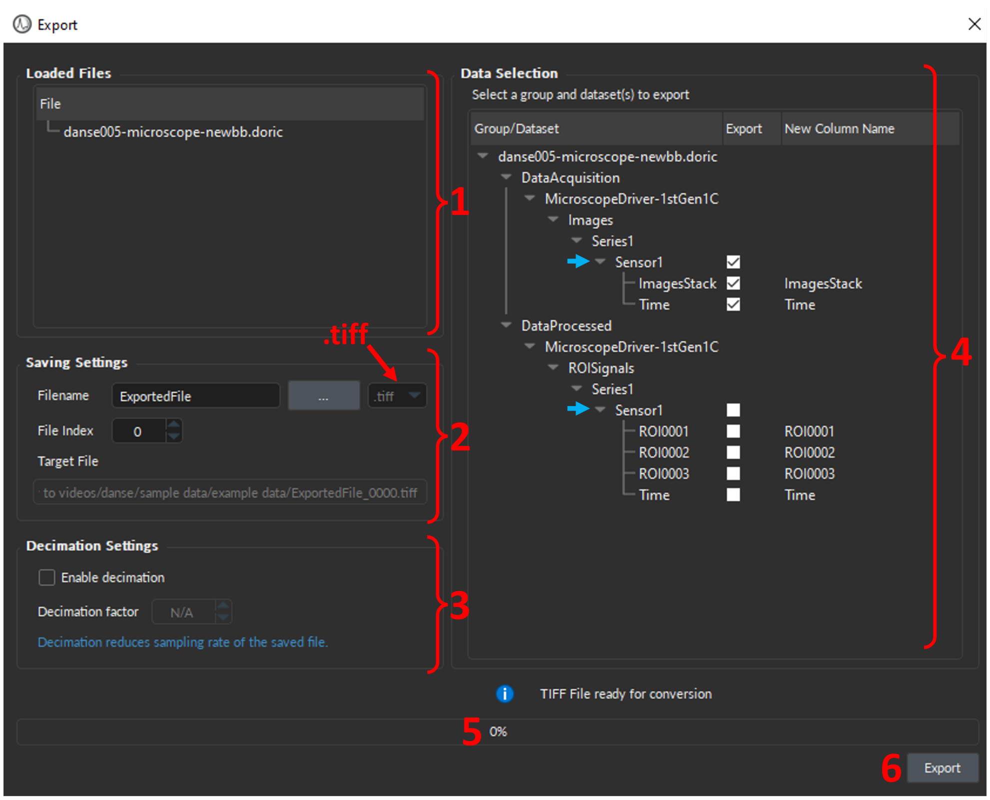
5. The **Loading Bar** (Fig. 4.21, 5) displays the percentage of the file that has been exported.

6. The **Export** button (Fig. 4.21, 6) will create a new file (either .csv or .tiff, depending on the **Sensor Type**) in the specified **Target File** directory.

[1] For a data set of 10 points, saved with a **Decimation Factor** of 2, only 1,3,5,7,9 will be kept while the remainder will be dropped, producing a file of 5 points of data.



(a) .csv



(b) .tiff

Figure 4.21: *Export Window*

4.3 File Structure Overview

The file structure begins with the data **File** itself, which is labeled as *FILENAME.doric* (Fig. 4.22, arrow), followed by two[2] branches (Fig. 4.22, 1-2):

1. The **Configurations** branch (Fig. 4.22, 1) contains all the recording parameters set during the recording, including *Saving*, *Global*, *Time series* and channel-specific folders (Section 4.4).

2. The **Data Acquisition** branch (Fig. 4.22, 2) contains and organizes the raw data (Section 4.5).

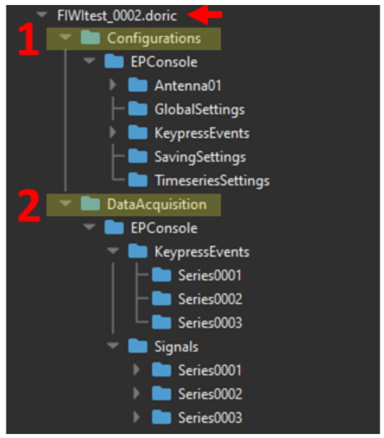
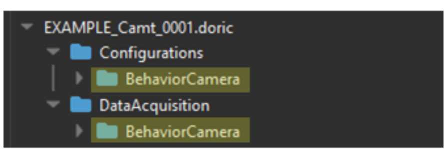
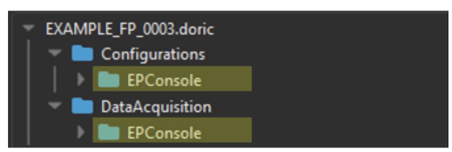


Figure 4.22: *General Structure of a .doric file*

Both the **Configuration** and **Data Acquisition** folders each contain an identical next layer (Fig. 4.23): a branch for every **Device** used during the recording. The **Device** folder contains different information depending on whether it is under the **Configuration** or **DataAcquisition** branches (Sections 4.4 and 4.5).



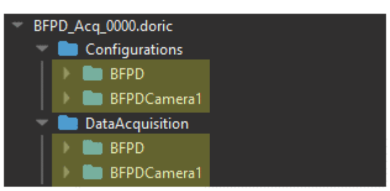
(a) *Device 1 - Camera*



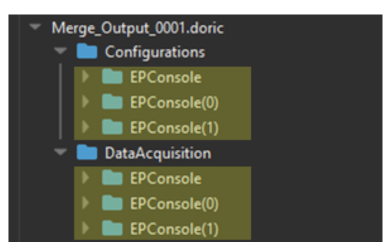
(b) *Device 2 - Console*

Figure 4.23: *Device branch in both Configuration and DataAcquisition paths*

If multiple devices are used during the recording, each device will have its own folder in both paths, labeled as the device name (Fig. 4.24a). If multiple *.doric* files were merged together (Section 4.2.1), identical **Device** folders from different recordings will remain separate, named **Device #** (Fig. 4.24b).



(a) *Multiple Devices*



(b) *Duplicate Devices after merge*

Figure 4.24: *Multiple Device folders*

[2]***If the **File** was processed and analysed using *danse*^{TM}, then the **DataProcessed** and **DataAnalyzed** folders will be contained within the **File**.***

Selecting any folder or Dataset within the data tree will display either **General Info** (Section 4.3.1) or the **Attributes** (Section 4.3.2) on the right side of **Doric File Editor** (Fig. 4.25, box), depending on which tab is enabled (Fig. 4.25, red arrow).

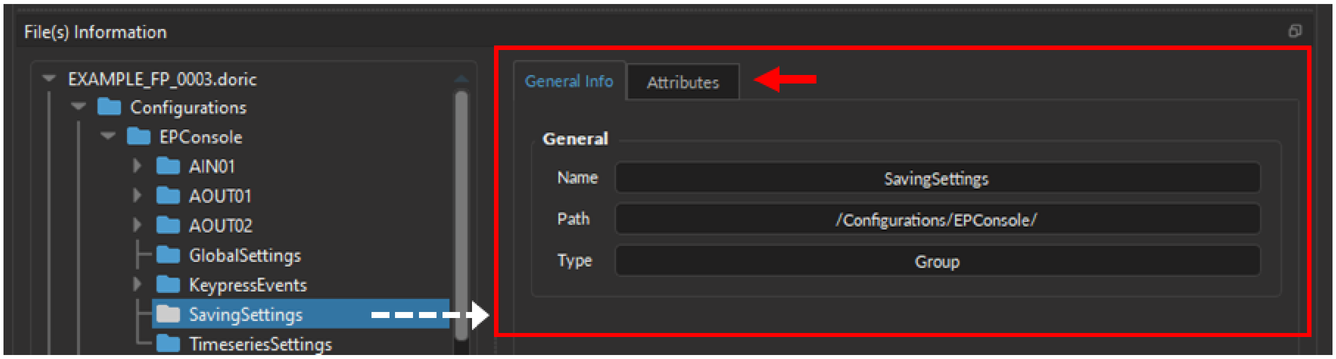


Figure 4.25: *Parameters are stored in both tabs*

4.3.1 General Info Tab

The **General Info** Tab stores information about the name of the branch, its path within the nested *.doric* file, and the type of branch. For datasets, this Tab additionally stores the raw data (see Section 4.5.1).

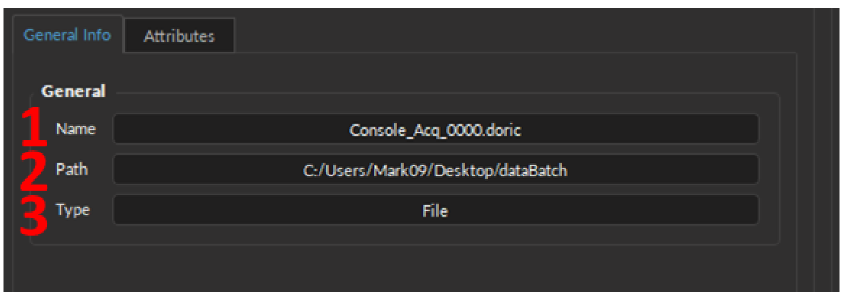


Figure 4.26: *General info tab - File & Group branches*

1. The **Name** (Fig. 4.26, 1) parameter displays the label given to the currently selected branch. For the **File** type, the **Name** is in fact the filename.
2. The **Path** parameter (Fig. 4.26, 2) displays the location of the selected branch (for **Group** and **Dataset** types) within the structure, following *Parent-Folder/Sub-Folder* syntax. For the **File** type, the **Path** is in fact the location of the computer where the *.doric* file is saved.
3. The **Type** parameter (Fig. 4.26, 3) displays which of three categories the selected branch belongs to:
   - *File* - This type is always the trunk of the **File Manager**. By definition, no other branch can be of the file type. In addition, information about the file path can be found in **General Info** tab (Fig. 4.26, 2) and information about the date of creation and software version can be found in the **Attributes** tab of this branch type.
     - **NOTE:** The date of creation corresponds to Time-point 0 of the time Datasets.
   - *Group* - This type of branch stores other branch points (either Group or Dataset types) and attributes (which can be viewed in the **Attributes** Tab, Section 4.3.2).
   - *Dataset* - This type is always an ending branch point and contains the raw data. The data can be viewed within the **General Info** Tab (Fig. 4.41, 5-6). The **General Info** tab of this type also stores information about the dataset (Fig. 4.41, 1-4; see below for details). This type also stores attributes (in **Attribute** tab), but can never store other nested folders.

4.3.2 Attributes Tab

The **Attribute** Tab contains a table where all the relevant attributes (if any) of the Datasets and Groups type branches are contained. These attributes include parameters and configuration set by the user in *Doric Neuroscience Studio* before a recording, in addition to default values collected by the software.

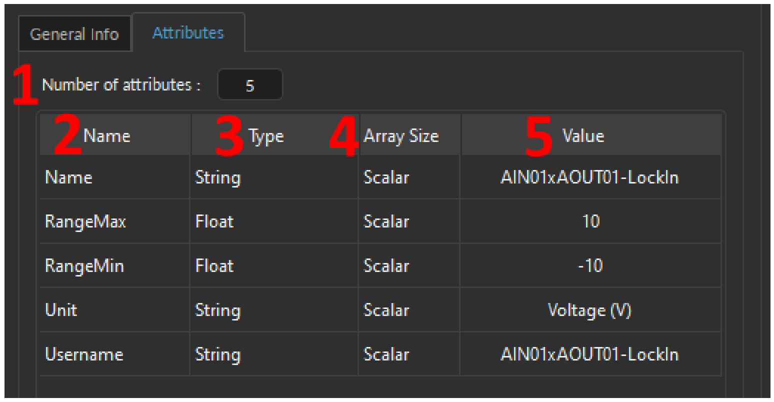


Figure 4.27: *Attribute tab - overview*

The **Attribute** tab is structured as a table. The rows of the table correspond to a single attribute, ordered alphabetically. Each attribute row is split into four standard columns (Fig. 4.27, 2-5):

1. The **Number of attributes** (Fig. 4.27, 1) defines the number of rows in the attribute table and corresponds to the number of parameters stored within the selected branch.
2. The **Name** column (Fig. 4.27, 2) contains the label given to the attribute. Most parameters set by the user within the software have identical names.
3. The **Type** column (Fig. 4.27, 3) displays what kind of value is stored within the attribute (e.g. float, integers, strings, etc.).
4. The **Array Size** column (Fig. 4.27, 4) displays the dimensions of the attribute value. Most values stored in **Attribute** tab are scalars.
5. The **Value** column (Fig. 4.27, 5) displays the numerical or text information associated with the attribute.

4.4 Configurations Structure

The **Configurations** folder contains a branch for every **Device**. As per Figure 4.28, each **Device** branch will, in turn, contain a branch for every channel used during the recording (storing channel-specific settings, Section 4.4.1) and three folders contain general parameters: **Global Settings** (Section 4.4.2), **Saving Settings** (Section 4.4.3) and **TimeSeries Settings** (Section 4.4.4).

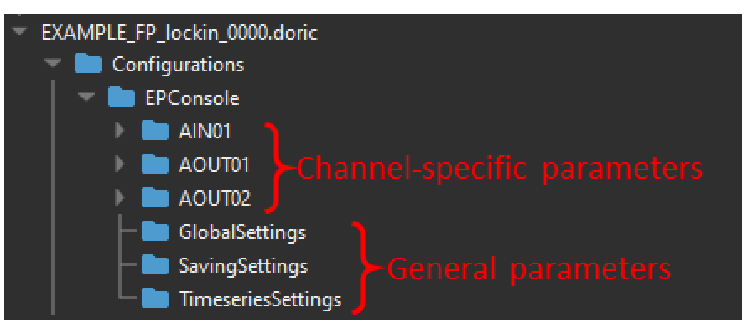


Figure 4.28: *Configuration Structure*

4.4.1 Channel-specific Settings

The **Channel-specific Settings** are stored within the **Attribute** tab. These attributes correspond to the parameters set by the user within the **Configuration Window** in the *Acquisition Console* module of the software. The order of the **Attributes** is sorted alphabetically, while the parameters in the **Configuration Window** are organized by function.

To illustrate the general principle of how **Channel-specific Settings** are structured, we will show how the organization of all the attributes of the Analog Out channel. This template can be applied to a large number of **Channel / Sensor Types** (Digital In, Analog Out, and Analog In, Antenna, Cam Ex). Note that Keypress Events, Camera, and Microscope channels have different structures, but the attributes all still come from the **Channel Configuration** window.

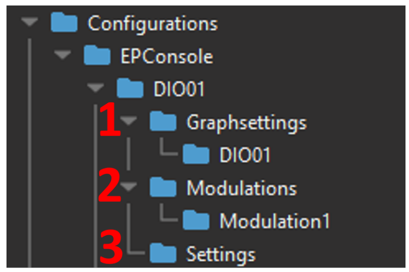


Figure 4.29: *Channel-specific Structure*

Each **Channel / Sensor Type** has different configuration attributes, however, they mostly can all be split into 2-3 sub-folders (Fig. 4.29):

1. The **Graphsettings** folder (Fig. 4.29, 1 & 4.31) contains the attributes concerning how the signals are displayed within the **Acquisition View** of the **Acquisition Console** module. Users can specify the trace name, color, style, size, and type of points within the **Graph(s) Options** window (Fig 4.30).

2. The **Modulations** folder (Fig. 4.29, 2 & 4.33a) is only included for output-type channels (Digital or Analog). This type of folder contains the user-specified attributes in the **Sequence Option(s)** of the **Channel(s) Configuration** window (Fig. 4.32, 2) (e.g. *Starting Delay*, *Frequency*, *Time ON*, *Pulse(s) Sequence*, etc.). Note that depending on the Channel type (Digital vs Analog) and Channel Mode (*CW*, *Square*, *Stairs*, etc.) the parameters of the **Sequence Option(s)** will change. However, all possible attributes, regardless of Channel Type and Mode, are included in the **Attribute** tab of the **GraphSettings** folder. If the attribute is unused, the value will be 0.

3. The **Settings** folder (Fig. 4.29, 3 & 4.33b) contains the attributes from the **Channel Options** box in the **Channel(s) Configuration** window (Fig. 4.32, 3).

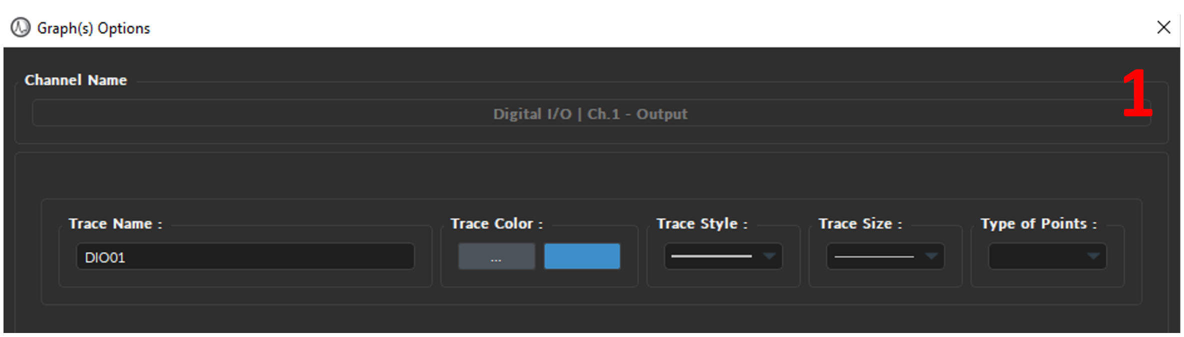
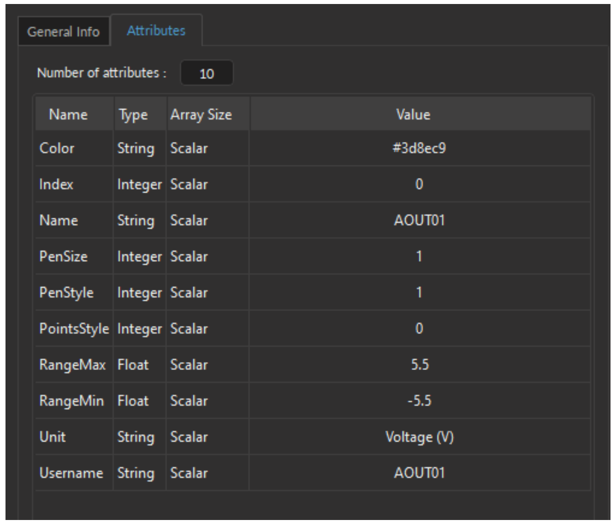


Figure 4.30: *Acquisition Console, Graph Option(s) window*



General Info | Attributes

Number of attributes : 10

Name	Type	Array Size	Value
Color	String	Scalar	#3d8ec9
Index	Integer	Scalar	0
Name	String	Scalar	AOUT01
PenSize	Integer	Scalar	1
PenStyle	Integer	Scalar	1
PointsStyle	Integer	Scalar	0
RangeMax	Float	Scalar	5.5
RangeMin	Float	Scalar	-5.5
Unit	String	Scalar	Voltage (V)
Username	String	Scalar	AOUT01

Figure 4.31: *Attribute Tab - Graphsettings*

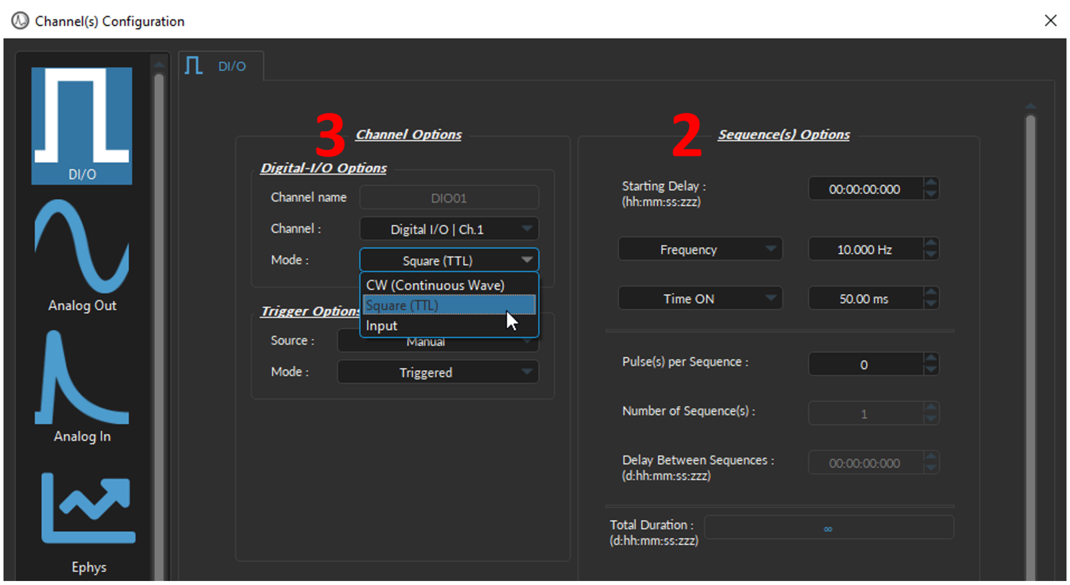
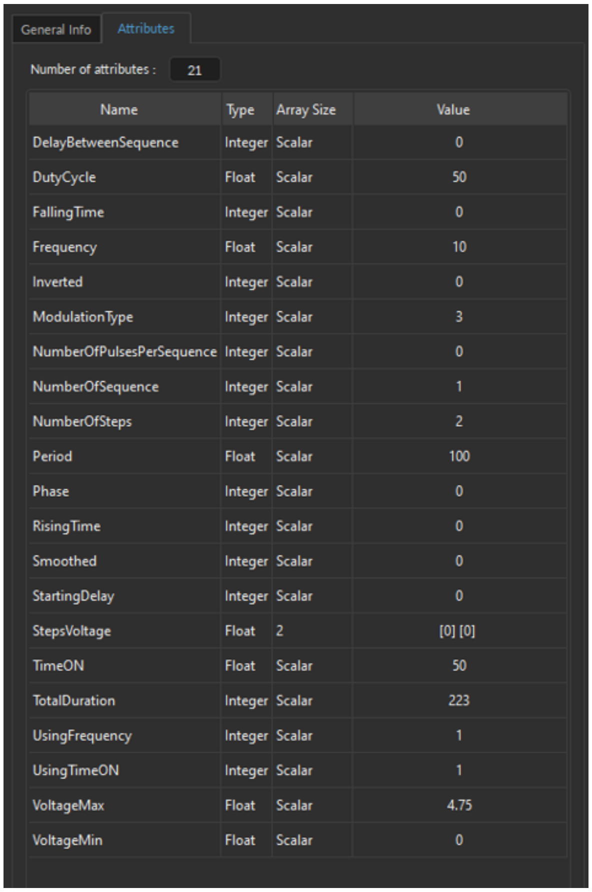


Figure 4.32: *Acquisition Console, Channel(s) Configuration window*

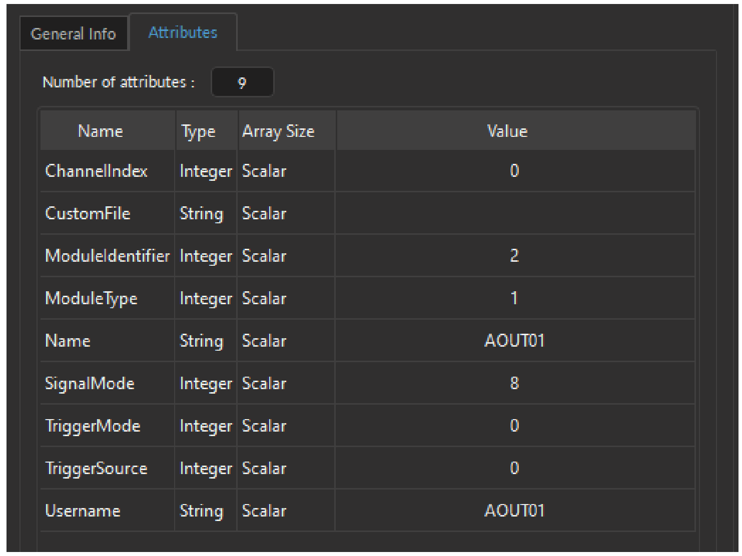


General Info | Attributes

Number of attributes : 21

Name	Type	Array Size	Value
DelayBetweenSequence	Integer	Scalar	0
DutyCycle	Float	Scalar	50
FallingTime	Integer	Scalar	0
Frequency	Float	Scalar	10
Inverted	Integer	Scalar	0
ModulationType	Integer	Scalar	3
NumberOfPulsesPerSequence	Integer	Scalar	0
NumberOfSequence	Integer	Scalar	1
NumberOfSteps	Integer	Scalar	2
Period	Float	Scalar	100
Phase	Integer	Scalar	0
RisingTime	Integer	Scalar	0
Smoothed	Integer	Scalar	0
StartingDelay	Integer	Scalar	0
StepsVoltage	Float	2	[0] [0]
TimeON	Float	Scalar	50
TotalDuration	Integer	Scalar	223
UsingFrequency	Integer	Scalar	1
UsingTimeON	Integer	Scalar	1
VoltageMax	Float	Scalar	4.75
VoltageMin	Float	Scalar	0

(a) *Modulation*



General Info | Attributes

Number of attributes : 9

Name	Type	Array Size	Value
ChannelIndex	Integer	Scalar	0
CustomFile	String	Scalar	
ModuleIdentifier	Integer	Scalar	2
ModuleType	Integer	Scalar	1
Name	String	Scalar	AOUT01
SignalMode	Integer	Scalar	8
TriggerMode	Integer	Scalar	0
TriggerSource	Integer	Scalar	0
Username	String	Scalar	AOUT01

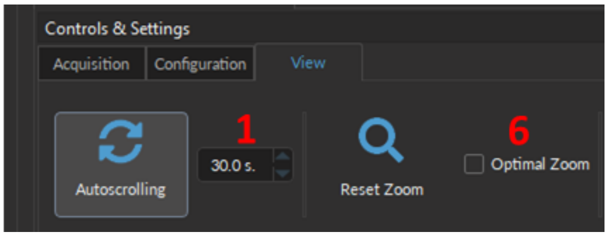
(b) *Settings*

Figure 4.33: *Attribute Tab, Channel-specific Settings - Analog Out example*

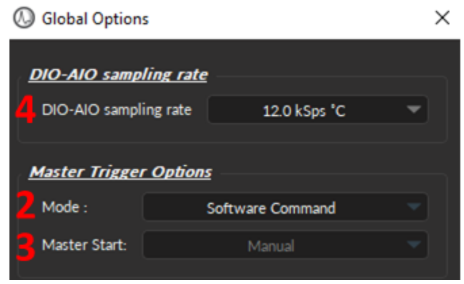
For more details on the workings of each attribute, see the *danse*^{TM} user manual, sections on the **Channel(s) Configuration** and the **Graph Options**.

4.4.2 Global Settings

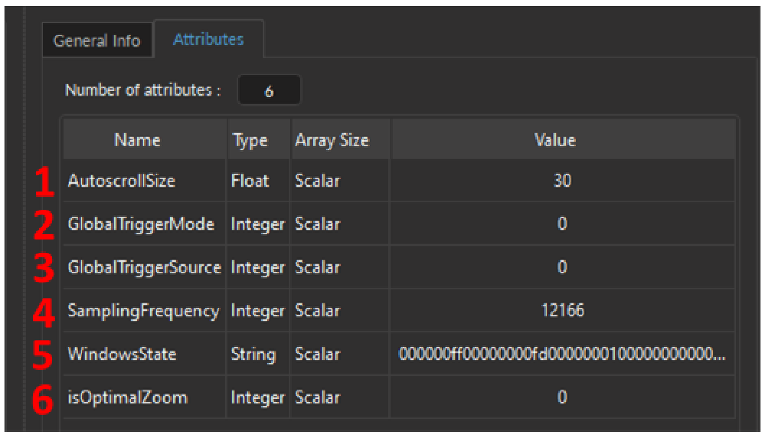
The **Global Settings** folder contains attributes from both the **View Tab** and **Global Options** window of the *Acquisition Console* module, as follow (Fig. 4.34c):



(a) *Acquisition Console, View Tab*



(b) *Acquisition Console, Global Options window*



General Info | Attributes

Number of attributes : 6

	Name	Type	Array Size	Value
1	AutoscrollSize	Float	Scalar	30
2	GlobalTriggerMode	Integer	Scalar	0
3	GlobalTriggerSource	Integer	Scalar	0
4	SamplingFrequency	Integer	Scalar	12166
5	WindowsState	String	Scalar	000000ff00000000fd0000000100000000000...
6	isOptimalZoom	Integer	Scalar	0

(c) *Doric File Editor, Attribute Tab*

Figure 4.34: *Configurations, Global Settings*

1. The **Autoscroll Size** attribute (Fig. 4.34c, 1) contains the user-specified value set in the **View** tab of the *Acquisition Console Module* in Fig. 4.34a, 1.

2. The **Global Trigger Mode** attribute (Fig. 4.34c, 2) contains the user-specified value set in the **Global Options** window in the *Acquisition Console Module* in Fig. 4.34b, 2.

3. The **Global Trigger Source** attribute (Fig. 4.34c, 3) contains the user-specified value set in the **Global Options** window in the *Acquisition Console Module* in Fig. 4.34b, 3.

4. The **Sampling Frequency** attribute (Fig. 4.34c, 4) contains the user-specified value set in the **Global Options** window in the *Acquisition Console Module* in Fig. 4.34b, 4.

5. The **Window State** attribute (Fig. 4.34c, 5) is a code that saves the channel graph set-up in the **Acquisition View** of the *Acquisition Console Module*. Sliding individual channel windows and changing how the input and output tabs are organized changes this code, which is also saved in the configuration file. Loading a configuration file will automatically reset the **Acquisition View** as it was when the configuration file was saved.

6. The **isOptimalZoom** attribute (Fig. 4.34c, 6) contains the user-specified value set in the **View** tab of the *Acquisition Console Module* in Fig. 4.34a, 6. This value is binary: 0 when the checkbox is unchecked, and 1 when it is checked.

For more details on the workings of each attribute, see the *danse*^{TM} user manual, sections on the **Global Options** and the **View** tab.

4.4.3 Saving Settings

The **Saving Settings** folder contains the following attributes (Fig. 4.35a):

1. The **Decimation Enabled** attribute (Fig. 4.35a, 1) contains the user-specified value set in the **Saving Parameters** window from the *Acquisition Console Module* in Fig. 4.35b, 1. This value is binary: 0 when the checkbox is unchecked, and 1 when it is checked.

2. The **Decimation Factor** attribute (Fig. 4.35a, 2) contains the user-specified value set in the **Saving Parameters** window from the *Acquisition Console Module* in Fig. 4.35b, 2. The default value of the decimation factor is 1.

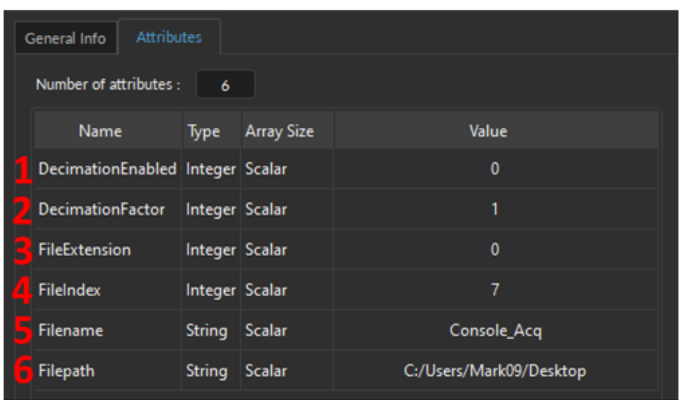
3. The **File Extension** attribute (Fig. 4.35a, 3) is always set to 0 since DNSv6 only offers *.doric* format in Fig. 4.35b, 3.

4. The **File Index** attribute (Fig. 4.35a, 4) contains the user-specified value set in the **Saving Parameters** window from the *Acquisition Console Module* in Fig. 4.35b, 4.

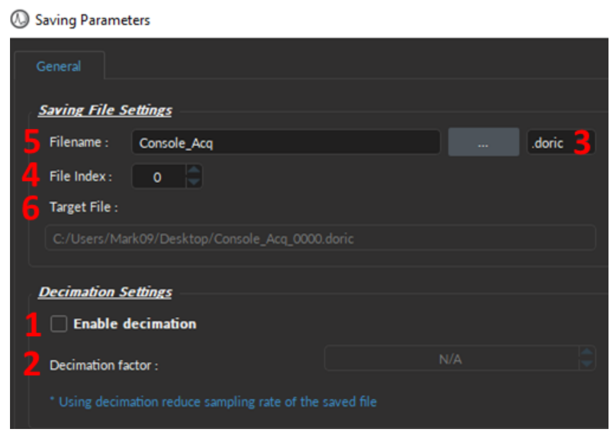
5. The **File Name** attribute (Fig. 4.35a, 5) contains the user-specified string set in the **Saving Parameters** window from the *Acquisition Console Module* in Fig. 4.35b, 5.

6. The **File path** attribute (Fig. 4.35a, 6) contains the generated **Target File** path created once the user selected the folder where the data will be saved in the **Saving Parameters** window from the *Acquisition Console Module* in Fig. 4.35b, 6.

For more details on the workings of each attribute, see the *danse*TM user manual, sections on the **Saving Options**.



(a) *Doric File Editor, Attribute Tab*



(b) *Acquisition Module, Saving Parameters window*

Figure 4.35: *Configurations, Saving Settings*

4.4.4 Time Series Settings

The **Time Series Settings** folder contains the following attributes (Fig. 4.36a):

1. The **Interval Between Series (ms)** attribute (Fig. 4.36a, 1) contains the user-specified value set in the **Time Series** window from the *Acquisition Console Module* in Fig. 4.36b, 1.

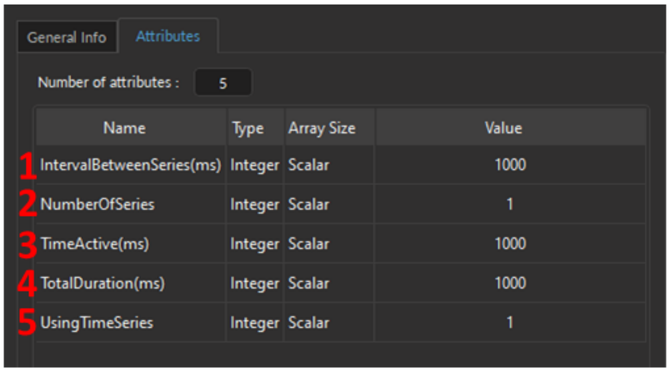
2. The **Number of series** attribute (Fig. 4.36a, 2) contains the user-specified value set in the **Time Series** window from the *Acquisition Console Module* in Fig. 4.36b, 2.

3. The **Time Active (ms)** attribute (Fig. 4.36a, 3) contains the user-specified value set in the **Time Series** window from the *Acquisition Module* in Fig. 4.36b, 3.

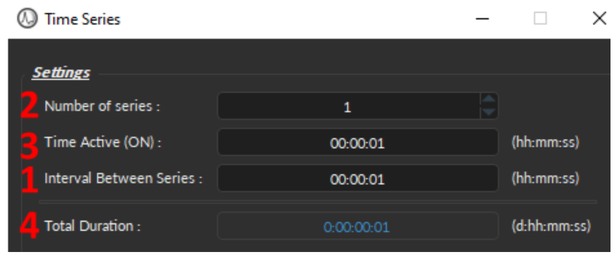
4. The **Total Duration (ms)** attribute (Fig. 4.35a, 4) contains the calculated value from the **Time Series** window from the *Acquisition Console Module* in Fig. 4.36b, 4.

5. The **Using Time Series** attribute (Fig. 4.36a, 5) will be 1 when the **Master Trigger Options** mode is set to **Time Series** in the **Global Options** window (Fig. 4.34b, 2) and 0 when set to any other mode.

For more details on the workings of each attribute, see the *danse*™ user manual, sections on the **Time Series** window and **Global Options** window.



(a) *Doric File Editor, Attribute Tab*



(b) *Acquisition Module, Time Series window*

Figure 4.36: *Configurations, Time Series Settings*

4.5 Data Acquisition Structure

The **Data Acquisition** folder is the meat of the file structure, where the raw data is stored in **Datasets**. These **Datasets** are organized into nested groups based on the **Device**, **Data Type**, **Time Series**, and **Sensor / Channel Type** (Fig. 4.37). Each dataset within the **Sensor / Channel Type** folder will be named based on the **Sensor / Channel number** (*e.g.* AOUT01, DIO03, ROI 5, Keypress01, Sensor1, Cam342093402, etc.), unless the channels were re-labeled by the user before acquisition.

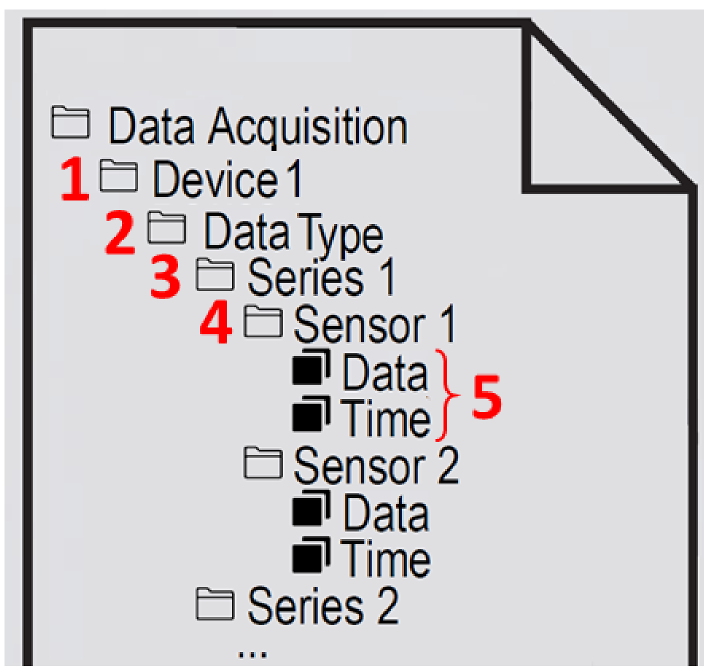
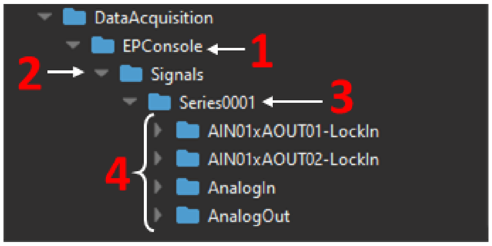


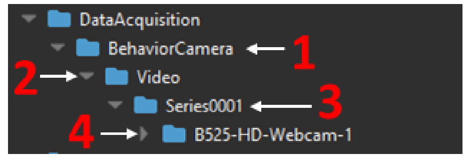
Figure 4.37: *Schematic of the Structure of a .doric file*

The following are the nested branches of the **Data Acquisition** folder (Fig. 4.37):

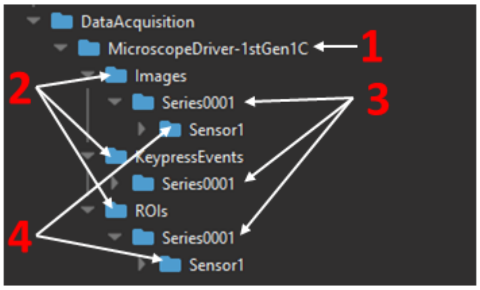
1. The **Device** folder (Fig. 4.37, 1) is the name of the equipment used to record the data, such as *FP/EP Console*, *BFPD* or *Behaviour Camera* to name a few. When multiple **Devices** are used during a recording, each will have a separate folder (Fig. 4.24a).



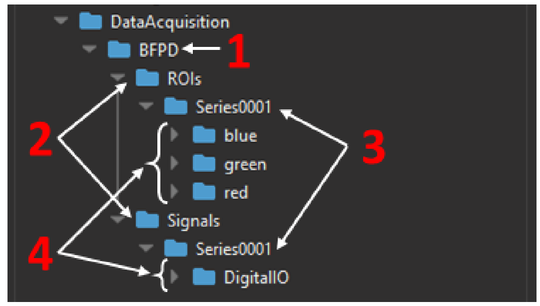
(a) *Console*



(b) *Behaviour Camera*



(c) *Microscope*



(d) *BFPD*

Figure 4.38: *Data Acquisition structure examples*

2. The **Data Type** folder (Fig. 4.37, 2) is named based on the kind of data that the **Device** recorded:

    - *Signals* (Figs. 4.38a, 4.38c & 4.38d) - vector data from either *Analog* or *Digital Inputs/Outputs*. This **Data Type** is used for LED triggers, Fiber Photometry & Electrophysiology systems.
    - *ROIs* (Fig. 4.38c & 4.38d) - vector data from ROIs in a video recording. This **Data Type** is used for Bundle Fiber Photometry and Fluorescent Microscopy.
    - *Keypress Events* (Fig. 4.38c) - vector data from keyboard inputs.
    - *Images* (Fig. 4.38c)- video data from the Fluorescent Microscopy Systems.
    - *Video* (Fig. 4.38b) - video data from a behavior camera or webcam.

    When multiple **Data Types** are used during a recording, each will have a separate folder (Figs. 4.38c & 4.38d, 2).

3. The **Series 0001** folder (Fig. 4.37, 3) is contained within a **Data Type** folder. By default, even if **Time Series** is not enabled, the entire recording is stored within **Series 0001** (of one). When **Time Series** is enabled, multiple **Series #** folders will be created one after another, for each **Data Type** and **Device** folders (Fig. 4.39). The structure within each repeated **Series** will be identical (*i.e.* Series0001, 0002, 0003...) when inside the same **Data Type** folder, but not between them. E.g. *Keypress Event* series and *Signal* series structure will be different (Fig. 4.40).

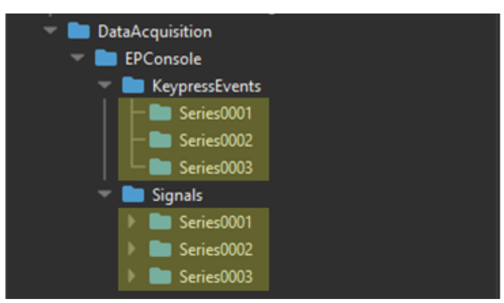
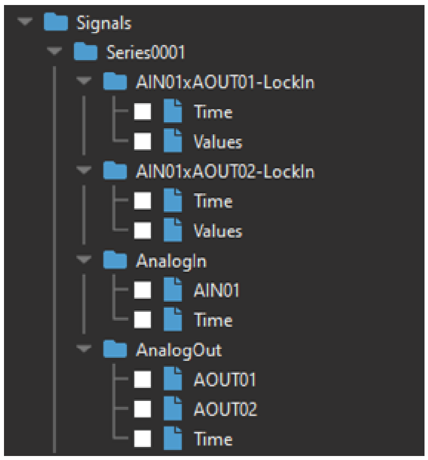


Figure 4.39: *Time Series structure*

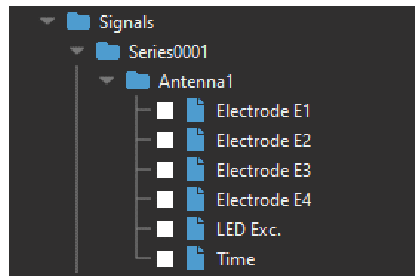
4. The **Channel / Sensor** folders (Fig. 4.37, 4) are the next set of branches within the **Series 0001** folder. If multiple **Channel / Sensor Types** are used, each will have its own branch, whose name will be the **Channel / Sensor Type** (e.g. AnalogIn, DigitalOut, Antenna1, KeypressEvent1, etc; Fig. 4.38a, 4) or user-specified names (Fig. 4.38d, 4).

5. The **Dataset** branches (Fig. 4.37, 5) are identified by their document button and contain the raw data. Every **Channel / Sensor** folder will at least have a *Signal / Value* **Dataset** (Fig. 4.40) and often a *Time* **Dataset** too (Figs. 4.40a, 4.40b & 4.40d, but not 4.40c). The **Dataset** names are commonly the name of the channel/sensor itself + an index value (if there are multiple channels), such as Fig. 4.40b. However, if the user specified a name for the **Channel / Sensor** within the software, that name is used instead. The raw data is stored within the **General Info** tab, under the **Dataset** box (Section 4.5.1), while the attributes related to the **Datasets** are located within the **Attributes** tab (Section 4.5.3).

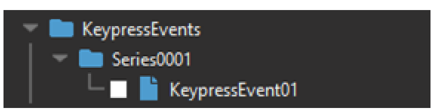
NOTE: The following **Group** type folders: **Data Acquisition**, **Device**, **Data Type**, **Series**, and **Channel Type** do NOT have any **Attributes** assigned to them, but do have **General Info** as per Section 4.3.1.



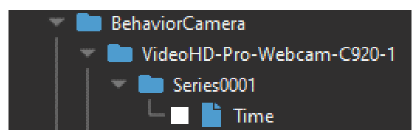
(a) *Analog In & Out (Lock-In)*



(b) *Antenna*



(c) *Keypress Event(s)*



(d) *Video*

Figure 4.40: *Data Acquisition, Dataset structure*

4.5.1 Dataset Viewer

To view the data stored within a **Dataset**, select it from the **File Manager** and click on the **General Info** tab (Fig. 4.25). In addition to having a **General** box (Section 4.3.1), **Dataset** have a **Dataset** box (Fig. 4.41), which displays the parameters and the button required to view either a subset or the entire raw data.

Note that video data from webcams/behavior cameras are the only data type not directly stored inside a **Datasets** element. Instead, the **Attribute** tab stores the path where the raw footage is saved (Section 4.5.3).

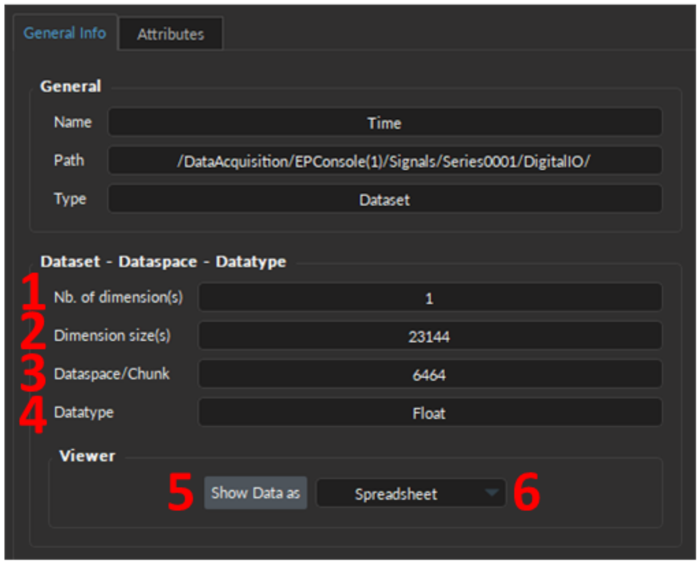


Figure 4.41: *General Info tab, Dataset Viewer*

The following elements are contained within the **Dataset Info** box (Fig. 4.41):

1. The **Number of dimension(s)** (Fig. 4.41, 1) describes the number of dimensions that form the dataset. A *vector* has a dimension of 1, *images Stack* and *video* have a dimension of 3.

2. The **Dimension size(s)** (Fig. 4.41, 2) describes the length of the dataset (*i.e.* the number of rows) for *vector* data. For *images Stack* and *video* it is the width x height x # frames.

3. The **Dataspace/Chunk** (Fig. 4.41, 3) is the value used to reduce the data when displaying it in the **Data Viewer** (Fig. 4.43) using the **Spreadsheet Chunked** setting (Fig. 4.41, 6). This setting speeds up the data loading process, which is particularly useful for quickly checking the values. The software automatically generates the **Dataspace/Chunk** value based on the dataset's size.

4. The **Datatype** (Fig. 4.41, 4) displays what kind of numerical value is used within the dataset (e.g. float, integers, etc.). The *float* type is the most common of *Doric Lenses* acquisition devices. *Integers* are used for images and videos.

5. The **Show Data as** button (Fig. 4.41, 5) opens the **Data Viewer** window (Fig. 4.43) for a quick view of the selected dataset. Section 4.5.2 describes the features of the window.

6. The **Spreadsheet** drop-down list (Fig. 4.41, 6) allows users to select the viewing mode, including:

   - *Spreasheet*- displays the entire dataset in the **Data Viewer** window (Fig. 4.42 & 4.43). Note this mode is only available for vector data.
   - *Spreadsheet Chunked* - displays a truncated dataset in the **Data Viewer** window (Fig. 4.42), which is cut-off at the Chunked value, specified in the **Dataspace/Chunk** (Fig. 4.42, 6) parameter. Images opened using this mode will view a matrix of pixel values instead of a picture.
   - *Image* - displays the raw images in the **Dataset Viewer** window (Fig. 4.43).

4.5.2 The Data Viewer Window

Depending on the **Data Type**, different features are available within the **Data Viewer** window.

For *vector* data (Fig. 4.42), a column vector with the number of rows matching either the **Dimension size(s)** (Fig. 4.41, 2) or the **Dataspace/Chunk** (Fig. 4.41, 3) parameter. The column header will always be the **Name** (Fig. 4.26, 1) of the **Dataset**. Note the **Frame Control** box (Fig. 4.43, 1) is inactive for data with a **Number of Dimension(s)** lower than three.

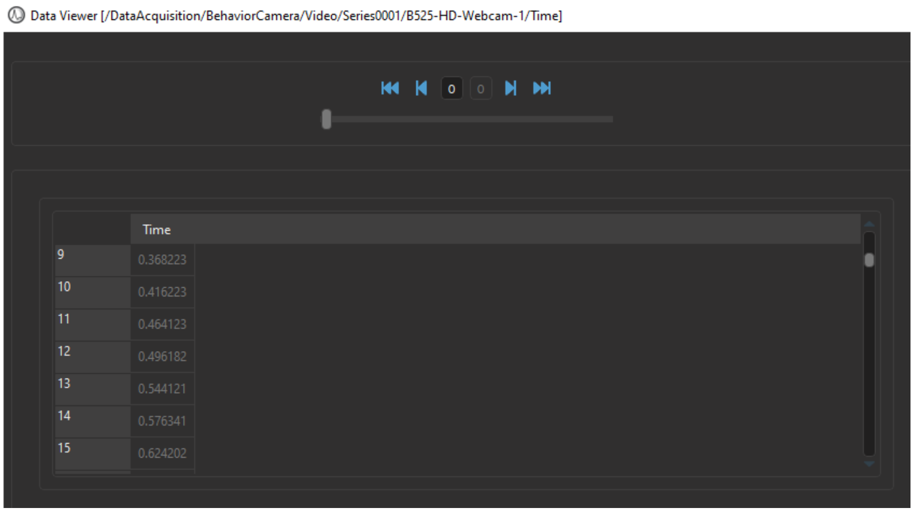


Figure 4.42: *Dataset Viewer: vector data*

For *image* data (Fig. 4.43):

1. The **Frame Control** box allows users to move easily between frames. The **Double Arrow** buttons will automatically hop to either the beginning or the end of the frame sequence, while the **Single Arrow** buttons will shift the frame by 1. The left text box displays the **Current Frame Number** and the right text box displays the **Total Frame Number**. The **Slider** allows users to quickly scroll between frames.

2. The **Image Options** box displays the following:

   a) The **Min. Count** (Fig. 4.43, 2a) sets the minimum pixel value of the gradient that will be displayed in the image. Thus, all pixel values lower than the **Min. Count** will be displayed as the minimum pixel value color.

   b) The **Max. Count** (Fig. 4.43, 2b) sets the maximum pixel value of the gradient that will be displayed in the image. Thus, all pixel values higher than the **Max. Count** will be displayed as the maximum pixel value color.

   c) The **Gradient** (Fig. 4.43, 2c) sets the type of color gradient the pixel values will represent in the image below. Twelve gradient options are available, including *Grayscale*, *Thermal*, and *Spectrum* to name a few.

   d) The **Cursor Coordinates** (Fig. 4.43, 2d) displays the x and y coordinates, and the pixel value of the cursor within the image (Fig. 4.43, 3 - arrow).

   e) The **Timestamp** text-box (Fig. 4.43, 2e) displays the time associated with the image (Fig. 4.43, 3). Changing the **Current Frame Number** using the **Frame Controls** (Fig. 4.43, 1) will automatically change the value of the **Timestamp**.

3. The **Raw Image**

   f) The **X & Y Axes** of the image (Fig. 4.43, 3f) displays the scale and resolution of the image. The axes begin at 0 and end at the value of the image pixel width and height (respectively) corresponding to the **Dimension size(s)**. The x and y coordinate of the cursor within the image will always be within this range of values (Fig. 4.43, 2d).

   g) The **Gradient bar** (Fig. 4.43, 3g) displays the color associated with the pixel value (Count(s)).

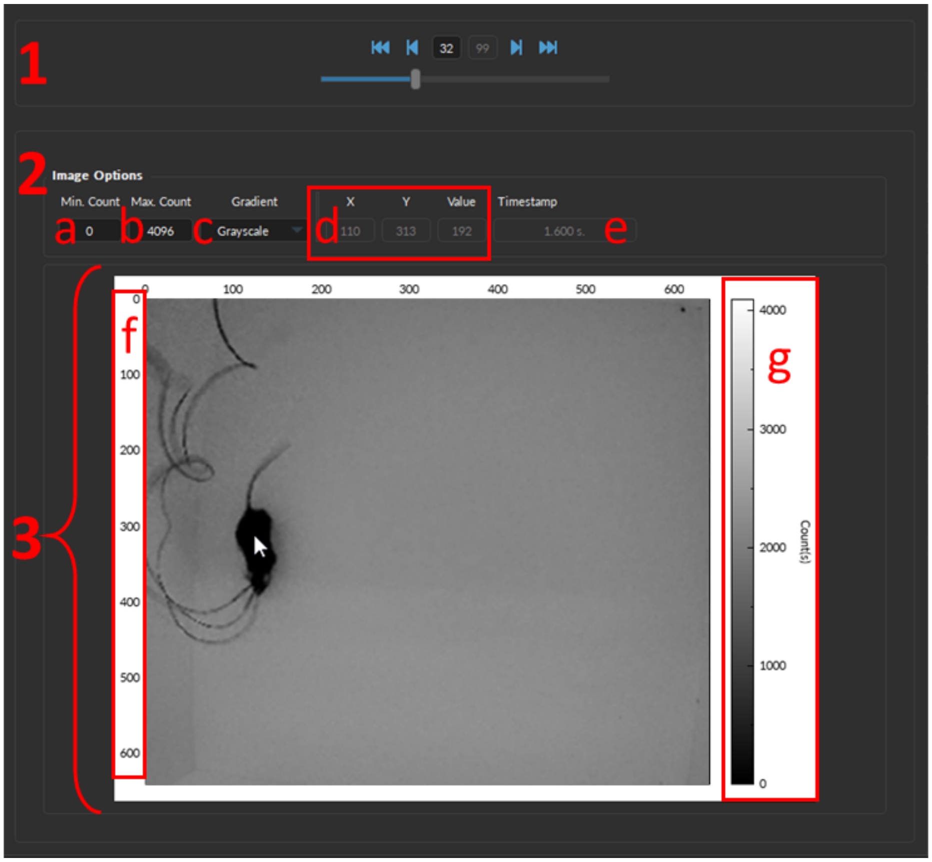


Figure 4.43: *Dataset Viewer: image data*

4.5.3 Dataset Attributes

Most **Dataset** (besides Time) store parameters within the **Attributes** tab. These attributes store parameters related to the value the data can take (such as Bit number, Min and Max range, unit, etc.) and, thus, the list of attributes included within the tab depends on the **Channel / Sensor Type** itself. Since the attribute names are self-explanatory, below are examples of different attributes based on their **Channel / Sensor Type**.

Digital & Analog In/Out Attributes:

Name	Type	Array Size	Value
Name	String	Scalar	AIN01xAOUT01-LockIn
RangeMax	Float	Scalar	10
RangeMin	Float	Scalar	-10
Unit	String	Scalar	Voltage (V)
Username	String	Scalar	AIN01xAOUT01-LockIn

Figure 4.44: *Digital & Analog In/Out Attributes*

Keypress Event(s) Attributes:

Name	Type	Array Size	Value
Color	String	Scalar	#56d9e8
Duration	String	Scalar	00:00:00
Index	Integer	Scalar	1
Information	String	Scalar	
Shortcut	String	Scalar	Space
Type	String	Scalar	Single
Username	String	Scalar	Keypress #01

Figure 4.45: *Keypress Attributes*

Images Attributes:

Name	Type	Array Size	Value
BitsCount	Integer	Scalar	10
Format	Integer	Scalar	28
Height	Integer	Scalar	600
Width	Integer	Scalar	600

Figure 4.46: *Images (Microscopy) Attributes*

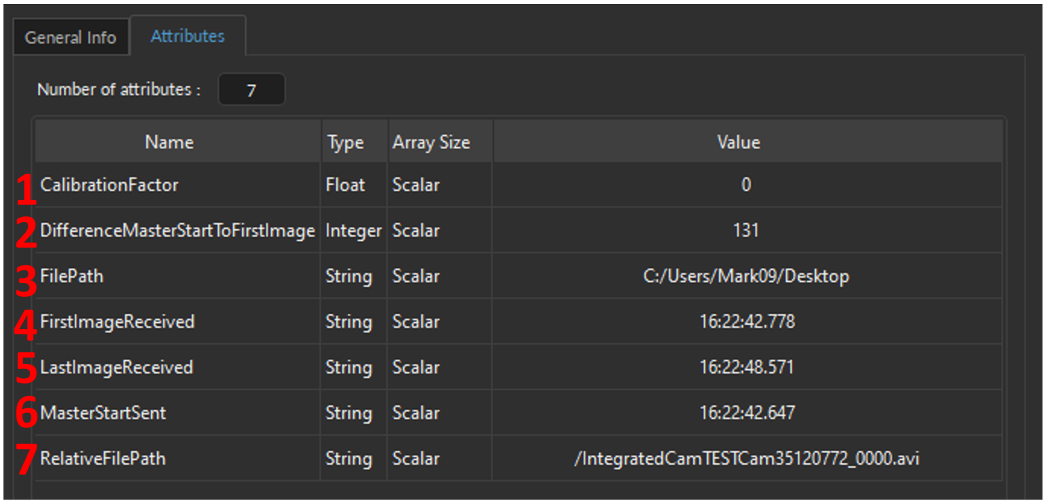
ROI Attributes:

Name	Type	Array Size	Value
BitsCount	Integer	Scalar	16
Username	String	Scalar	ROI 1 - (CAM1 EXC1)

Figure 4.47: *ROI Attributes*

Video Attributes:

When recording behavior data using a webcam or other external (non-Doric) cameras, the video files are stored separately from the *.doric* file. However the **File Path** (Fig. 4.48, 3) of that folder and the name of the raw video footage (**Relative File Path**; Fig. 4.48, 7) are stored within the attribute. The timestamps of when the **First** and **Last Image Received** are also displayed (Fig. 4.48, 4-5).



General Info | Attributes

Number of attributes : 7

	Name	Type	Array Size	Value
1	CalibrationFactor	Float	Scalar	0
2	DifferenceMasterStartToFirstImage	Integer	Scalar	131
3	FilePath	String	Scalar	C:/Users/Mark09/Desktop
4	FirstImageReceived	String	Scalar	16:22:42.778
5	LastImageReceived	String	Scalar	16:22:48.571
6	MasterStartSent	String	Scalar	16:22:42.647
7	RelativeFilePath	String	Scalar	/IntegratedCamTESTCam35120772_0000.avi

Figure 4.48: *Video Attributes*

Since the **Video** folder attributes are significantly different and values from this folder will be likely used during data analysis, the following describes these attributes in greater detail:

1. The **Calibration Factor** (Fig. 4.48, 1) stores the value of the conversion factor used to convert pixel dimension to real length measures (mm, cm or inches). This value is required for when using the **Animal Tracking** algorithm in the **Behavior Analyzer** module.

2. The **Difference Master Start to First Image** (Fig. 4.48, 2) contains the calculated difference between when the Master Start timestamp, which was sent to trigger the camera, and the First Image Received timestamp. This value is useful when using the **Web Camera** module, which doesn't have *External TTL* mode to synchronize the camera and recording software. This value can be used to align the behavior and neural activity data together when doing data analysis.

3. The **File Path** (Fig. 4.48, 3) contains the directory where both the *.doric* file and the video file are saved.

4. The **First Image Received** (Fig. 4.48, 4) contains the timestamp (hh:mm:ss:zzz) when the first frame was obtained from the camera.

5. The **Last Image Received** (Fig. 4.48, 5) contains the timestamp (hh:mm:ss:zzz) when the last camera frame was obtained.

6. The **Master Start Sent** (Fig. 4.48, 6) contains the timestamp (hh:mm:ss:zzz) when the **Record** button was selected.

7. The **Relative File Path** (Fig. 4.48, 7) is the name of the behavior video file itself (in .avi).

5 Light Sources

Doirc Neuroscience Studio expands the *Doric* **Light Sources** control options. All Light Source Drivers, including *LED Modules*, *Laser Diode Modules* and ⋆*LISER*^{TM} *Light Source*[1], are compatible with the software.

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.

5.1 Channel Configuration Window Overview

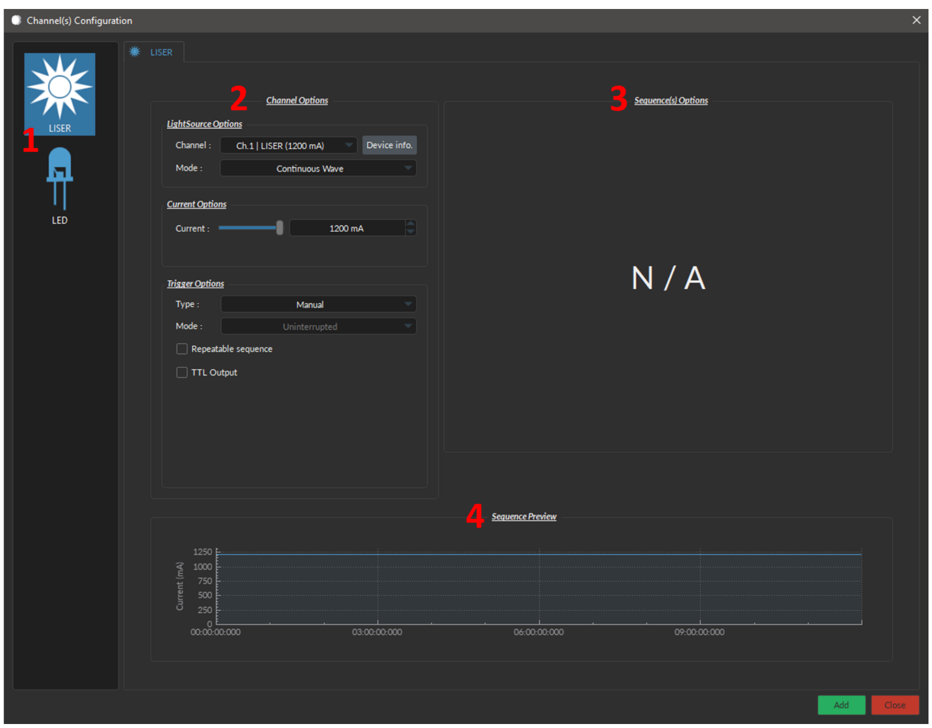


Figure 5.1: *Channels Configuration Main Interface*

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 5.1 that are defined below.

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

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.

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 5.1.1.

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 5.1.2.

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.

5.1.1 Channel Options Section

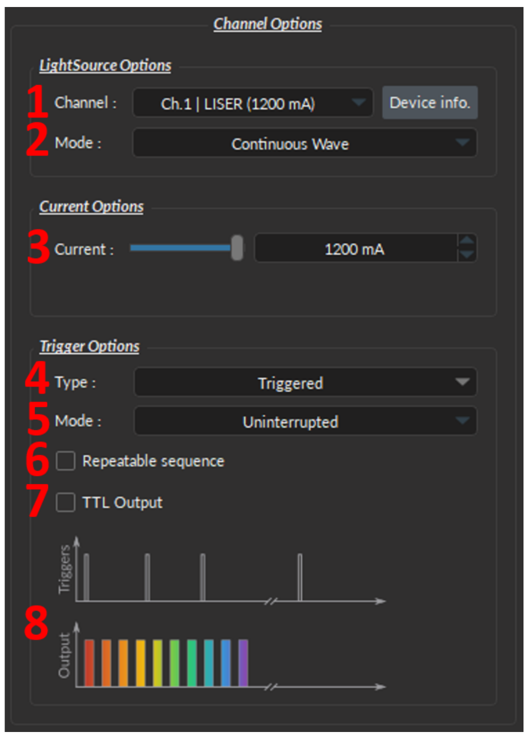


Figure 5.2: *Channel Options of the Channel Configuration Window*

The Channel Option section (Fig. 5.2) is separated into 3 sub-sections, the **LightSource Options** section that defines the channel and its mode, the **Current Options** and the **Trigger Options** section that controls the trigger method of the selected channel.

5.1.1.1 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 5.1.2.

5.1.1.2 Current Options

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

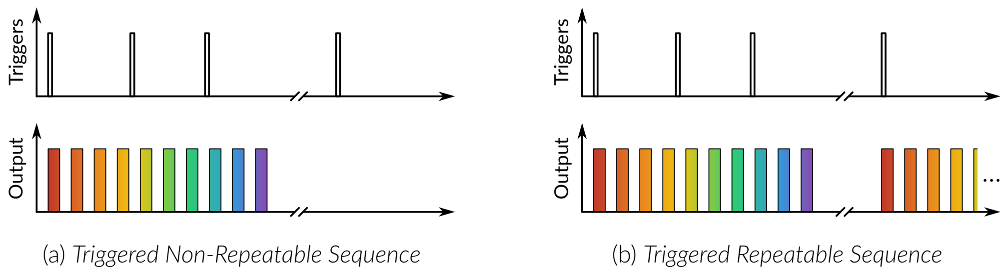
   - When using some *LED* module, the **Overdrive** checkbox will appear. When selected, this allows the system to exceed the normal safe current limit of the light source. **THIS SHOULD ONLY BE USED WITH PULSED SIGNALS, AS IT CAN OTHERWISE DAMAGE THE LIGHT SOURCE.**
   - When using a *CLED* module, the **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.

5.1.1.3 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 5.7.

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 5.7 shows the different available combinations for the different Trigger Modes. Four Modes are available and are the following:

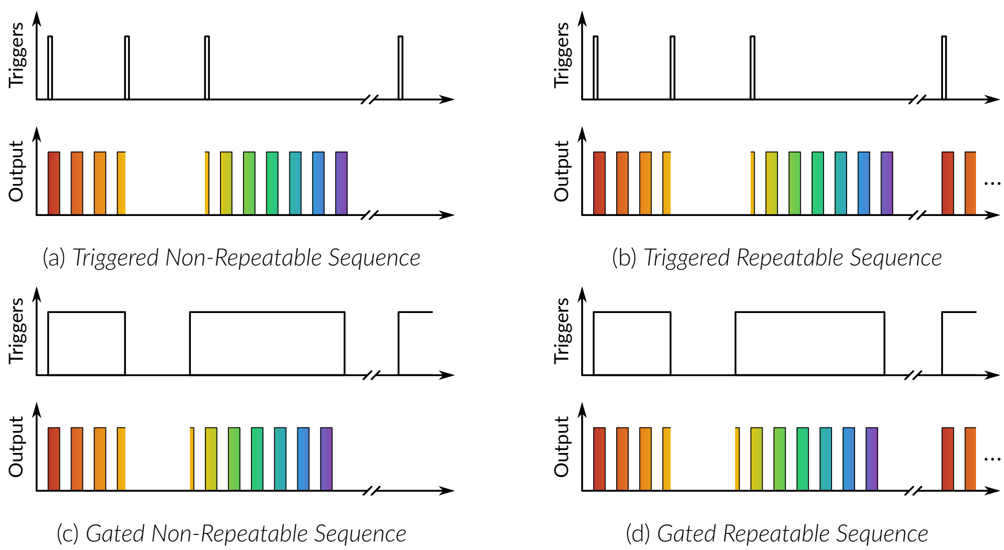
   - **Uninterrupted**: This mode activates the channel sequence when an input greater than 3.3 V is detected by the BNC input. Following input pulses will be ignored while the sequence is running (Fig. 5.3). When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Fig. 5.3b). 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. 5.4). Following input pulses (when *Triggered*, Fig. 5.4a) or falling edge (when *Gated*, Fig. 5.4c) 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. 5.4b and 5.4d).
   - **Continue**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 5.5). The following input pulse (when *Triggered*, Fig. 5.5a) or a falling edge (when *Gated*, Fig. 5.5c) 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. 5.5b and 5.5d).
   - **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. 5.6a) or falling edge (when *Gated*, Fig. 5.6b) 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 5.3: *Uninterrupted Sequence Mode*



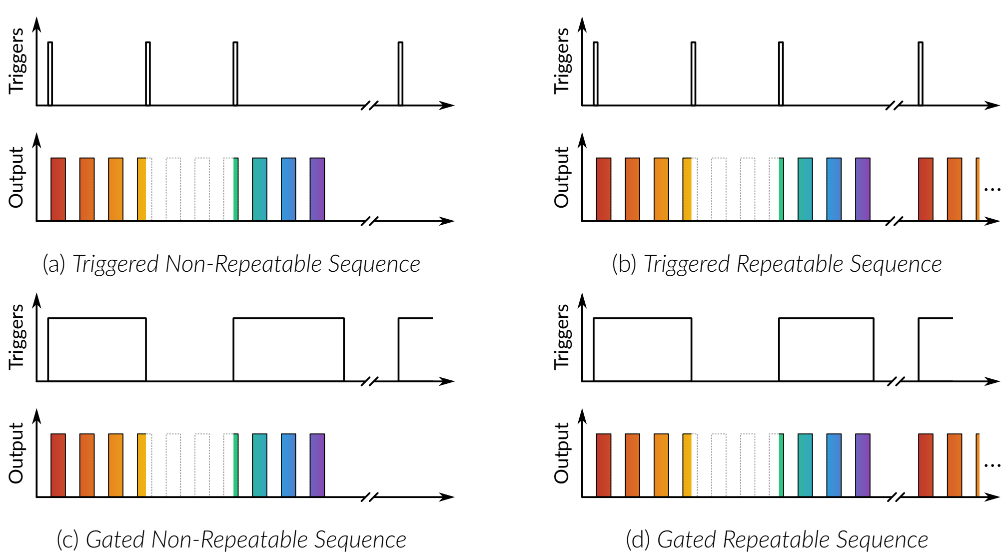
(a) *Triggered Non-Repeatable Sequence*

(b) *Triggered Repeatable Sequence*

(c) *Gated Non-Repeatable Sequence*

(d) *Gated Repeatable Sequence*

Figure 5.4: *Pause Sequence Mode*



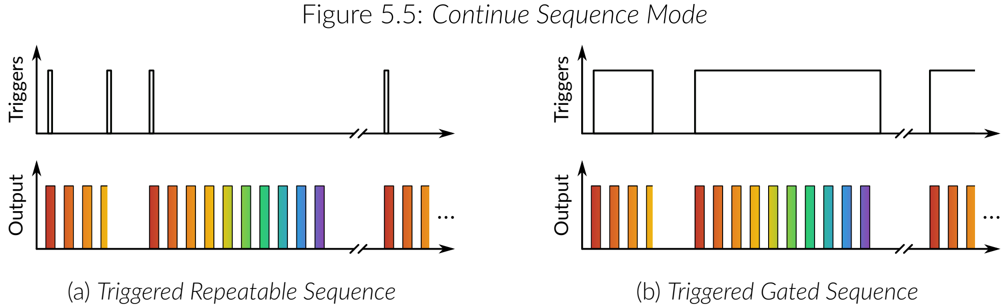
(a) *Triggered Non-Repeatable Sequence*

(b) *Triggered Repeatable Sequence*

(c) *Gated Non-Repeatable Sequence*

(d) *Gated Repeatable Sequence*

Figure 5.5: *Continue Sequence Mode*



(a) *Triggered Repeatable Sequence*

(b) *Triggered Gated Sequence*

Figure 5.6: *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 5.7 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 5.7: *Trigger options possibilities*

5.1.2 Sequence(s) Options Section

5.1.2.1 Continuous Wave

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

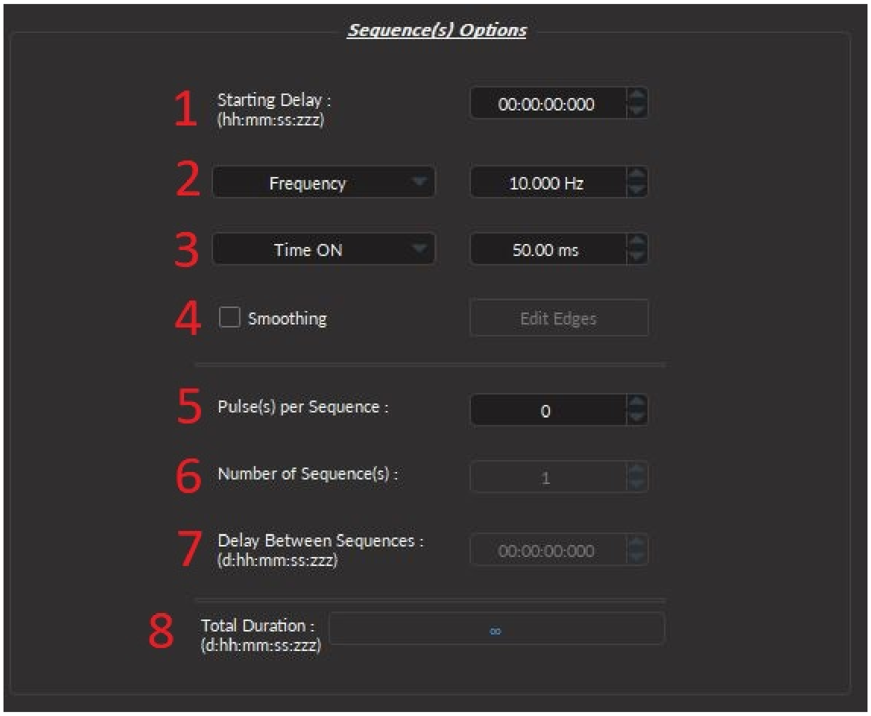
5.1.2.2 External TTL

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.

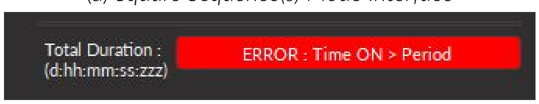
5.1.2.3 External analog

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.

5.1.2.4 Square Sequence(s)



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



(b) *Exemple of Error*

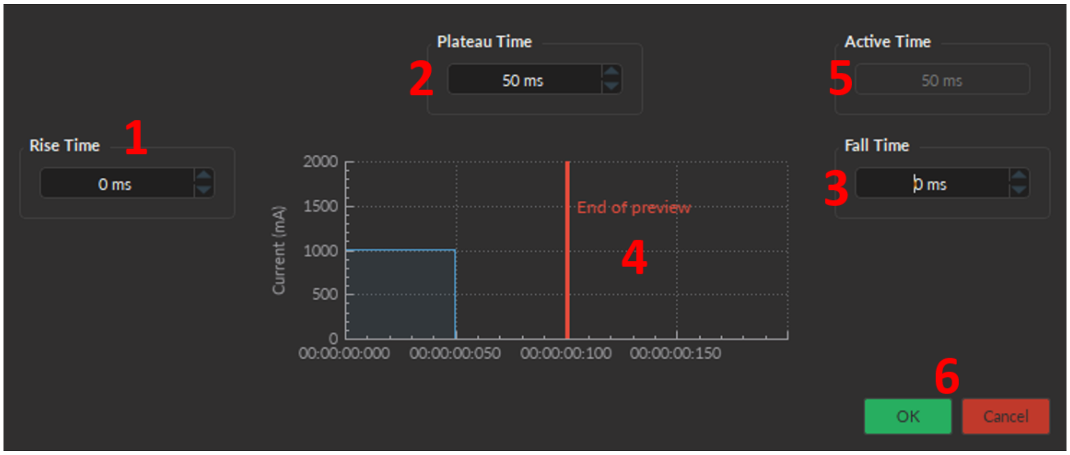
Figure 5.8: *Sequence Options of the Square Sequence(s) Mode.*

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 5.8a and are explained below.

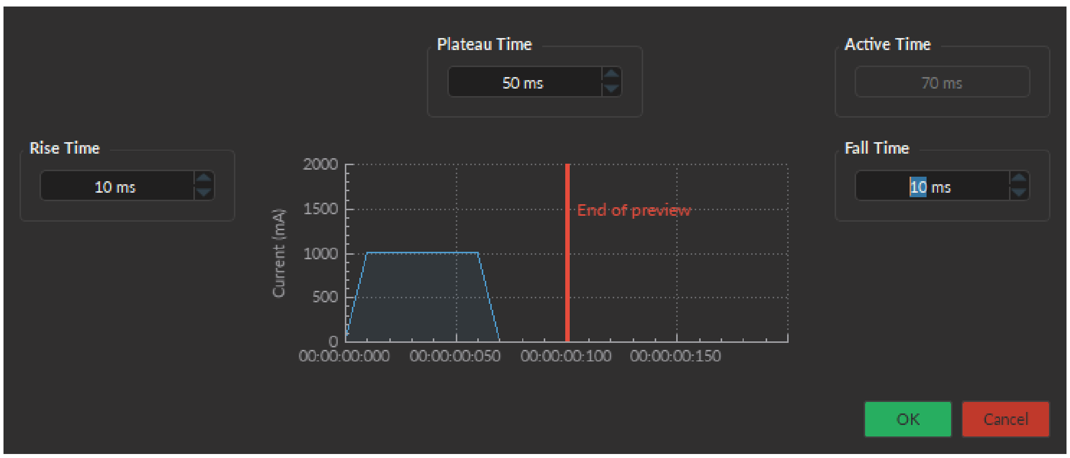
1. The **Starting Delay** defines the time between the activation of the pulse sequence and the beginning of the first light illumination.
2. The **Frequency** sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period**. For example, a light 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 5.8b).

5.1.2.5 Smoothing Edge(s)

The Smoothing Edge(s) window (Fig. 5.9) 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 5.9: *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.

5.1.2.6 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. 5.10).

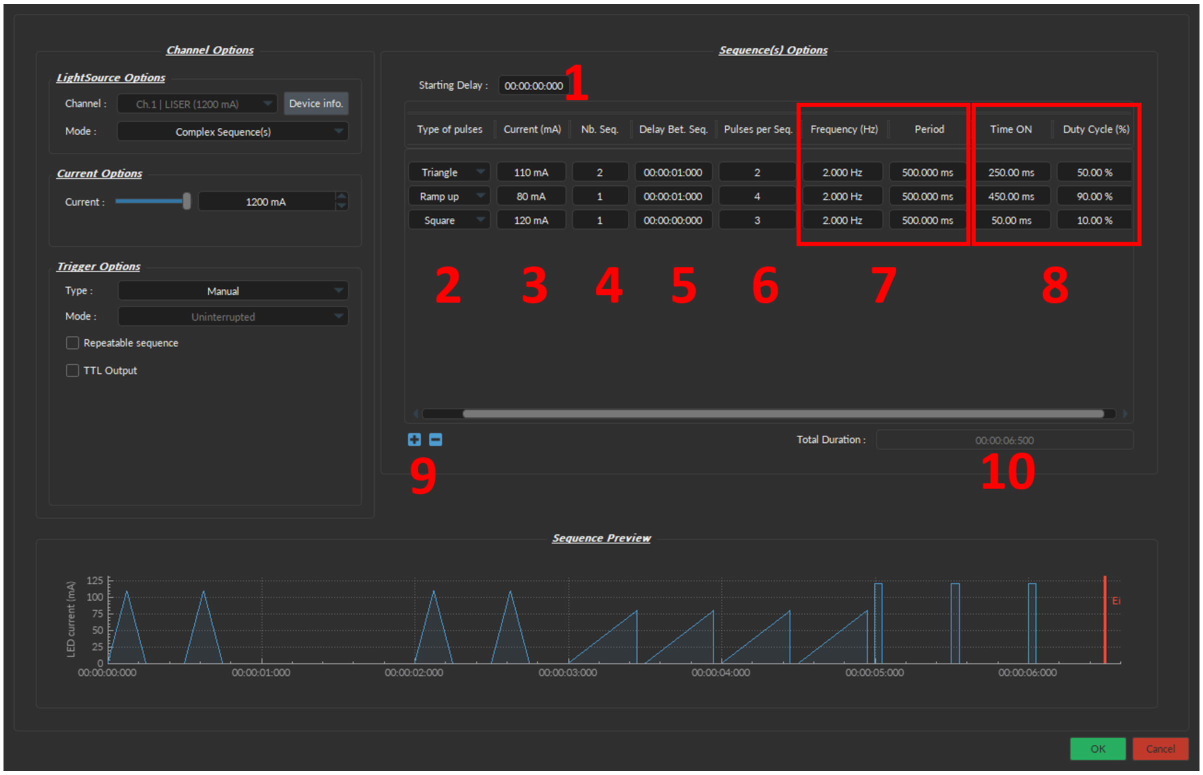


Figure 5.10: *Complex Sequences Window*

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**.

5.1.2.7 Custom File Sequence(s)

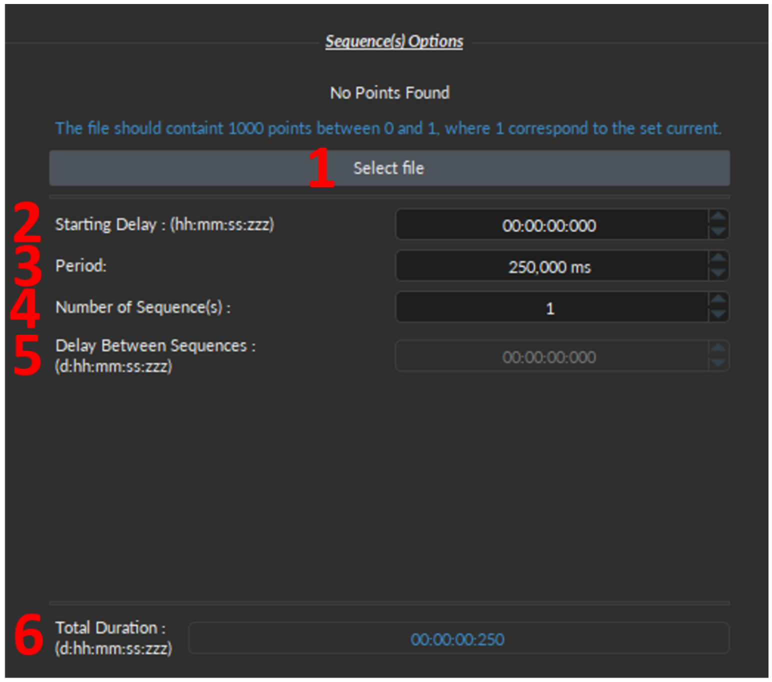


Figure 5.11: *Custom File Sequence*

1. The **Select file** button allows to import a CSV file with 1000 values between 0 and 1 contained in one column. Notes:
   - If the file contains less than 1000 values, the missing data will be replaced by 0 to reach the 1000 values.
   - If the file contains more than 1000 values, the extra values will be ignored.
   - If the file contains negative values, they will be set to 0.
   - If the file contains values greater than 1, they will be reduced to 1.
2. The **Starting Delay** (Fig. 5.11) sets the delay (in hh:mm:ss:zzz format) before the first pulse sequence.
3. The **Period** (Fig. 5.11) sets the period (in ms) for the pulse sequence contained in the file.
4. The **Number of Sequences** (Fig. 5.11) sets the number of times that the sequence will be repeated, with a minimum of 1.
5. The **Delay Between Sequences** (Fig. 5.11) sets the delay (in hh:mm:ss:zzz format) between each sequence if the **Number of Sequences** is greater than 1.
6. The **Total Duration** (Fig. 5.11) displays the total time of the experiment. The displayed values can be *Inf* for infinite or a valid time value.

5.1.3 Preview

The **Preview** box (Fig. 5.1, number 4) displays a preview of the chosen sequence while in the **Continuous Wave**, **Square Sequences**, **Complex Sequences** and **Custom File Sequence(s)** mode.

5.2 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.

5.2.1 Acquisition Tab

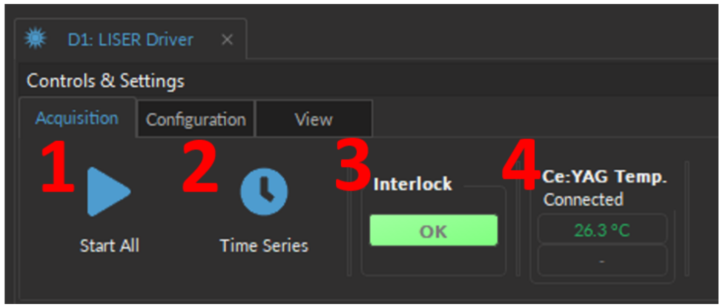


Figure 5.12: *Acquisition Tab*

The different buttons of the **Acquisition Tab** are shown in Figure 5.12 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. 5.13). This tool allows all channels to share the same timing.
3. The **Interlock** indicator displays 

 when the interlock is correctly connected, and 

 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.

5.2.1.1 Time Series

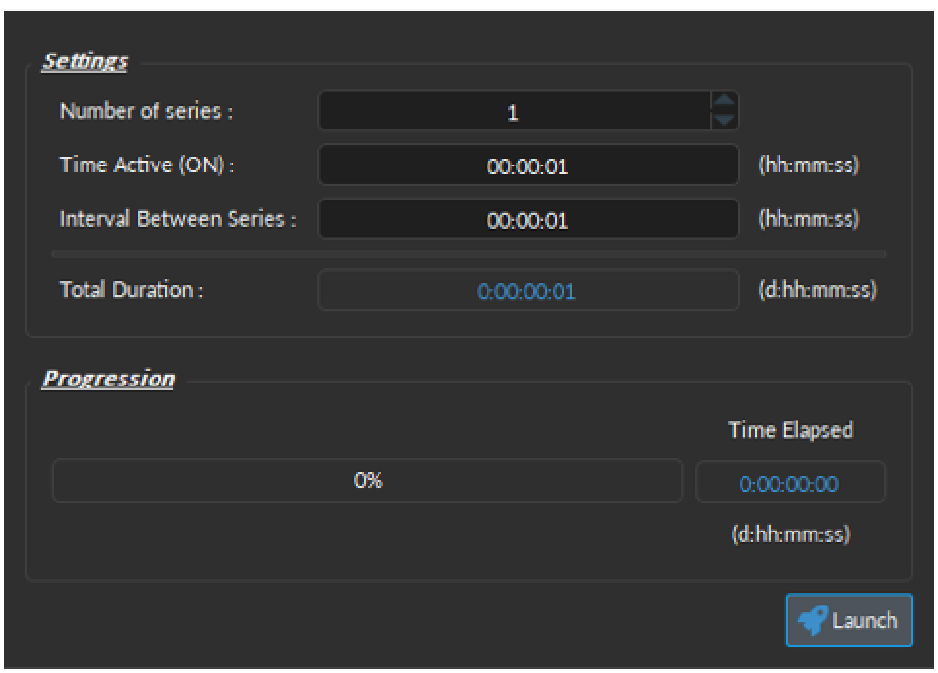


Figure 5.13: *Time Series Window*

- 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.

5.2.2 Configuration Tab

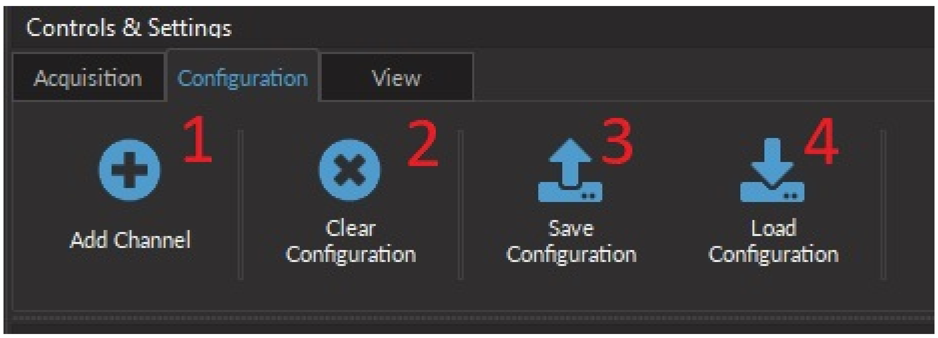


Figure 5.14: *Configuration Tab*

The different buttons of the **Configuration Tab** are shown in Figure 5.14 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 5.1.
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.

5.2.3 View Tab

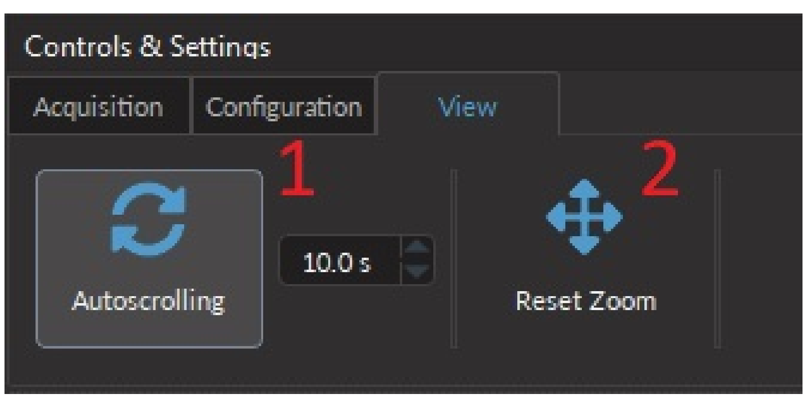


Figure 5.15: *View Tab*

The different buttons and fields of the **View Tab** are shown in Figure 5.15.

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.

5.3 Acquisition View

5.3.1 Acquisition View Overview

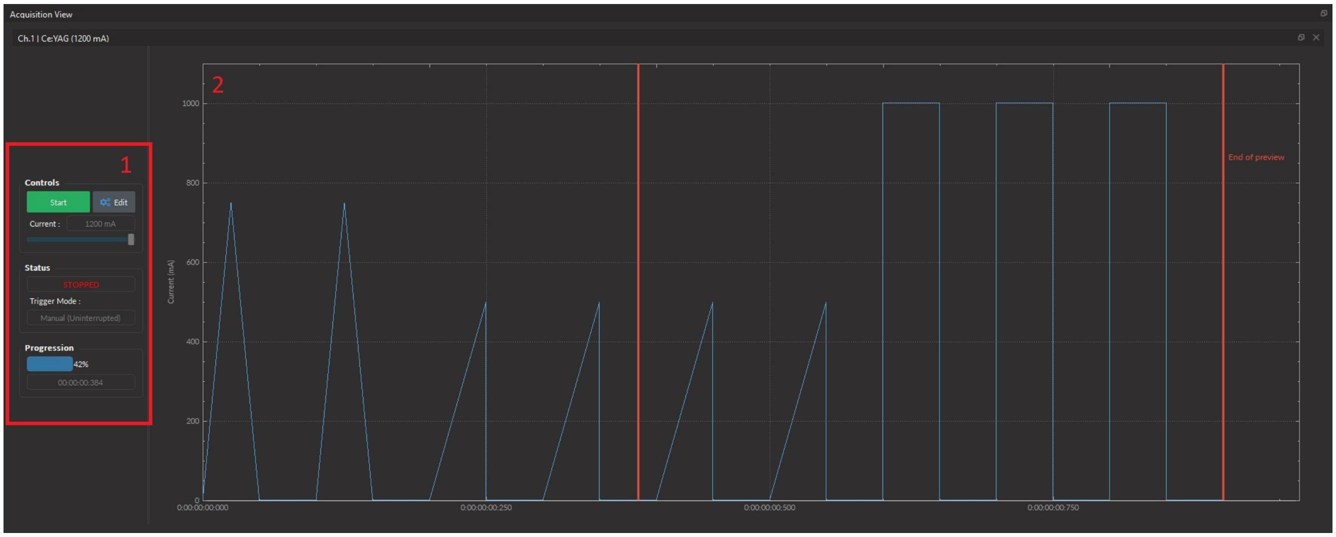


Figure 5.16: *Experiment View*

The Acquisition View (fig:LS- 5.16) 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 5.3.2.

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

5.3.2 Acquisition View Control

Figure 5.17: *Control of the Acquisition View*

The different buttons of the **Control of the Acquisition View** are shown in Figure 5.17 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 5.1.
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 5.1.1.3.
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.

6

Fiber Photometry

The Fiber Photometry Console module controls the *Fiber Photometry Console*, an FPGA-based data acquisition unit that 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 allows 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 & Settings** tabs. Separate channel windows are used to define output/input specifications.

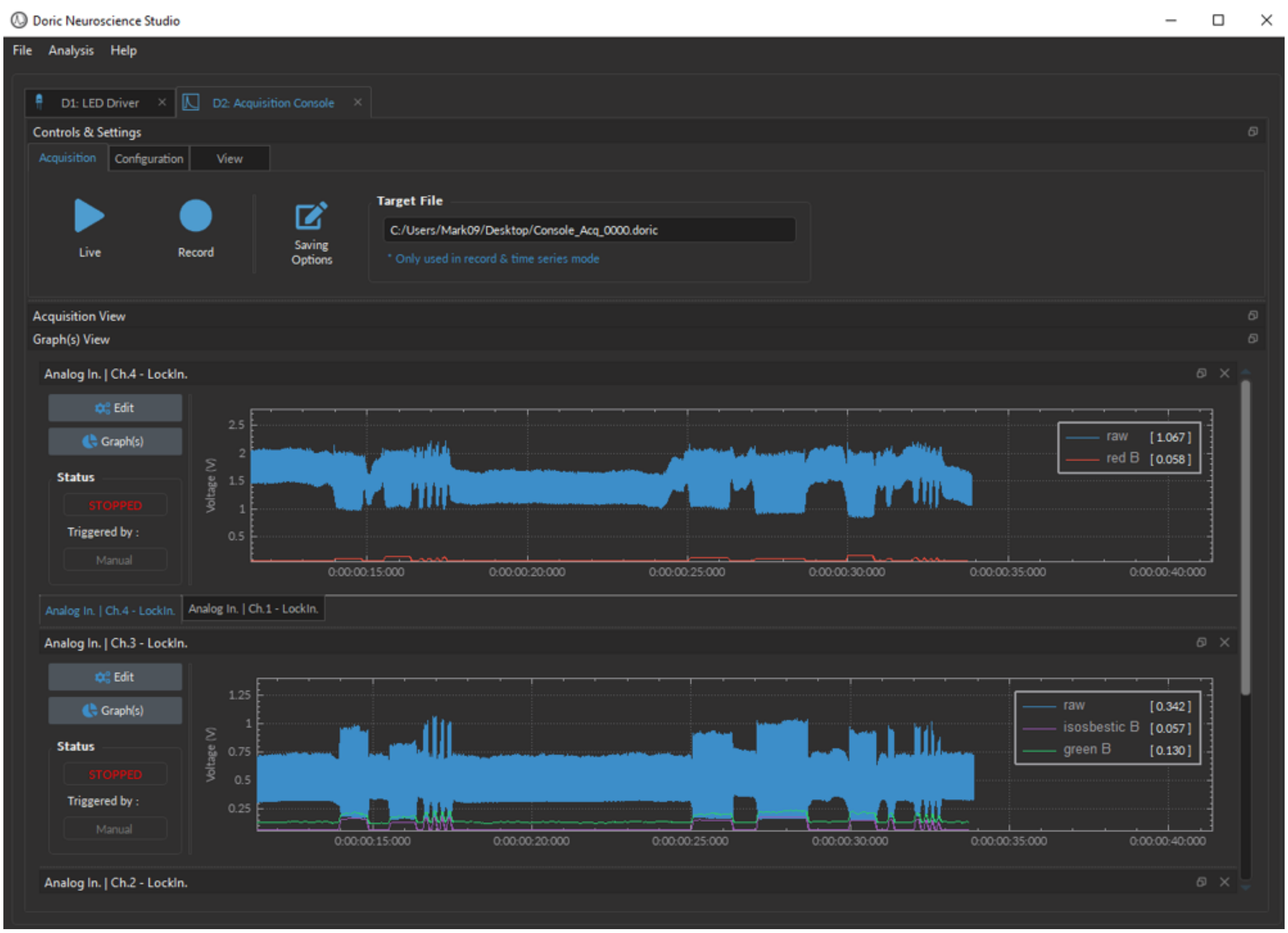


Figure 6.1: *Acquisition Console interface*

6.1 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 a USB port (as in Fig. 6.2).

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. 6.2).

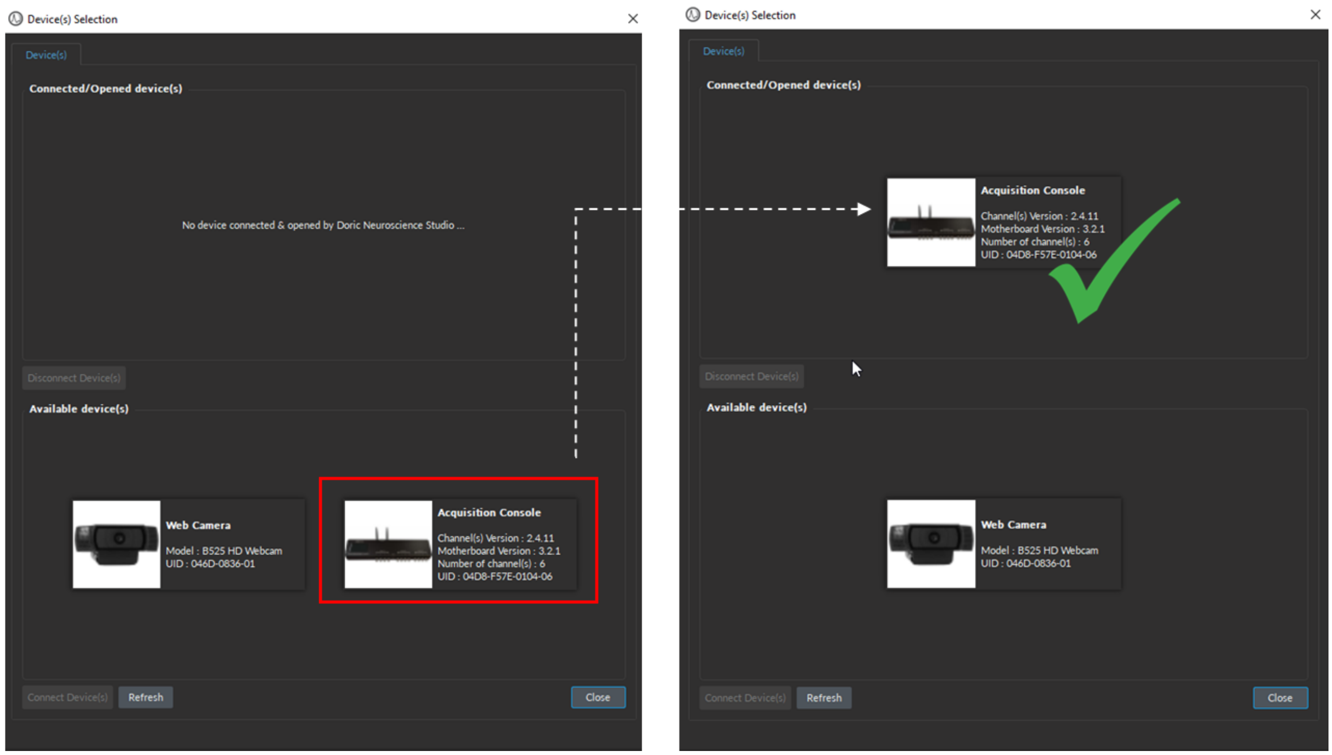


Figure 6.2: *Double click on the device of choice to connect it to Doric Neuroscience Studio*

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* menu, then *Device Selection* (as per Fig. 6.3) or use the hot key: *Ctrl+N*.

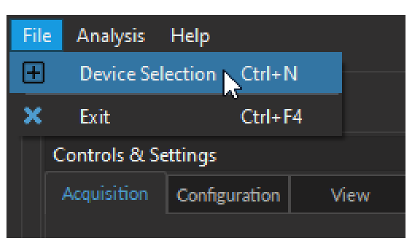


Figure 6.3: *Open Device Selection Window*

6.2 Overview

The **Acquisition Console** interface of *Doric Neuroscience Studio* software is split into two sections: **(1) Control & Settings** tabs (Section 6.3) are used to manage different elements of the software (Acquisition, Configuration, and View); **(2) Acquisition view** (Section 6.5) displays the input and output traces for visualization.

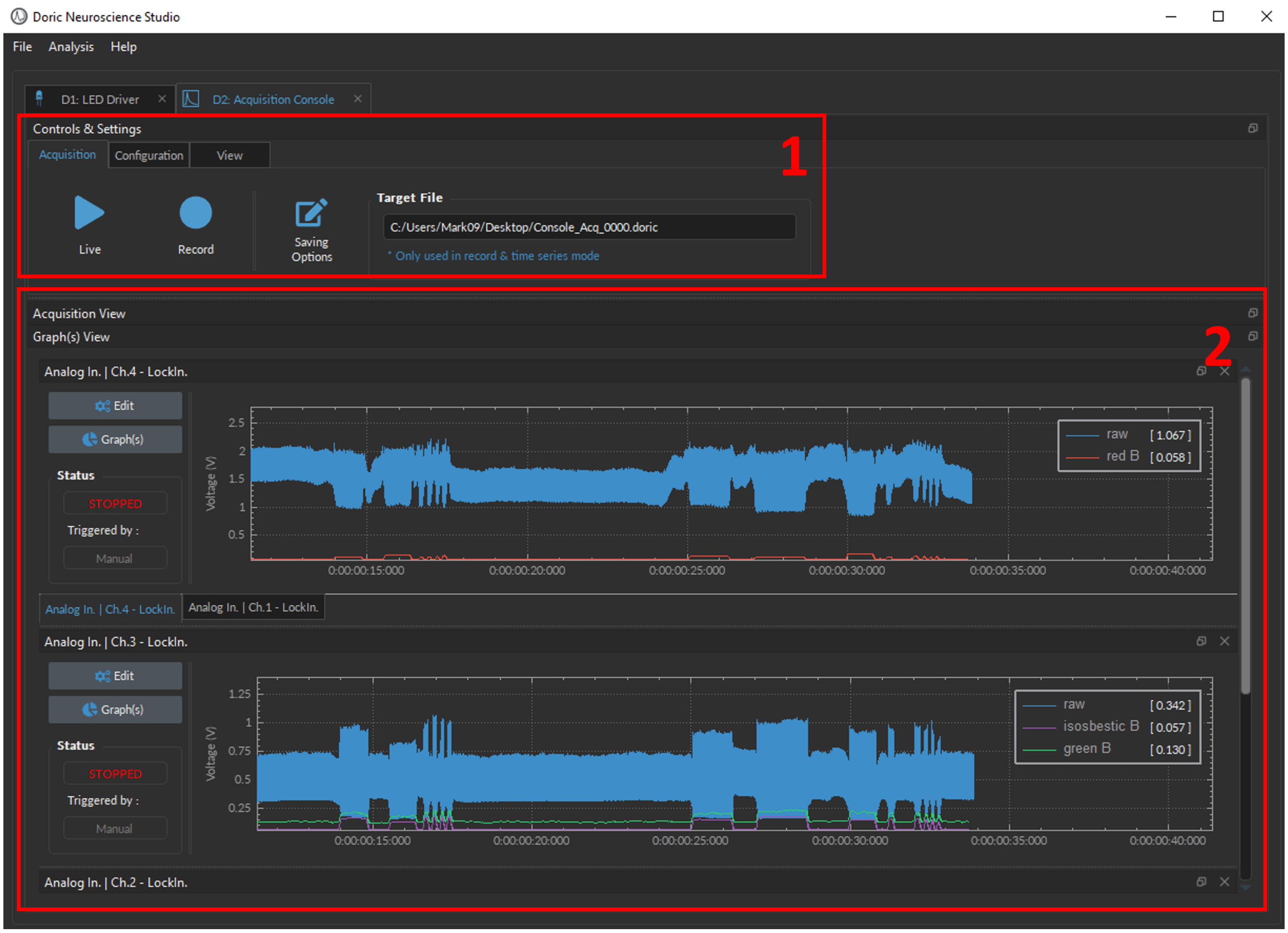


Figure 6.4: *Acquisition Console User Interface*

6.3 Control & Settings tabs

The three **Control & Settings tabs** are used to manage the different parts of the software. There are three tabs: **Acquisition** (Section 6.3.1), **Configuration** (Section 6.3.2), and **View** (Section 6.3.3).

6.3.1 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 6.4.1 to configure channels for recording.

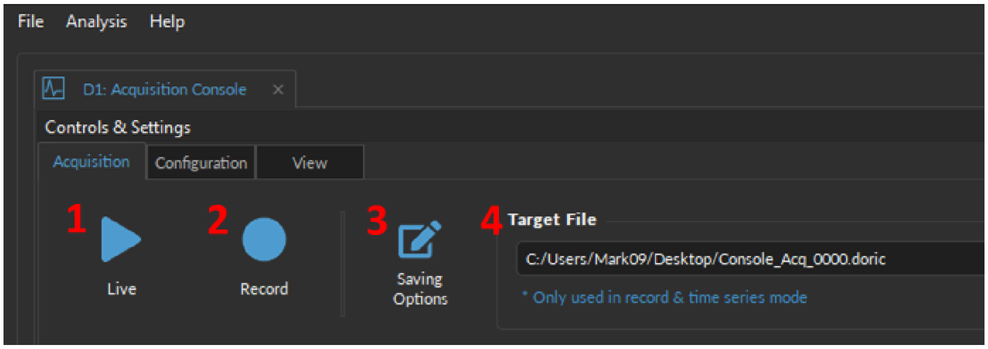


Figure 6.5: *Acquisition Tab*

1. The **Live** button (Fig. 6.5, 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. 6.5, 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. 6.5, 3) button opens the **Saving Parameters** window (Fig. 6.6). See section 6.3.1.1 for more details.
4. The **Target File** (Fig. 6.5, 4) displays the path and file name where the data will be stored once the **Record** button is pressed. Select the **Saving Options** button to change the path and file name.

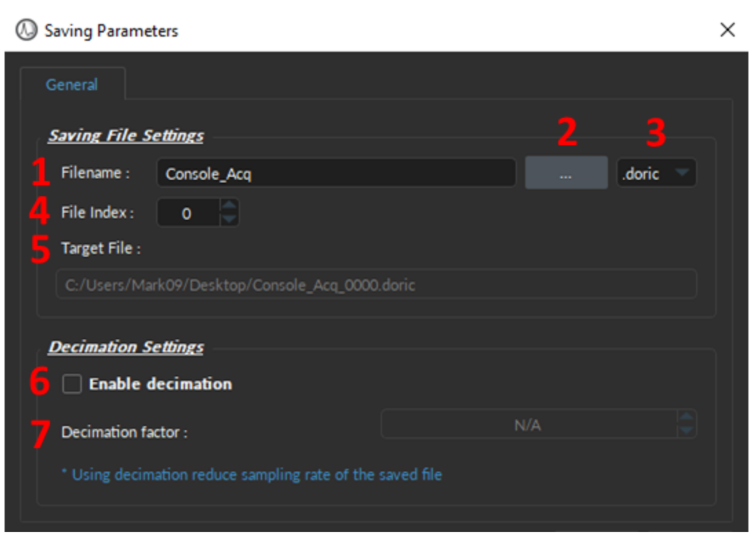


Figure 6.6: *Saving Menu Window*

6.3.1.1 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. 6.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 6.6, 1).
2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 6.6, 2).
3. The **File format** (Fig. 6.6, 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. 6.6, 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. 6.6, 5) displays the absolute path and filename where the data will be saved.
6. The **Enable decimation** checkbox (Fig. 6.6, 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. 6.6, 7) is used to define the number of points saved.[1]

6.3.2 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.

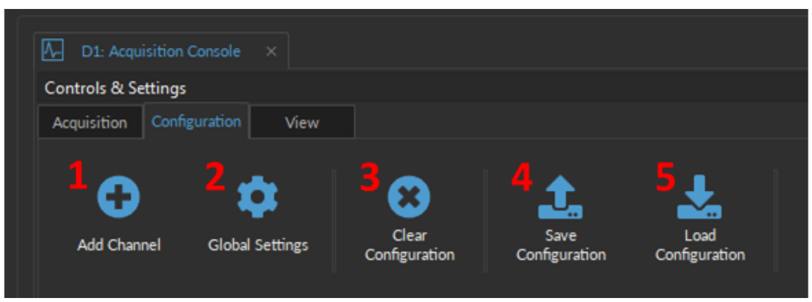


Figure 6.7: *Configuration Tab*

1. The **Add Channel** button (Fig. 6.7, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 6.4. Table 6.1 describes different types of channels available, their use cases and their individual sections.
2. The **Global Settings** (Fig. 6.7, 2) opens the **Global Options** window in Fig. 6.8, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 6.3.2.1 for more details.
3. The **Clear configuration** button (Fig. 6.7, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.
4. The **Save configuration** button (Fig. 6.7, 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. 6.7, 5) imports a pre-configured **.doric** file into the module.

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

6.3.2.1 Global Settings

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

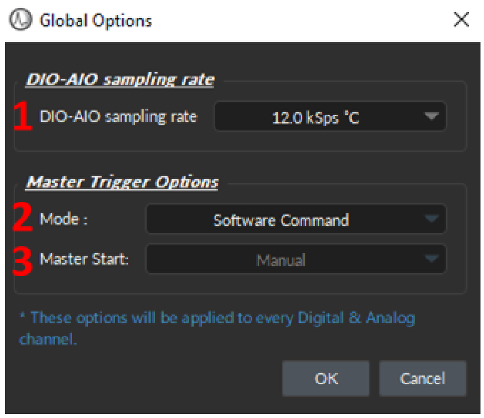


Figure 6.8: *Global Options Window*

1. The **DIO-AIO sampling rate** (Fig. 6.8-1) 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 6.3.1.1 to enable the *Decimation* and effectively reduce the saving sampling rate and restrict the data file size.

2. The **Mode** (Fig. 6.8, 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:

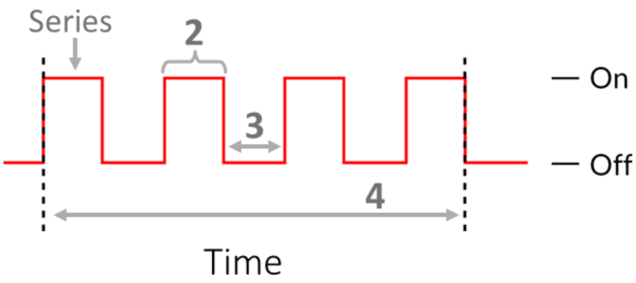
   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 6.5, 2). The **Master Start** is, by definition, always **Manual**.
   - *Triggered* - The recording session starts when a trigger signal is received (from the **Master Start**, either 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. 6.9). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig. 6.9b) pops up. See section 6.3.2.2 for more details.

3. The **Master Start** (Fig. 6.8, 3) defines the source that automatically starts the recording. This source can either be:

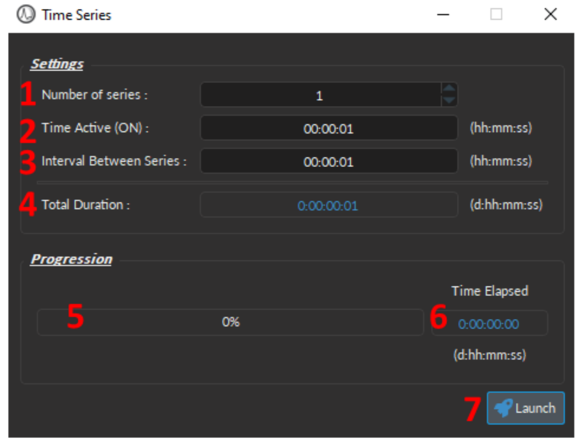
   - *Manuel* - the user ultimately starts the recording session by clicking **Record** within *Doric Neuroscience Studio*.
   - *Digital I/O Channel (1-4)* - The specified channel will automatically begin the recording session when it receives a digital trigger pulse from an external device. ***However,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

6.3.2.2 Time Series

The **Time Series** Window (Fig. 6.9) can be opened by clicking on the **Record** button (Fig. 6.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 6.8, 2). Every **Time series** sequence is automatically saved to the *.doric* file defined in **Saving Options** (Section 6.3.1.1).



(a) *Time Series Acquisition Timing Diagram*



(b) *Time Series Window*

Figure 6.9: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 6.9) sets the following parameters:

1. The **Number of series** (Fig. 6.9, 1) defines the total number of time periods (*i.e.* serie, Fig. 6.9a, 1) when the recording will be ON.
2. The **Time Active (ON)** (Fig. 6.9, 2) defines the duration of a series.
3. The **Interval Between Series** (Fig. 6.9, 3) defines the amount of time between each series if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 6.9, 4) displays the total amount of time that the timeseries recording will take place.
5. The **Progression bar** (Fig. 6.9, 5) indicates the progression of the time series (in %).
6. The **Time Elapsed** (Fig. 6.9, 6) counter indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** (Fig. 6.9, 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.

6.3.3 View Tab

The **View Tab** (Fig. 6.10) is used to modify the presentation of graphs in the **Acquisition view**.

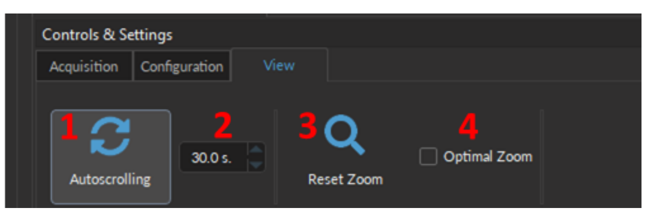


Figure 6.10: *View Tab*

The **View** parameters are as follows:

1. The **Autoscrolling** button (Fig. 6.10, 1), when selected, automatically scrolls as new data appears.
2. The **Zoom range** (Fig. 6.10, 2) sets the **Autoscrolling** time axis to the value of choice, specified in the text-box.
3. The **Reset Zoom** button (Fig. 6.10, 3) readjusts the graph Y-axis to the default value, or the **Optimal Zoom** value if the Optimal Zoom check-box is selected.
4. The **Optimal Zoom** check-box (Fig. 6.10, 4) automatically adjusts the graph Y-axis range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

6.4 Channel Configurations

6.4.1 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. 6.11). This will open the **Channel(s) Configuration** window (Fig. 6.12).

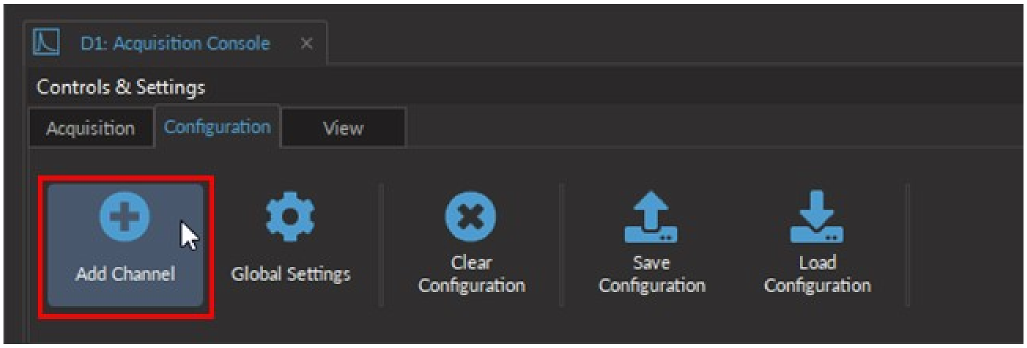


Figure 6.11: *Add Channel button opens the Channel Configuration window*

To generate a new **Channel** using the **Channel(s) Configuration** window (Fig. 6.12):

1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 6.12). Table 6.1 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 6.1 for hyperlinks to the relevant sections.

3. Select the **Add** button (Fig. 6.12) 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.

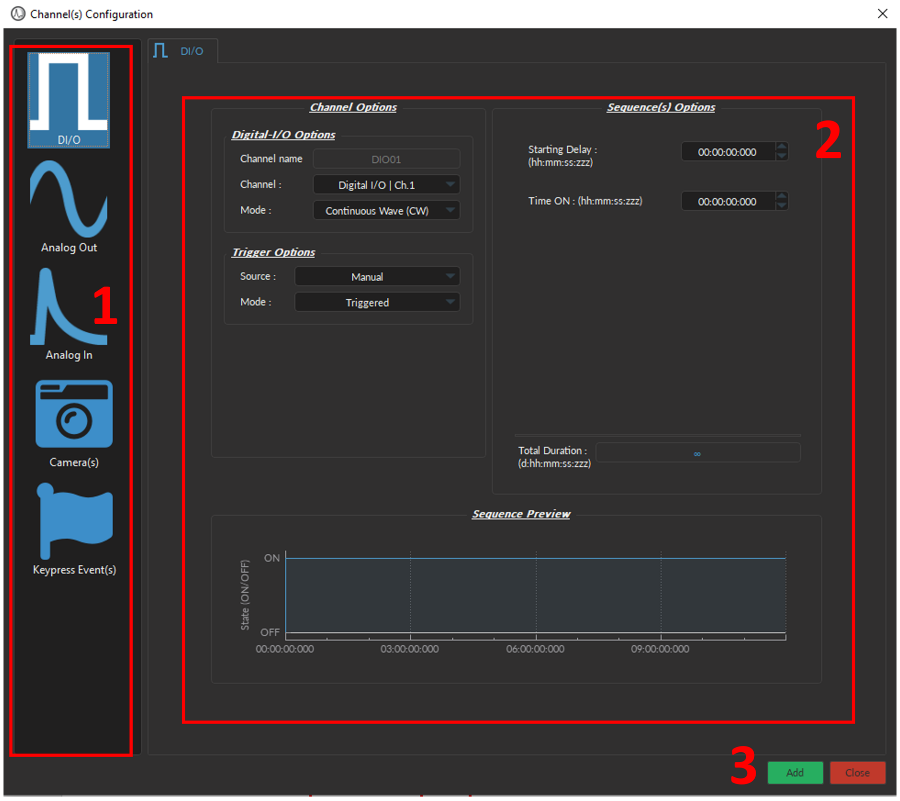
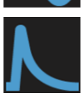


Figure 6.12: *Channel(s) Configuration, Digital I/O input*

6.4.2 Channels Types

Different types of input and output can be configured for the specifics of the experiment by creating a new Channel in the Configuration tab or editing an existing one (Fig. 6.11). Table 6.1 details the types of inputs and output the console and the software can handle and gives quick access to their sections.

Table 6.1: *Types of channels and their use cases*

Icon	**Channel Type**	**Use Case**	**Section**
	Digital I/O	For input and output of TTL signals	6.4.3
	Analog Output	For the output of analog signals, such as sine, stair or customized	6.4.4
	Analog Input	To collect the fluorescent signal (such as GCamp, RCamp, Isosbestic or FRET)	6.4.5
	Camera(s)	To collect images for behaviour experiments	6.4.6
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	6.4.7

6.4.3 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 externally driven device used during the experiment (such as the timing of a displayed stimulus or a measured behavior).

The *Channel(s) Configuration* window for the **Digital I/O** Channel is divided into three sections (Fig. 6.13): (1) the **Channel Options** (Section 6.4.3.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 6.4.3.2).

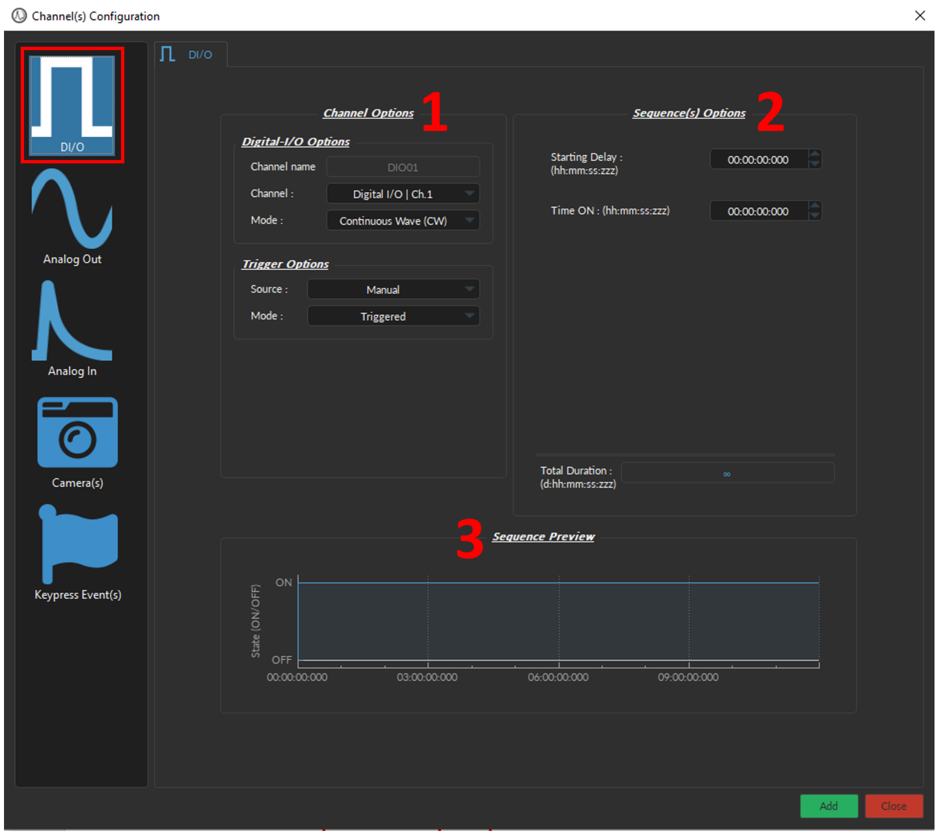


Figure 6.13: *Channel(s) Configuration, Digital I/O*

6.4.3.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.

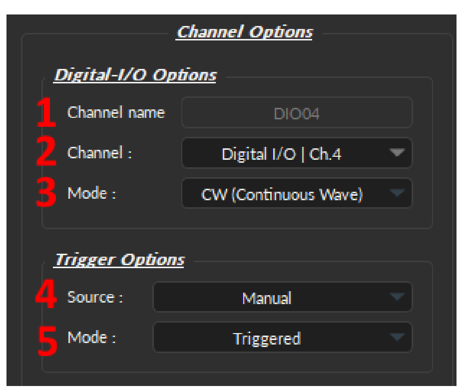
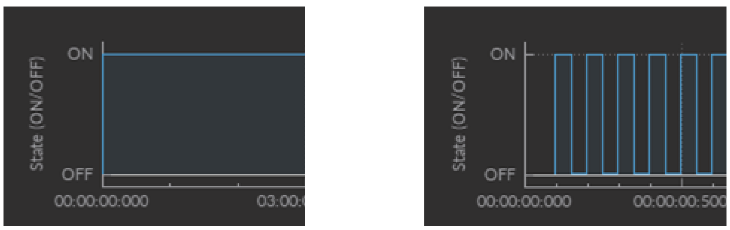


Figure 6.14: *Channel(s) Configuration, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel Name** (Fig. 6.14, 1) allows the user to specify a label for each channel.
2. The **Channel** (Fig. 6.14, 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 digital channels within the software.
3. The **Mode** (Fig. 6.14, 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. 6.15a);
   - The **Square (TTL)** Mode (Fig. 6.15b);
   - 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 6.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.

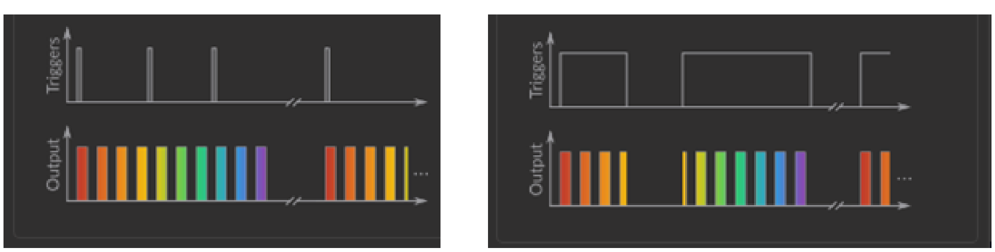


(a) *Continuous* (b) *Square*

Figure 6.15: *Channel Options - Output Modes*

Trigger Options:

1. The **Source** trigger option (Fig. 6.14, 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. 6.14, 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. 6.16a), 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. 6.16b), the sequence will start once the voltage reaches 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*

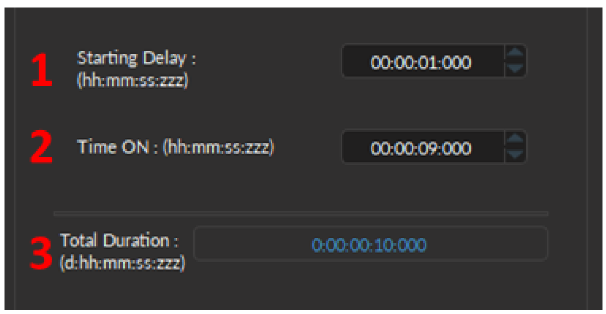
Figure 6.16: *Trigger Options Modes*

6.4.3.2 Sequence Options & Preview

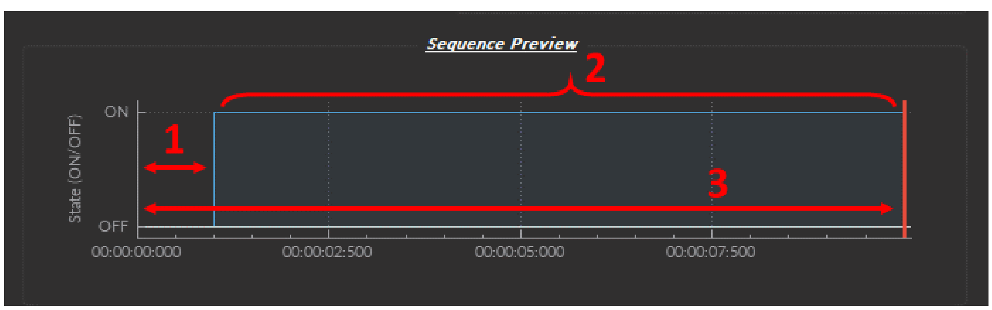
The **Sequence options** section (Fig. 6.17a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 6.17b) displays the corresponding shape and timing of the sequence. Should a parameter chosen be impossible to apply to a sequence (for example, a **Time ON** greater 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. 6.14), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 6.15a) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.
  1. The **Starting Delay** (Fig. 6.17, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Time ON** (Fig. 6.17, 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. 6.17, 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*

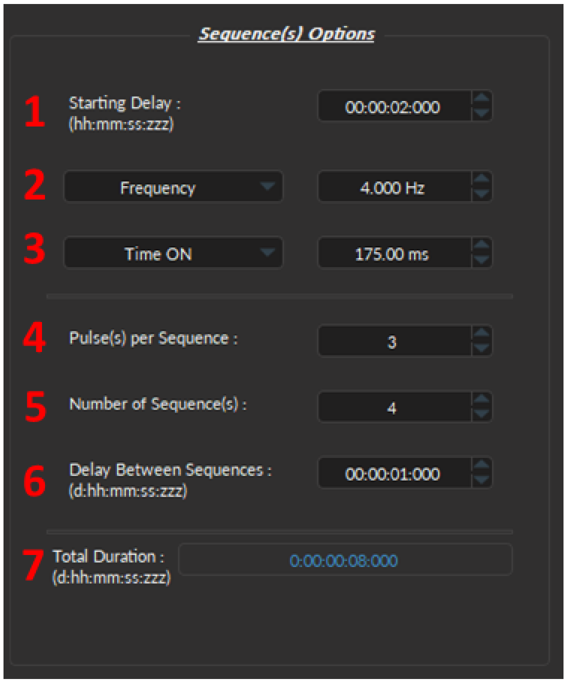


(b) *Sequence Preview*

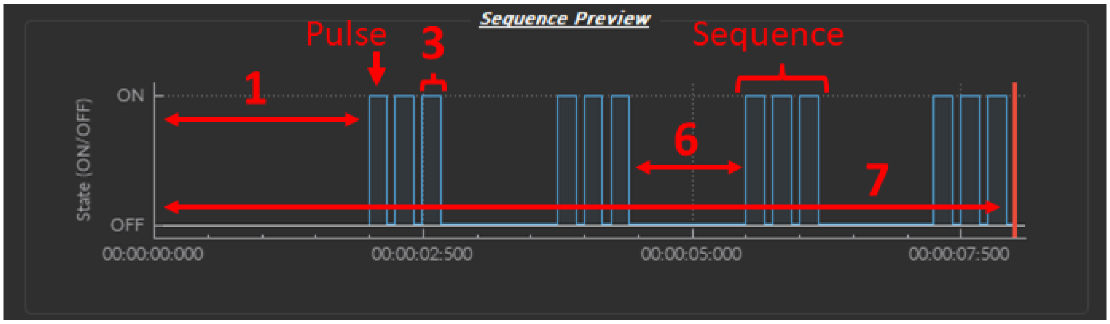
Figure 6.17: *Channel(s) Configuration, Digital I/O - CW Mode*

- The **Square** channel mode (Fig. 6.15b) allows the creation of a square TTL pulse sequence and includes the following elements:
  1. The **Starting Delay** (Fig. 6.18, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Frequency** (Fig. 6.18a, 2) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period** (Fig. 6.18a, 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. 6.18, 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. 6.18, 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. 6.18a, 5) sets the number of times that the sequence will be repeated.
6. The **Delay between sequences** (Fig. 6.18, 6) sets the amount of time separating any two sequences (excluding the **Starting Delay**).
7. The **Total Duration** (Fig. 6.18, 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*



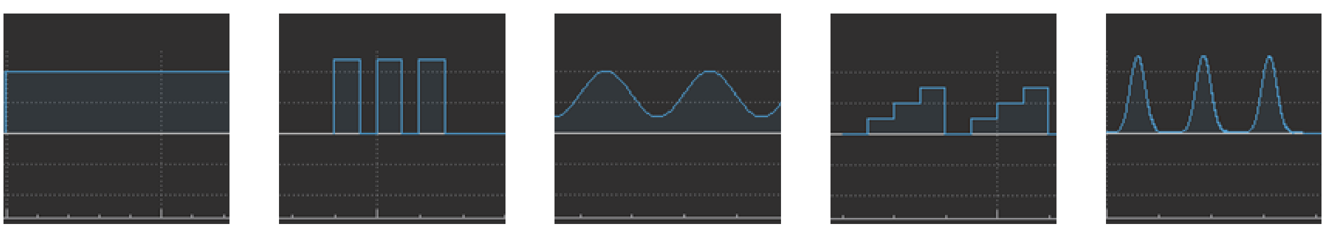
(b) *Sequence Preview*

Figure 6.18: *Channel(s) Configuration, Digital I/O - Square Mode*

6.4.4 Analog Output

Figure 6.19: *Channel(s) Configuration, 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. 6.20).



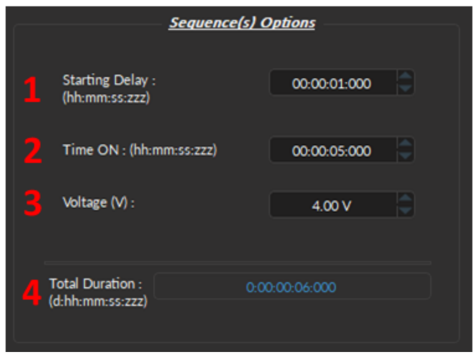
(a) *Continuous* (b) *Square* (c) *Sine* (d) *Stair* (e) *Custom*

Figure 6.20: *Analog Output Modes*

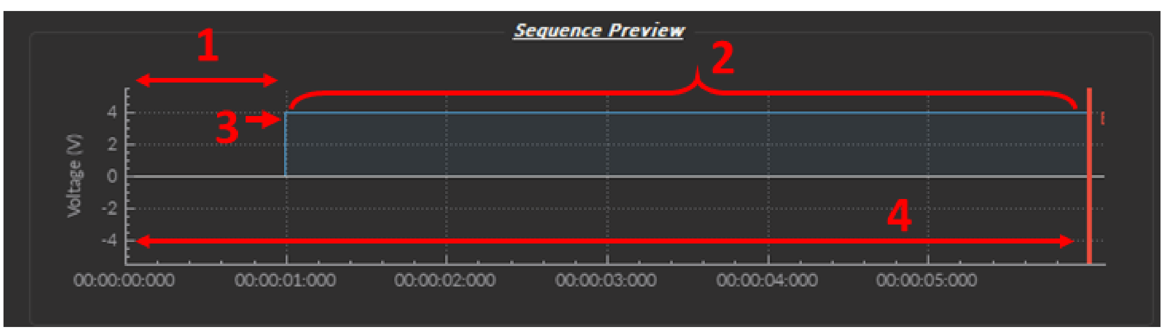
6.4.4.1 Continuous Wave (CW) Mode

The **CW (Continuous wave)** channel mode (Fig. 6.21) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box (Fig. 6.21a).

1. The **Starting Delay** (Fig. 6.21, 1) defines the time between the activation of the sequence and the beginning of the signal.
2. The **Time ON** (Fig. 6.21, 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 **Voltage** (Fig. 6.21, 3) defines the voltage of the continuous signal, in volts. The signal cannot go beyond ±4.75 V.
4. The **Total Duration** (Fig. 6.21, 4) shows the total expected duration of the 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 6.21: *Channel(s) Configuration, Analog Output CW*

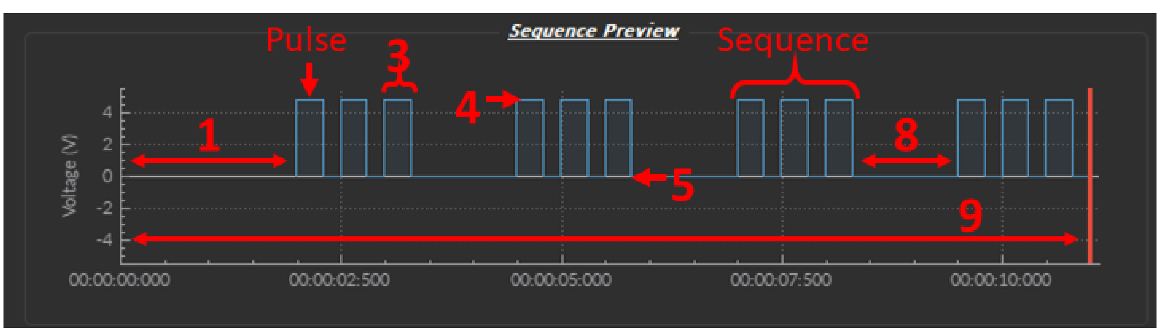
6.4.4.2 Square Mode

The **Square** channel mode (Fig. 6.22) 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. 6.22, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
2. The **Frequency** (Fig. 6.22, 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. 6.22, 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. 6.22, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.
5. The **Minimum Voltage (V-)** (Fig. 6.22, 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. 6.22, 6) sets 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. 6.22, 7) sets the number of times that the sequence will be repeated.
8. The **Delay between sequences** (Fig. 6.22, 8) sets the delay between each sequence.
9. The **Total Duration** (Fig. 6.22, 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*

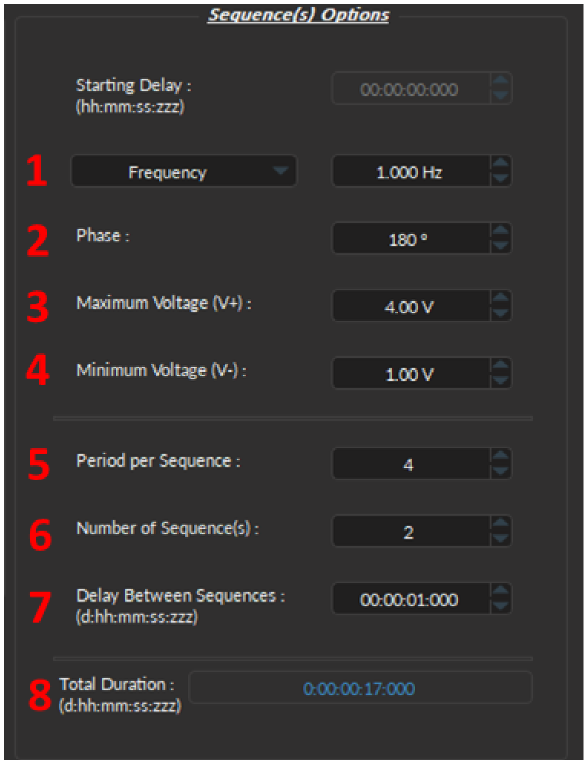
Figure 6.22: *Channel(s) Configuration, Analog Output Square*

6.4.4.3 Sine Mode

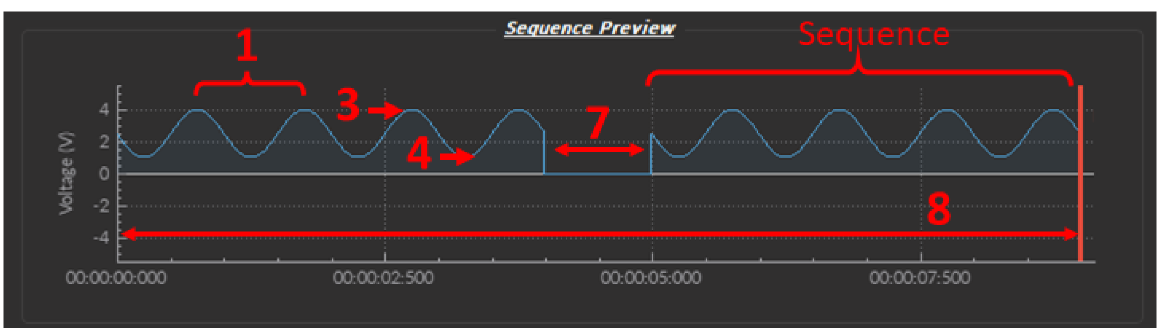
The **Sine** mode (Fig. 6.23) creates a sinusoidal pulse sequence with peaks at **V+** and **V-**.

Note: The **Starting Delay** is not available for this mode (Fig. 6.23a).

1. The **Frequency** (Fig. 6.23, 1) 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 sine wave every 100 ms (period), whereas a signal at 0.5 Hz (frequency) will output one sine wave every 2 seconds (period).
2. The **Phase** option (Fig. 6.23, 2) replaced **Time ON** (Fig. 6.22, 3). This allows the choice of the sine wave phase, in degrees.
3. The **Maximum Voltage (V+)** (Fig. 6.23, 3) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.
4. The **Minimum Voltage (V-)** (Fig. 6.23, 4) defines the minimum voltage of each pulse, in volts. The signal cannot go below -4.75 V.
5. The **Period per Sequence** (Fig. 6.23, 5) is similar to the **Pulse per Sequence** parameter in Square mode (Section 6.4.4.2, Square), but where the period is a single sine wave from peak to peak (Fig. 6.23b, 1).
6. The **Number of Sequence(s)** (Fig. 6.23, 6) sets the number of times that the sequence will be repeated.
7. The **Delay Between Sequences** (Fig. 6.23, 7) sets the delay between each sequence.
8. The **Total Duration** (Fig. 6.23, 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*

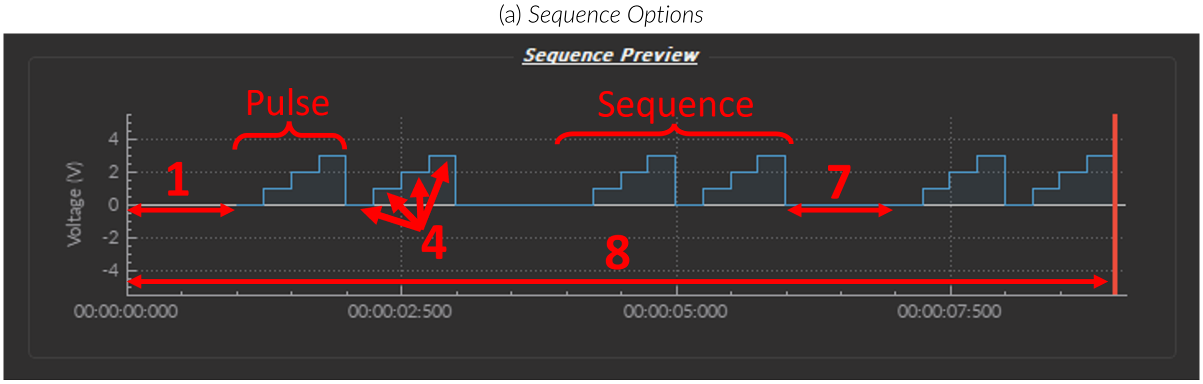
Figure 6.23: *Channel(s) Configuration, Analog Output Sine*

6.4.4.4 Stairs Mode

The **Stairs** mode (Fig. 6.24) creates a stepwise pulse sequence with peaks at several different Voltage levels **V+**.

1. The **Starting Delay** (Fig. 6.24, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
2. The **Frequency** (Fig. 6.24, 2) option replaces the **Time ON**. This parameter applies to a whole pulse, which includes 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. 6.24b, 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. 6.24, 4).
5. The **Pulse(s) per Sequence** (Fig. 6.24, 5) sets 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. 6.24, 6) sets the number of times that the sequence will be repeated.
7. The **Delay Between Sequences** (Fig. 6.24, 7) sets the delay between each sequence.
8. The **Total Duration** (Fig. 6.24, 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 6.24: *Channel(s) Configuration, Analog Output Stairs*

6.4.4.5 Custom Mode

The **Custom** mode (Fig. 6.25) provides a way to import a customized pulse sequence with a non-standard shape to fit experimental needs.

1. The **Select File** button (Fig. 6.25a, 1) is used to input a custom .csv file containing the data for the 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. 6.25b. The values can be any value between **-4.75V** and **+4.75V**.

2. The **Starting Delay** (Fig. 6.25, 2) defines the time between the activation of the sequence and the beginning of the signal.

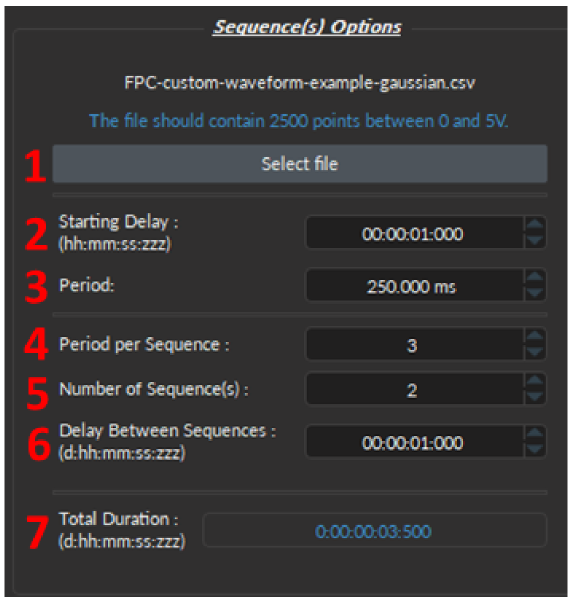
3. The **Period** option (Fig. 6.25, 3) replaces the **Time ON** option (Fig. 6.22, 3). This option will stretch or shrink the 2500 value sequence to fit the specified amount of time.

4. The **Period per Sequence** (Fig. 6.25, 4) is similar to the **Pulse per Sequence** field found in **Square** modes (Fig. 6.4.4.2, 6), where the pulse is replaced by the period sequence (Fig. 6.25c, Sequence).

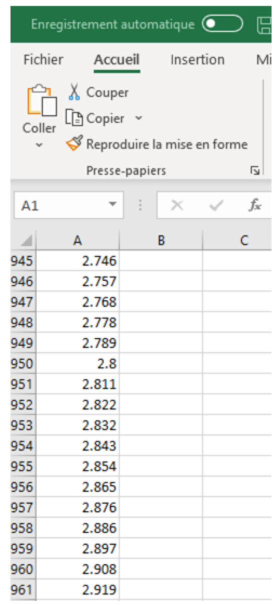
5. The **Number of Sequence(s)** (Fig. 6.25a, 5) sets the number of times that the sequence will be repeated.

6. The **Delay Between Sequences** (Fig. 6.25, 6) sets the delay between each sequence.

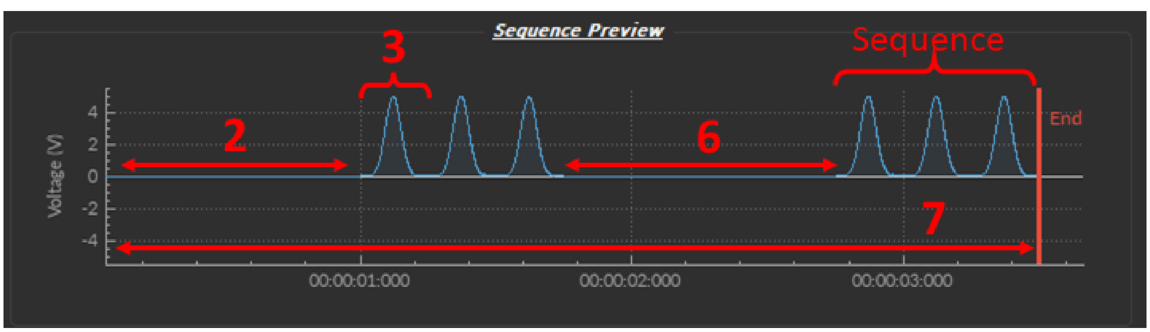
7. The **Total Duration** (Fig. 6.25, 7) shows the total expected duration of the pulse sequence. Should the duration be infinite, the box will display ∞. If there is an error in parameter selection, this box will display **N/A**.



(a) *Sequence Options*



(b) *Example .csv file*



(c) *Sequence Preview*

Figure 6.25: *Channel(s) Configuration, Analog Out - Custom*

6.4.5 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. 6.26): (1) the **Channel Options** (Section 6.4.5.1) and (2) the **Mode-specific Options** (*Linear*, Section 6.4.5.2; *Interleaved*, Section 6.4.5.3; *Lock-In*, Section 6.4.5.4).

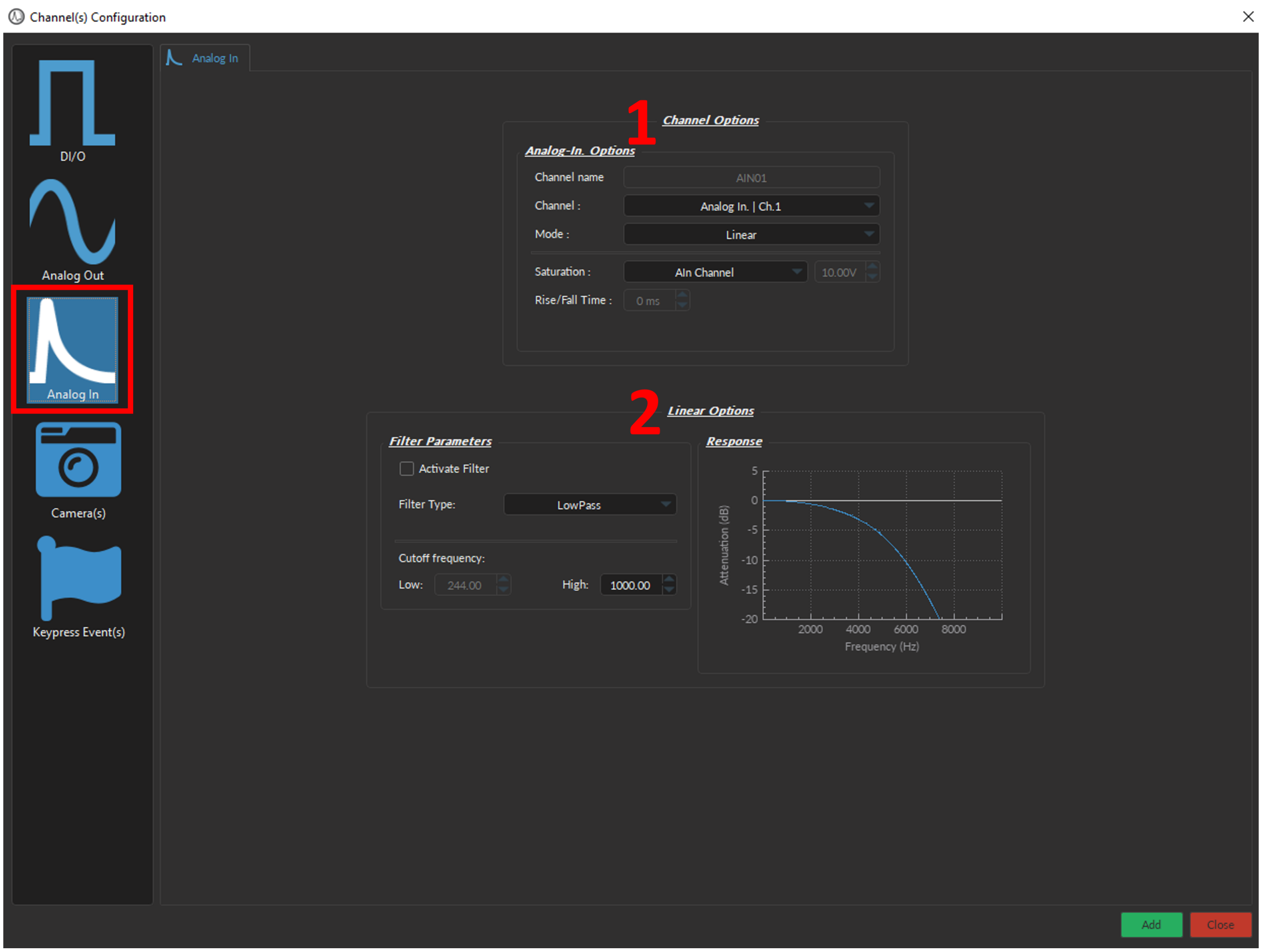


Figure 6.26: *Channel(s) Configuration, Analog Input*

6.4.5.1 Channel Options

The **Channel Options** (Fig. 6.27) defines the channel, source, and mode of the digital signal, as follows:

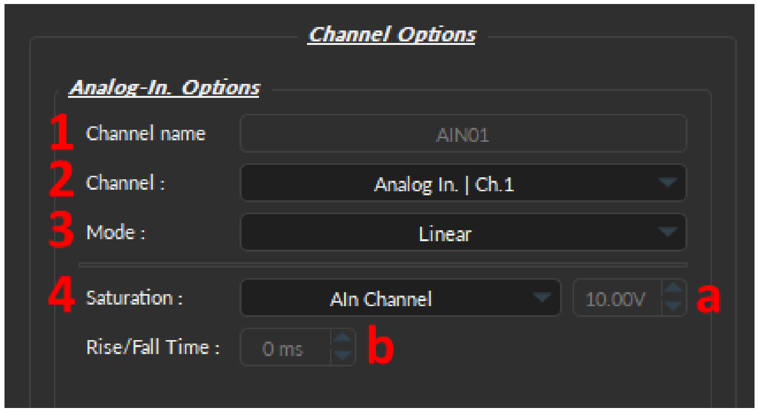


Figure 6.27: *Channel(s) Configuration, Analog Input - Channel Options*

1. The **Channel Name** (Fig. 6.27, 1) allows the user to specify a label for each channel.
2. The **Channel** (Fig. 6.27, 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 digital channels within the software.
3. Three **Mode** (Fig. 6.27, 3) are available to record the input signal, each of which has its own defined parameters in the Options box below **Channel Options**:
   - *Linear* - Section 6.4.5.2;
   - *Interleaved* - Section 6.4.5.3;
   - *Lock-In* - Section 6.4.5.4.
4. The **Saturation** (Fig. 6.27, 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. 6.27, 4a)
   b) The **Rise/Fall Time** (Fig. 6.27, 4b)

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

6.4.5.2 Linear

The **Linear** channel mode (Fig. 6.28) allows the direct measurement of signal received by a channel. The linear mode-specific parameters are as follows:

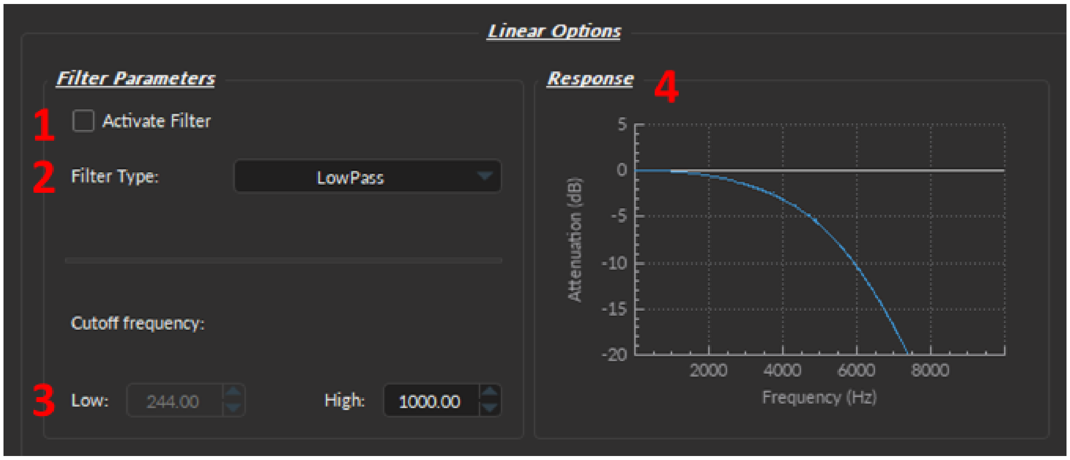


Figure 6.28: *Channel(s) Configuration, Analog Input Linear*

1. When the **Activate Filter** checkbox (Fig. 6.28, 1) 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 (Fig. 6.28, 2) allows the choice of a filter type from **High-Pass**, **Low-Pass**, **Band-Pass** and **Band-Stop**.
3. The **Cutoff Frequency** boxes (Fig. 6.28, 3) 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 cutoff value is, by definition, always 3 dB below (Low Cutoff) or above (High Cutoff) the specified value.
4. The **Response** box (Fig. 6.28, 4) displays the Frequency (Hz) vs Attenuation (dB) trace of the filter according to both the filter type and the cutoff values.

6.4.5.3 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. 6.29).

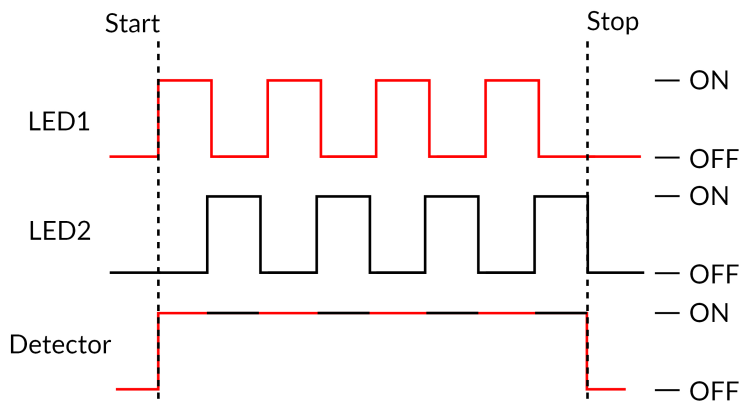


Figure 6.29: *Interleaved Acquisition Timing Diagram*

The interleave preset is using 50% duty cycle for each LED, without delay between them (Fig. 6.29). Thus, depending on the Rise/Fall time of the detector in use (Fig. 6.27, 4b, Detector Rise/Fall Time), there will be more or less crosstalk between the interleaved channels (Fig. 6.30).

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 turned ON or OFF (Fig. 6.30). Figure 6.30 shows how the Digital Output channel of LED 1 has a small increase in voltage when 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. 6.30).

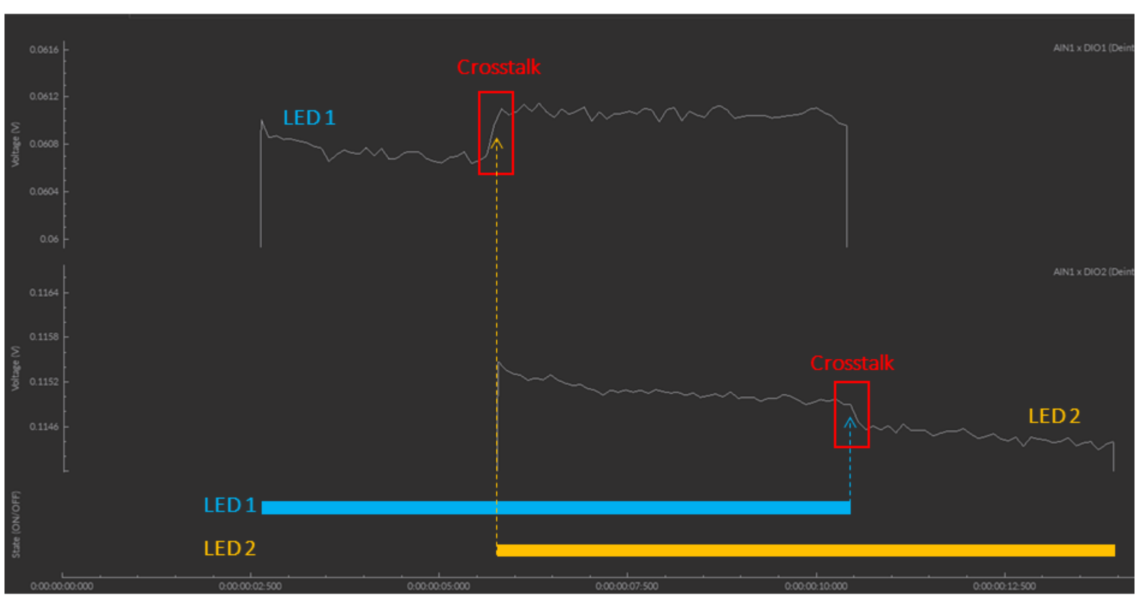


Figure 6.30: *Interleaved Cross-talk*

Strategies to Mitigate Crosstalk:

1. If the sampling rate of the triggered device(s) is high enough (>120Hz), use the **Lock-In mode** (Section 6.4.5.4) 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 a real signal during data analysis.**

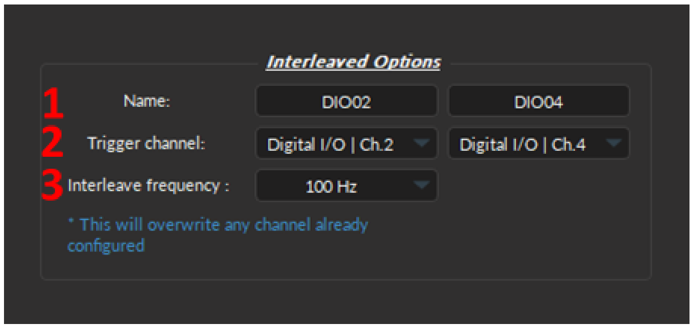


Figure 6.31: *Channel(s) Configuration, Analog Input Interleaved*

To use the **interleaved mode**, specify the parameters in the **Interleaved Options** section of the *Channel Configuration* window (Fig. 6.31):

1. The **Name** (Fig. 6.31, 1) lets users customize the label of the channel to increase the clarity of the acquisition system.

2. The **Trigger channel** (Fig. 6.31, 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. 6.31, 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.

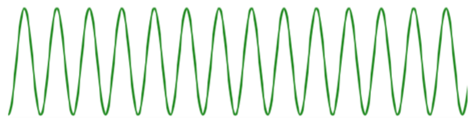
WARNING:
Specifying the interleave frequency will **overwrite** any channel already configured.

6.4.5.4 Lock-In

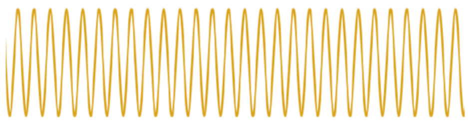
The **Lock-In** mode can detect fluorescence signals embedded in strong noise (e.g. Isosbestic and a fluorophore) or separate multiple signals from a single input during fiber photometry.

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).

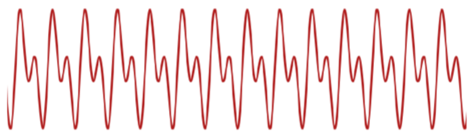
Each *LED light source* emits a sinusoidal illumination at a given frequency (Fig. 6.32a & 6.32b). The detector collects the fluorescent data at a frequency corresponding to the summation of the LED frequencies (Fig. 6.32c).



(a) *LED 1 frequency*



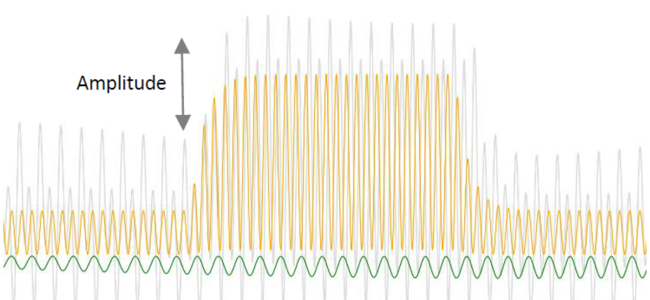
(b) *LED 2 frequency*



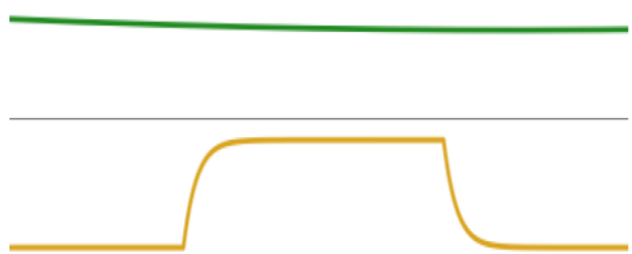
(c) *Raw signal*

Figure 6.32: *Lock-In Acquisition Timing Diagram*

The amplitude changes of the raw signal are due to the collected fluorescence and are dependent on the frequency (Fig. 6.33a). 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. 6.33). The result is separated from the ambient noise that occurred at different frequencies (Fig. 6.33b). The same principle can be applied to demodulate two fluorescent signals.



(a) *Amplitude changes (signal) are frequency dependent*



(b) *Separated signals*

Figure 6.33: *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. 6.34):

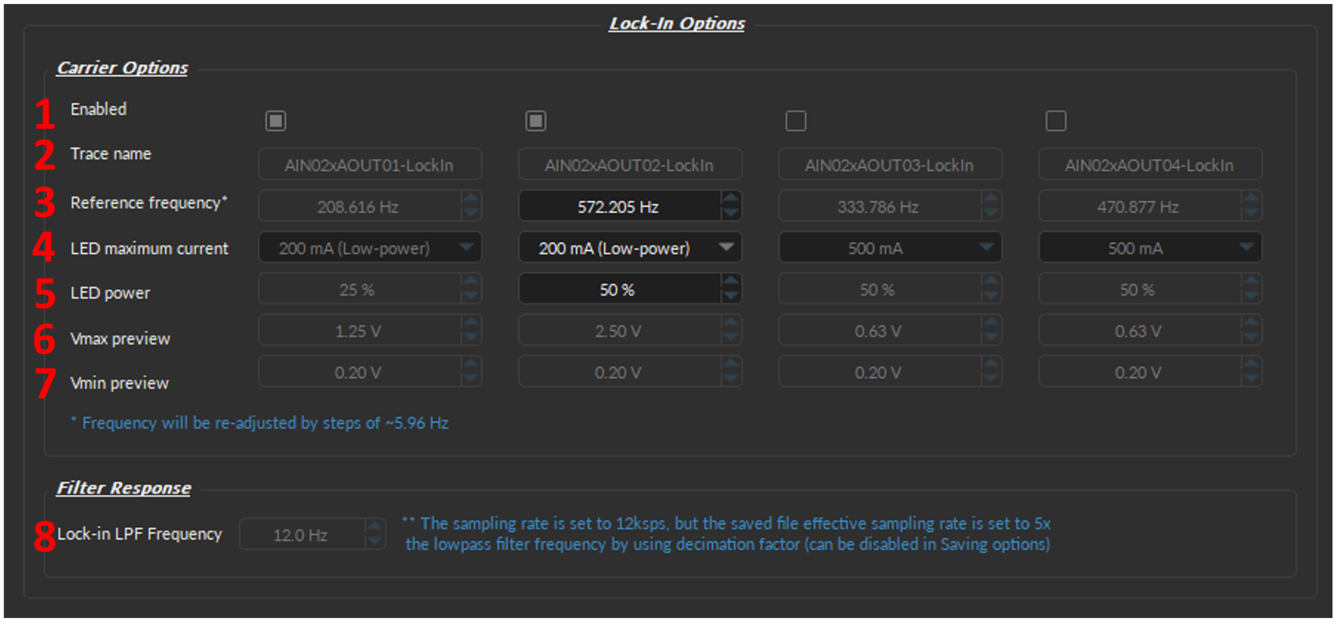


Figure 6.34: *Channel(s) Configuration, Analog Input Lock-In*

1. The **Enable** (Fig. 6.34, 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 enable the output(s) channels that will be driving the input specified in Fig. 6.27, 2.

2. **Trace Name** (Fig. 6.34, 2) is the identity of the Input and Output Channel(s) enabled for this Lock-In configuration.
   - The **AIN #** corresponds to 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. 6.27).
   - The **AOUT #** number corresponds to the Analog Out port on the console that sends electrical 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 Options** (Fig. 6.34), changing which port is enabled using the checkbox (Fig. 6.34, 1) 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. 6.34, 3) is the oscillating trigger signal that drives the LED (or device(s) of choice). We recommend using the default values since they are optimized 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 of known noise frequency (e.g. 50 and 60 Hz) or a multiple of another reference frequency.

4. **LED maximum current** (Fig. 6.34, 4) 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 on 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 the light source's 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 & 420 nm.
   - **1000 mA** - the **LED maximum current** for most *Doric* LEDs, except the four mentioned above.

- **Custom** - this setting allows users to manually adjust the Vmax and Vmin of the LED, regardless of LED's 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. 6.35).

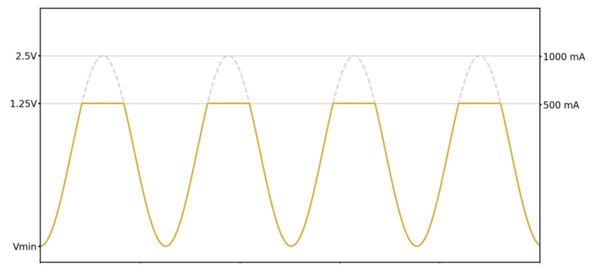


Figure 6.35: *Cropped LED excitation signal*

5. **LED power** (Fig. 6.34, 5) 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. 6.34, 6). **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. 6.34, 5).

7. **Vmin Preview** (Fig. 6.34, 7) - the default value is set to 0.2 V but can be changed if the **Custom** LED maximum current mode is selected. The **Vmin** should never be below 0.1 V.

8. **Lock-in LPF Frequency** (Fig. 6.34, 8) defines 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. 6.36).
   - **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.

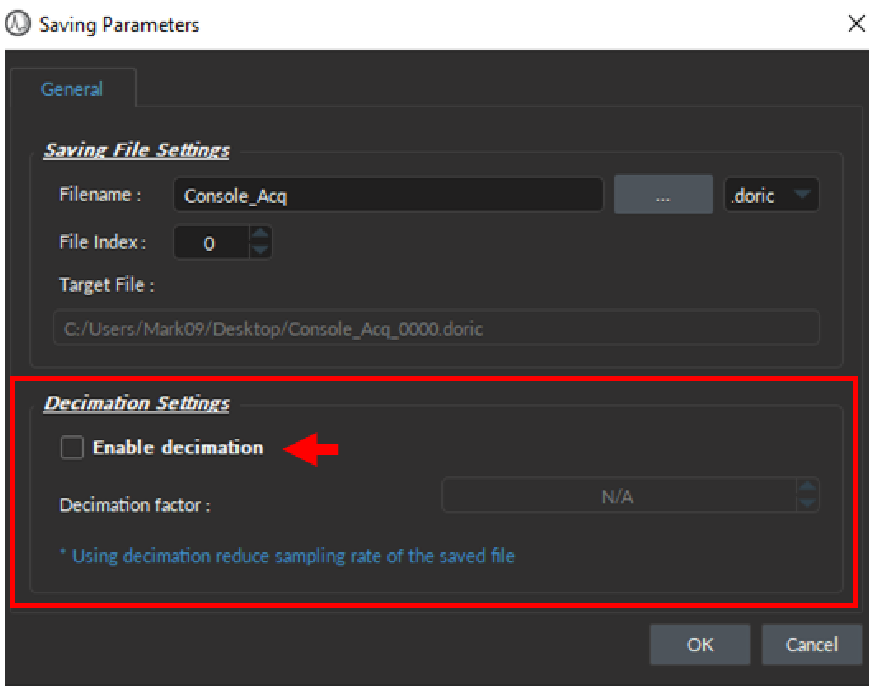


Figure 6.36: *Enable/Disable the decimation factor that reduces the Sampling Rate of the saved files*

6.4.6 Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for their measurement.

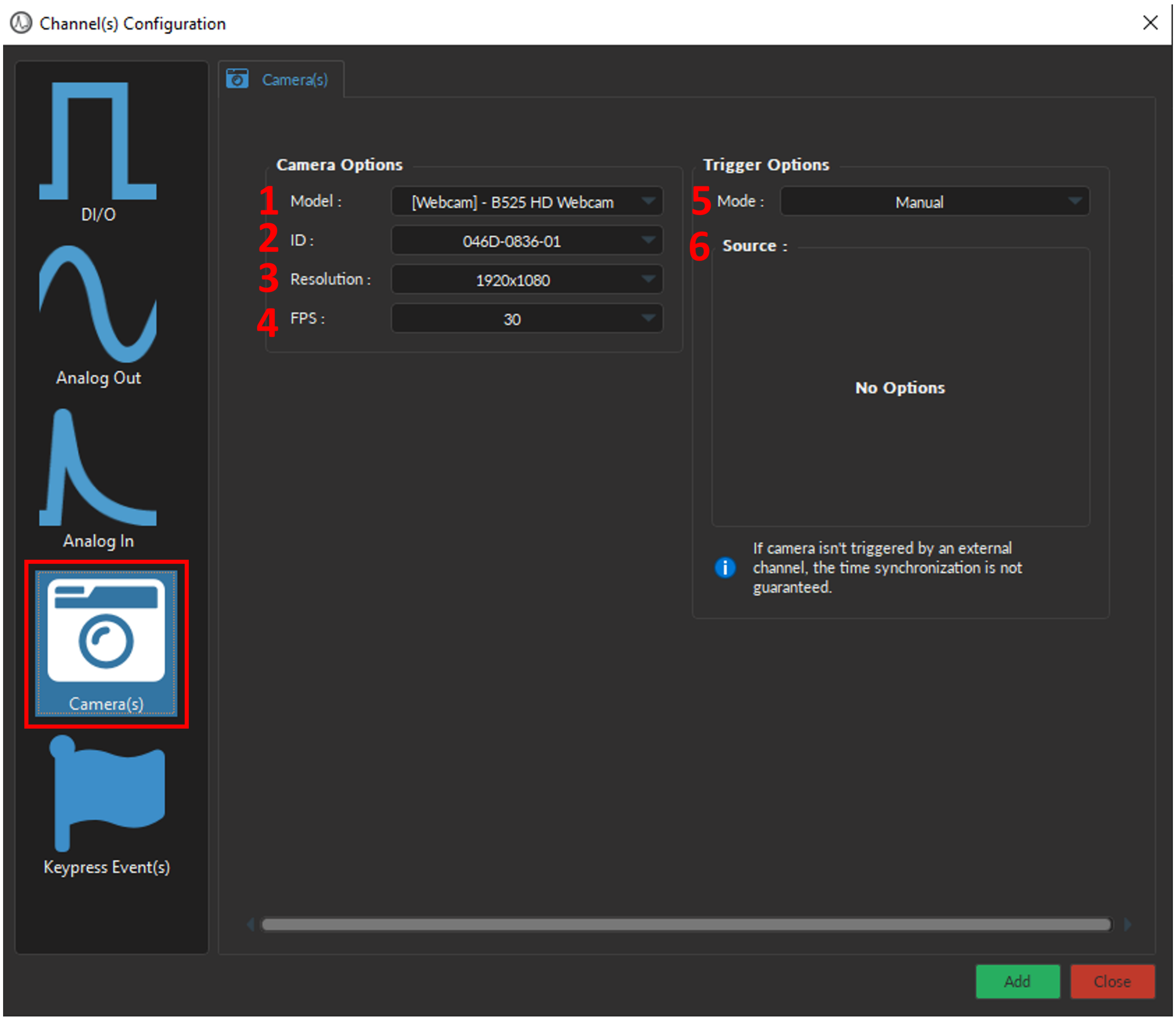


Figure 6.37: *Channel(s) Configuration, Camera*

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

Camera Options:

1. The **Model** (Fig. 6.37, 1) allows you to select the camera of choice based on the type of camera.
2. **ID** drop-down list (Fig. 6.37, 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 **Resolution** (Fig. 6.37, 3) is used to set the image size. The larger 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. 6.37, 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 5-30 for web cameras and up to 60 FPS for the *Doric Behavior Camera*.

Trigger Options:

5. The **Mode** (Fig. 6.37, 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 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. 6.38). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

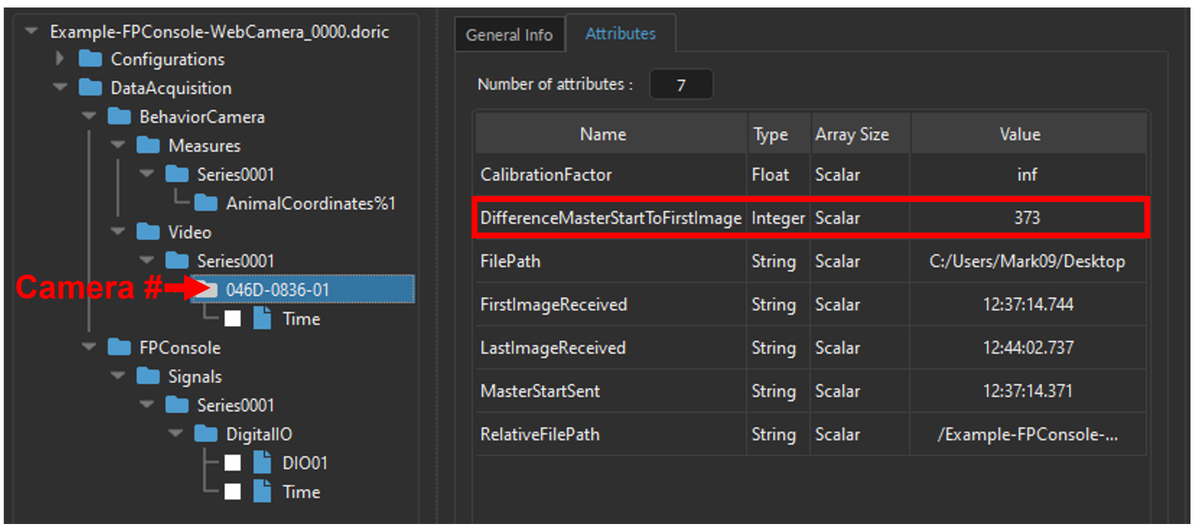


Figure 6.38: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **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. 6.37, 6 & Fig. 6.39) 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. 6.39). For a detailed description of each Digital I/O parameter see the corresponding section in the Fiber Photometry System Manual. Briefly, key parameters include:

   a) The **Channel** (Fig. 6.39, 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. 6.39, 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. 6.39, 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. 6.39, d) is by default 50%. The frame will be taken at the start of each square pulse.

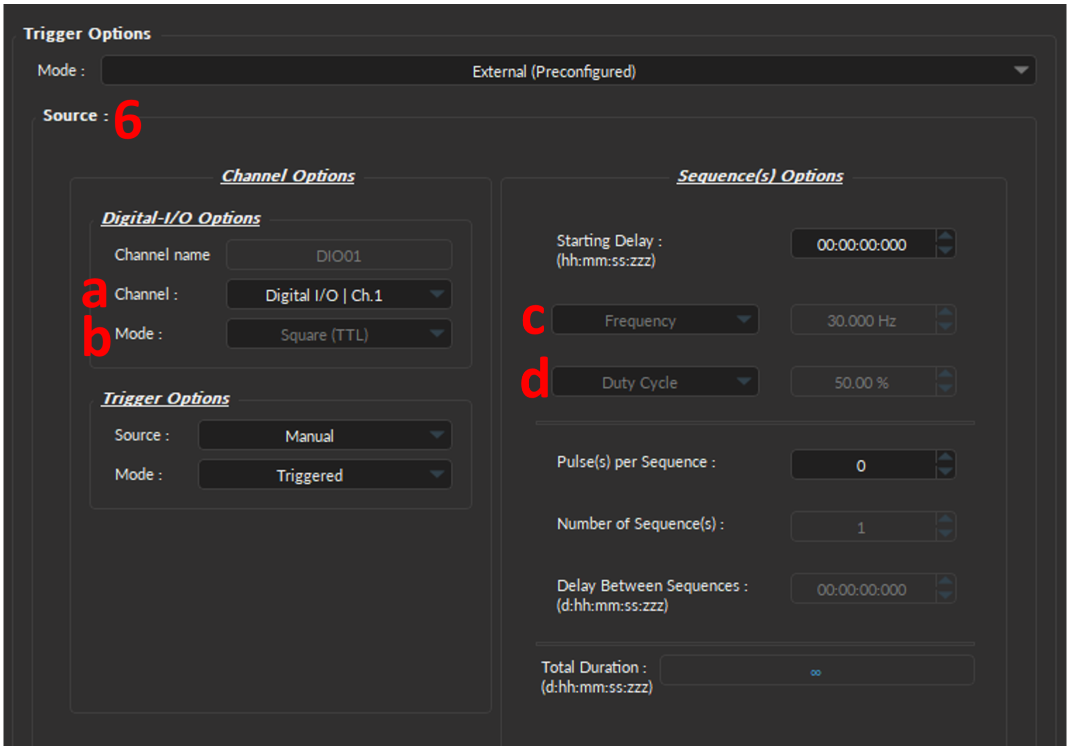


Figure 6.39: *Channel(s) Configuration, Camera - External (Preconfigured)*

6.4.7 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, door opening, construction noise, etc.
- Records 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.

6.4.7.1 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the bottom of the window (Fig. 6.40, left). To remove a KeyPress, use the - button (Fig. 6.40, 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.6.40).

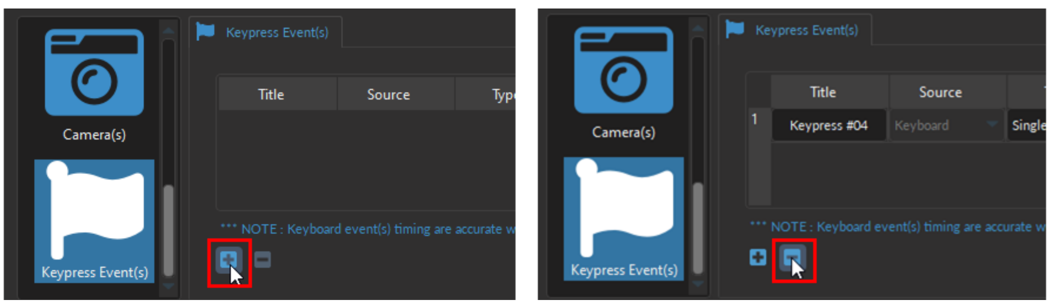


Figure 6.40: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 6.41) in the **Acquisition View**.

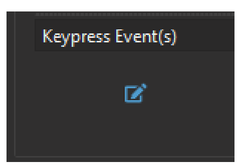
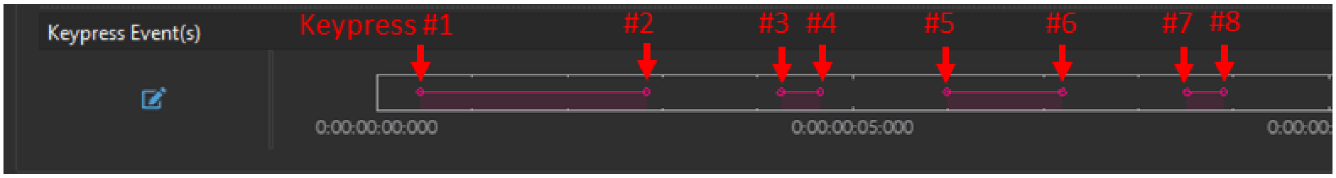


Figure 6.41: *Edit Keypress Event(s) Channel*

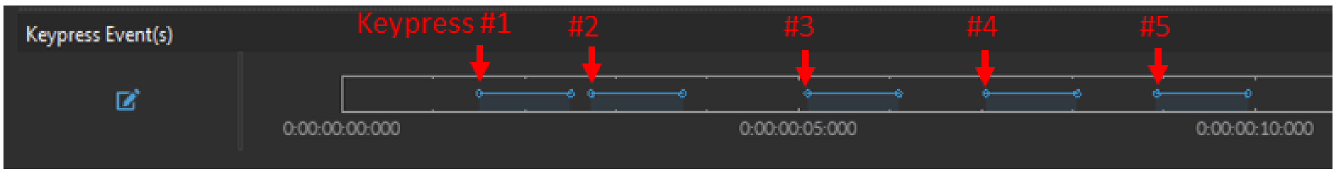
The following are the configurable parameters of a **Keypress Event,** per Fig. 6.43:

1. The **Title** (Fig. 6.43, 1) allows you to give a name for the Keypress event.
2. The **Source** Fig. 6.43, 2) is by default *Keyboard*.
3. Three **Types** of Keypress Event(s) Fig. 6.43, 3) can be specified with the drop-down list:
    - **Single** - Records single event at the touch of a key (Fig. 6.42a).
    - **Toggled** - Records the start and end of an event using the same key. The first press denotes the start of the event while the second press denotes the end of it (Fig. 6.42b).
    - **Timed** - Records an event for a predetermined duration of time (Fig. 6.42c). Every keypress is a new event, with the start of the event occurring when the key was depressed.

(a) *Single*



(b) *Toggled*



(c) *Timed*

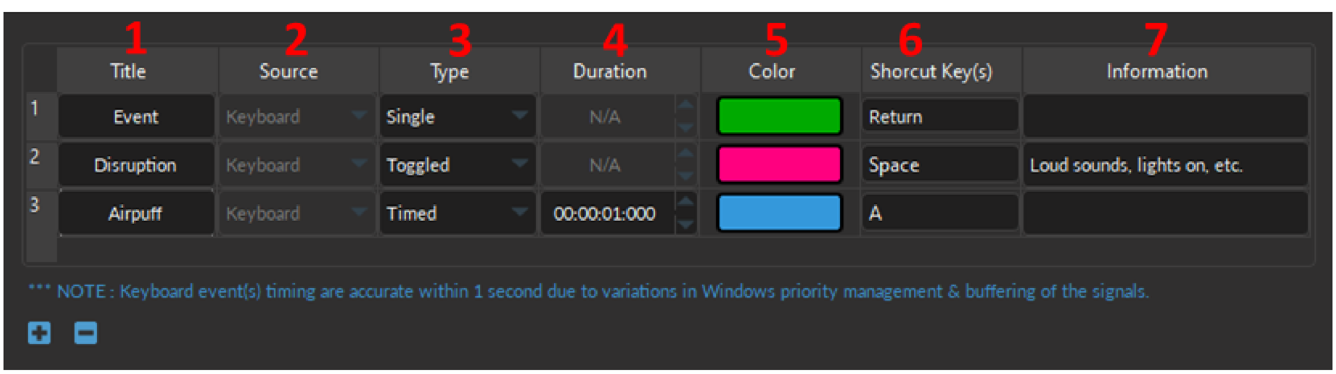
Figure 6.42: *Three types of Keypress Event(s)*

4. The **Duration** Fig. 6.43, 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** field Fig. 6.43, 5) 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. 6.43, 6) can be any keyboard key, including space bar, enter, backspace, any letters, number,s 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. 6.43, column 6).

7. The **Information** column Fig. 6.43, 7) provides space to make notes or write a short description of the Keypress Event.



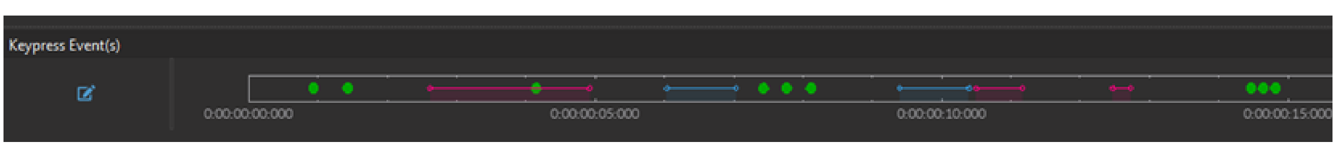


Figure 6.43: *Channel(s) Configuration, KeyPress Event(s)*

6.5 Acquisition View

The **Acquisition View** displays all the information concerning active channels: **Control box** (Fig.6.44) and the **Graphs** (Fig.6.45).

If neither **Control Box** nor **Graphs** are displayed in the **Acquisition View**, this means channels have yet to be configured. The user can either use the **Load Configuration** button (see Section 6.3.2) to load a *.doric* file with previously saved channel parameters, or the user can manually add channels using the **Add Channel** button (see Section 6.3.2).

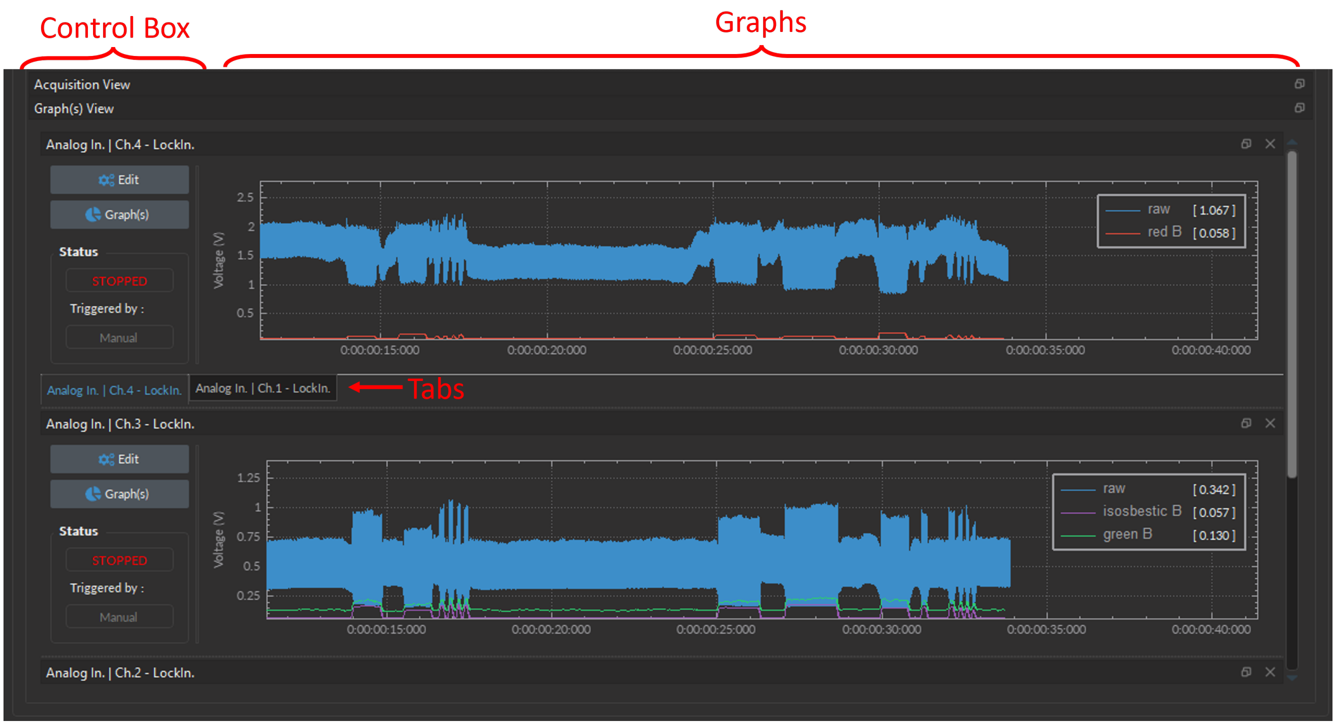


Figure 6.44: *Acquisition View Interface*

6.5.1 Channel Control Box

Each channel **Control box** shows the following basic elements (Fig.6.45), with additional elements available for specific channel types:

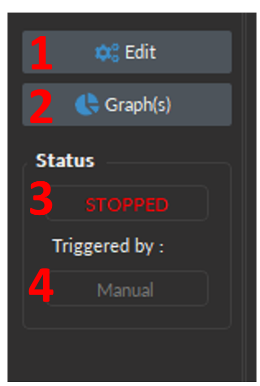


Figure 6.45: *Control Box*

1. The **Channel Name** (Fig. 6.45, 1) is located on the upper left of the **Control box**, identifying the type of channel and its number, corresponding to that on the console. This name can be modified in the **Graph options** window (Fig. 14.36).

2. The **Edit** button (Fig. 6.45, 2) opens the **Channel Configuration** window, where channel parameters can be modified (See section 6.4.1).

3. The **Graph(s)** button opens the **Graph Options** window (Fig. 14.36) corresponding to the channel whose graph will be modified. This window allows users to configure the visualization and naming parameters of each channel graph (Fig. 14.36). If a channel has multiple traces, parameters to configure each trace individually will appear automatically on different rows. **Graph(s) Options** parameters (Fig. 14.36) are as follows:

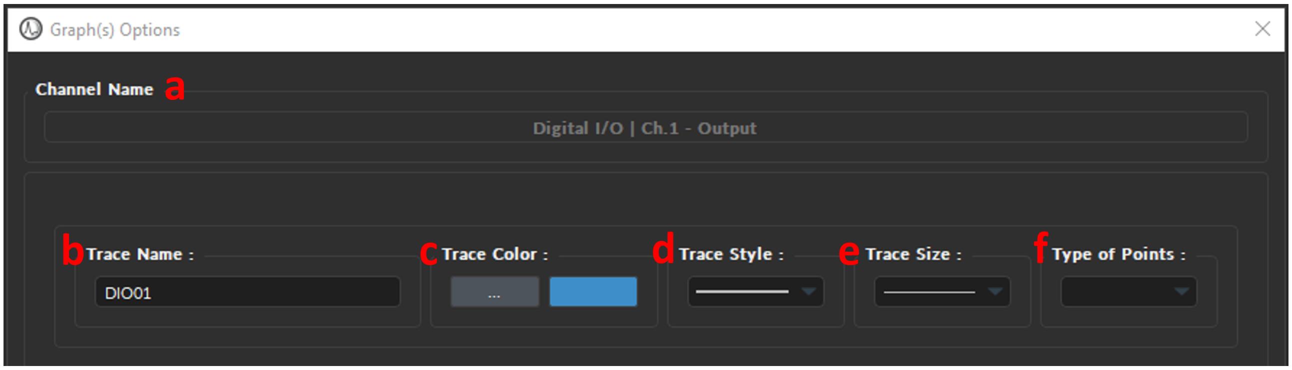


Figure 6.46: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 14.36, a) 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. 14.36, b) allows users to specify a name for the trace, instead of the default name generated by the software.
c) The **Trace Color** button (**...**) (Fig. 14.36, c) opens the **Color Select** window (Fig. 14.37), 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. 14.36, d) 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. 14.36, e) 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. 14.36, f) allows the selection of what type of point is used to indicate data points on the trace. Using different point types than the default (none) may result in slower display and performance degradation.

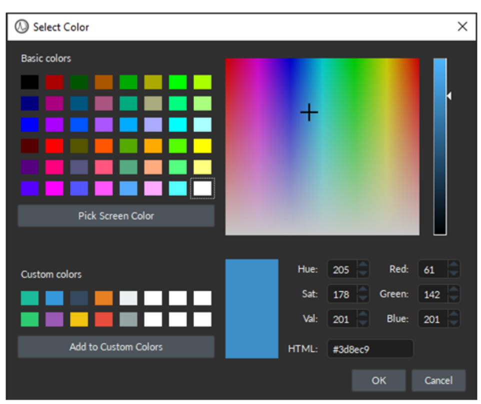


Figure 6.47: *Select Color Window*

4. The **Status** bar (Fig. 6.45, 4) displays acquisition status. **STOPPED** is displayed when the acquisition is inactive, and **STARTED** when acquisition is active.

5. The **Triggered by:** (Fig. 6.45, 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.

6.5.2 Channel Graph Visualisation

Besides editing the trace of the channel **Graph**, which can be done through the **Edit** button of the **Control box** (section 6.5.1), 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.

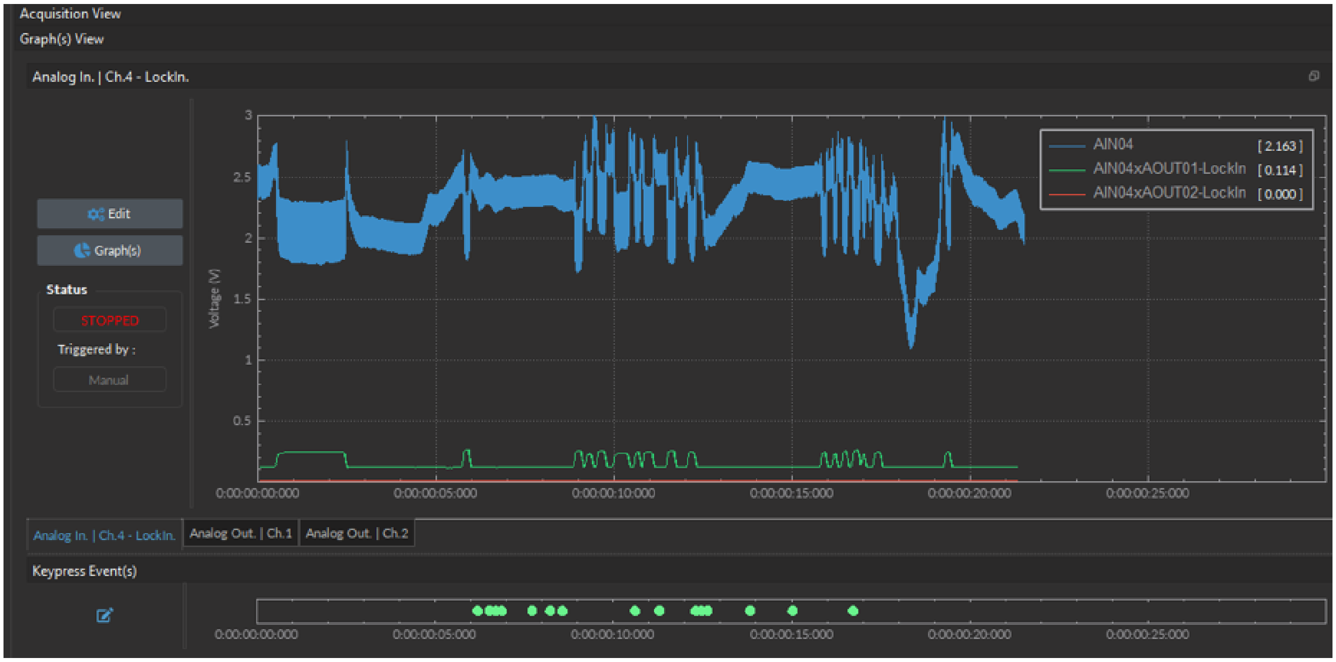


Figure 6.48: *Acquisition View - Graph*

- *Axis Options* - Each **Graph** 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** (Fig. 14.39). Any changes done on a horizontal axis will change the axis limits for every channel.

Figure 6.49: *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. 14.40). 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 an instantaneous value, double-click on the dot.

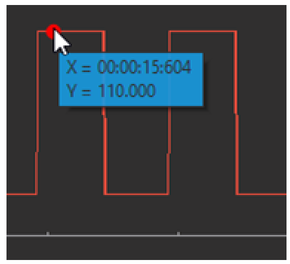


Figure 6.50: *Acquisition View - Instant values*

- The **Channel tabs** (Fig. 6.44, 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 un-docking one channel and moving it above another until it turns blue, then releasing it.
- Analog output channels display an **Active state** graph (Fig. 6.51, 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.6.51, right panel), showing a preview of the pulse sequence.

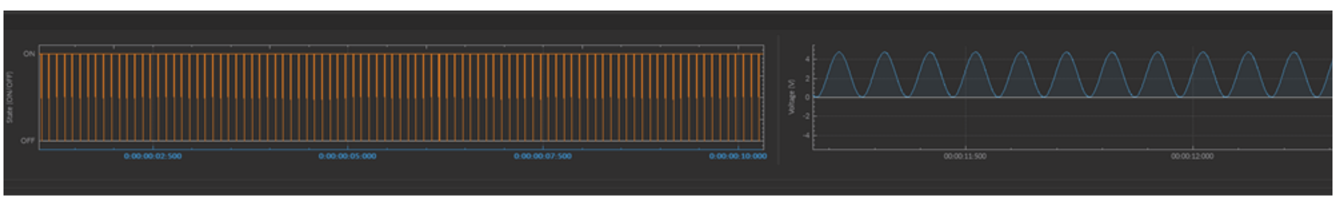


Figure 6.51: *Acquisition View - Output graph*

7

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 into the **Acquisition Console** module as a **Camera Channel**, providing a simultaneous view of the fiber photometry and behavior recordings (Fig. 7.14). See section 7.2 for more details.

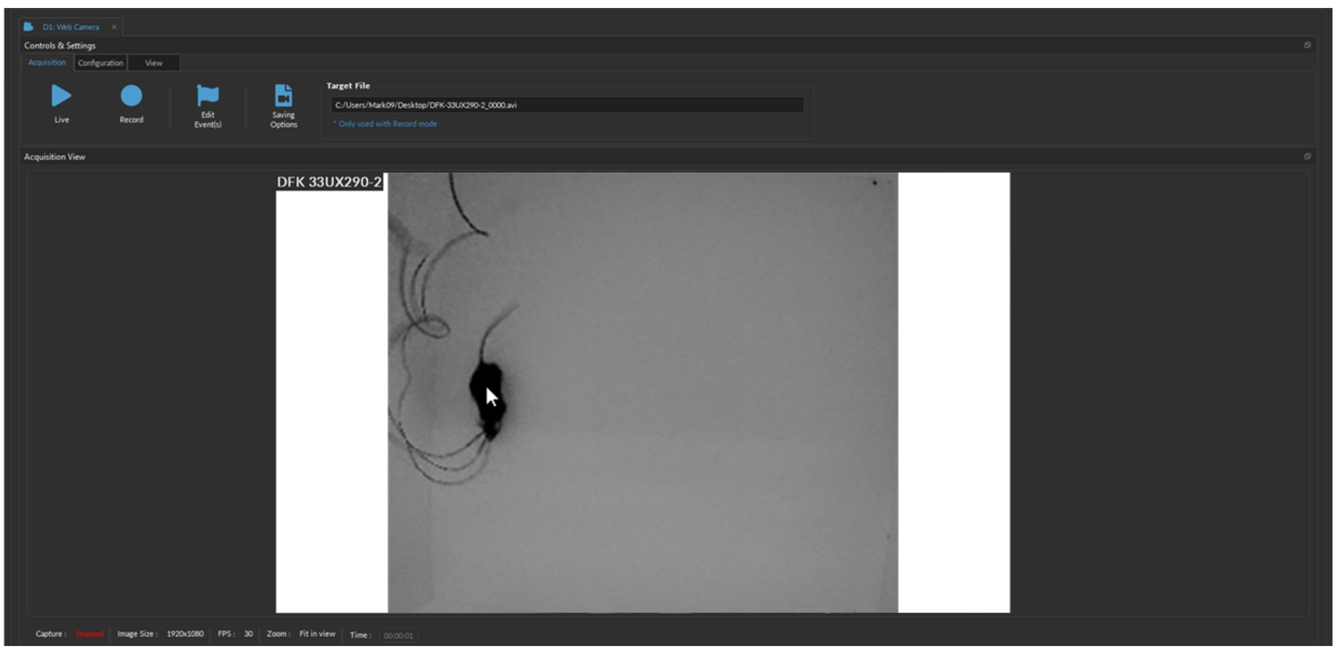


Figure 7.1: *Web Camera Module*

While most of the features and the user interface layout are identical between both modules, there are a few key differences (Table 7.1).

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. 7.2). If the camera name is under the *Camera* device tab (Fig. 7.2), then in most cases it will be compatible with the **Web Camera** module.

Table 7.1: *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

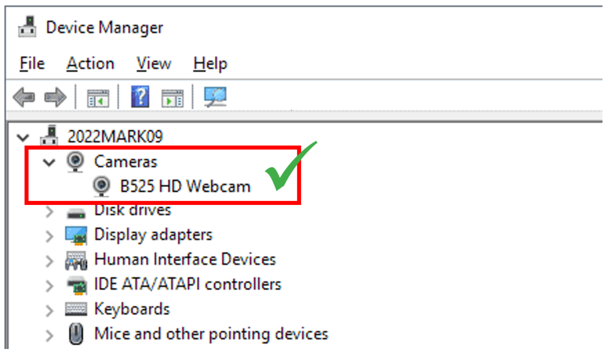


Figure 7.2: *Compatible Web Cameras located under Device Manager Camera tab*

7.1 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 7.1.1
2. The **Configuration** tab - Section 7.1.2
3. The **View** tab - Section 7.1.3

7.1.1 Acquisition Tab

The **Acquisition** tab (Fig. 7.3) contains the controls to start, stop, and save behavior footage, and includes the following elements:

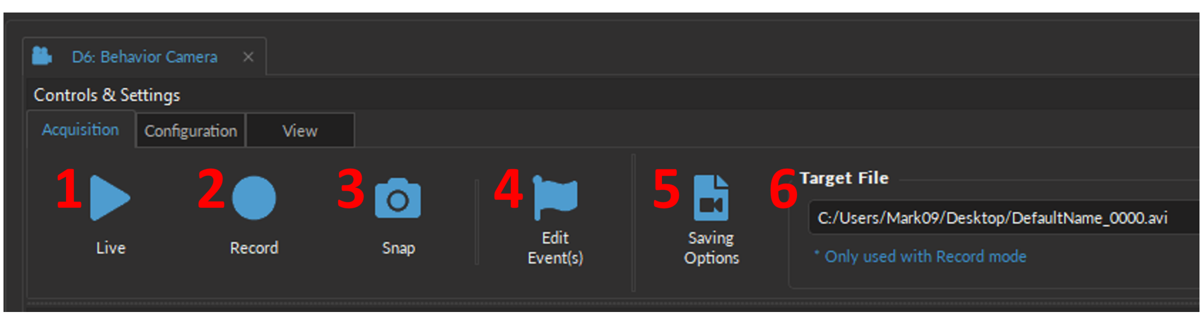
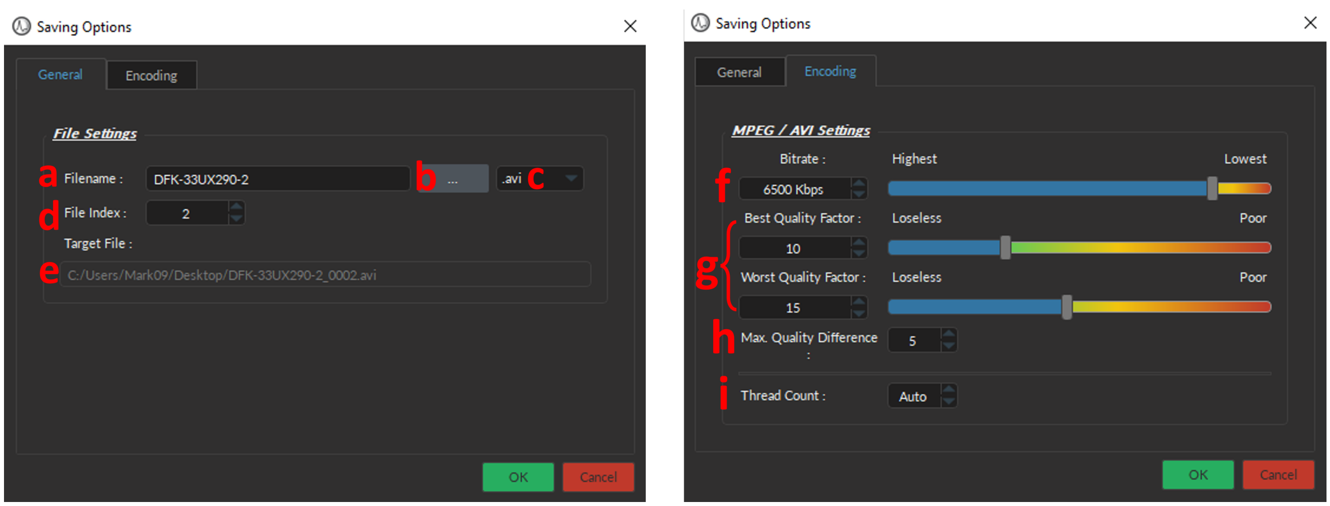


Figure 7.3: *Control & Settings, Acquisition tab*

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

1. The **Live** button (Fig. 7.3, 1) acquires images and displays them. These images are for display only and cannot be saved.
2. The **Record** button (Fig. 7.3, 2) acquires a continuous image stream and saves it to a user-defined file as one .AVI file.
3. The **Snap** button (Fig. 7.3, 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. 7.3, 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 7.1.5.
5. The **Saving Options** button (Fig. 7.3, 5) opens the **Saving Options** window (Fig. 7.4), which is split into two tabs. The **General** tab (Fig. 7.4a) is used to set file saving parameters, while the **Encoding** (Fig. 7.4b) is used to adjust the camera acquisition parameters.



(a) *General tab* (b) *Encoding tab*

Figure 7.4: *Saving Options window*

Specifically, using the **General** tab user can specify the following (Fig. 7.4a):

a) The **Filename** box (Fig. 7.4a, a) is used to define the name of the recorded video file.
b) The **[...]** button (Fig. 7.4a, 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. 7.4a, c).
d) The **File Index** box (Fig. 7.4a, 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. 7.3, 5).

The *Encoding* tab is used to choose video encoding quality (Fig. 7.4b). Most elements can be changed using the appropriate **Text Box** or **Slider**.

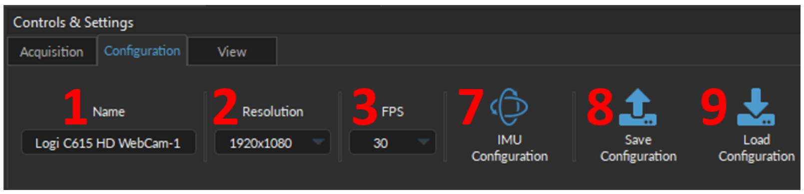
f) The **Bitrate** (Fig. 7.4b, f) sets the number of bits recorded per second. Larger resolution images require a higher **Bitrate**.
g) The **Best Quality Factor** and **Worst Quality Factor** (Fig. 7.4b, g) are used to define the compression of saved video, with a factor of 1 implying no compression, 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. 7.4b, h) indicates the maximal compression difference between two subsequent video frames.

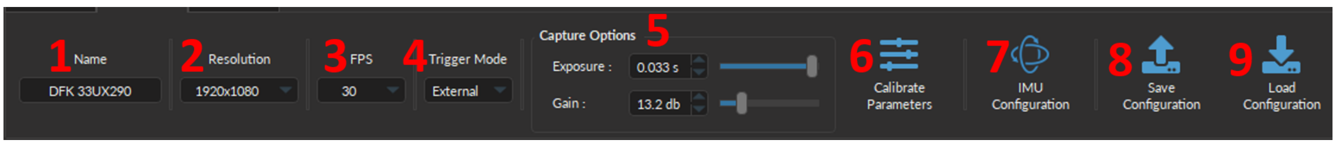
i) The **Thread Count** (Fig. 7.4b, i) defines the number of processing threads (real and virtual) used on the CPU. There is a maximum of 16 threads. Using more threads can provide better resolution and FPS, though it is more demanding on the CPU.

7.1.2 Configuration Tab

The **Configuration** tab is useful to save and load camera settings and includes the following options (Fig. 7.5):



(a) *Web Camera*



(b) *Behavior Camera*

Figure 7.5: *Camera Module Comparison, Configuration Tab*

1. The **Name** (Fig. 7.5, 1) displays the serial number of the camera currently in use.

2. The **Resolution** (Fig. 7.5, 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 for the **Behavior Camera**.

3. The **FPS** (Fig. 7.5, 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. 7.5b, 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. 7.5b, 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.

6. The **Calibrate Parameters** button (Fig. 7.5b, 6 - NOT available for *Web Camera* module) will automatically set either setting based on the current live detection. It also applies a White Balance, which 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. We recommend using the Calibrate Parameters. We recommend using the Calibrate Parameters option.

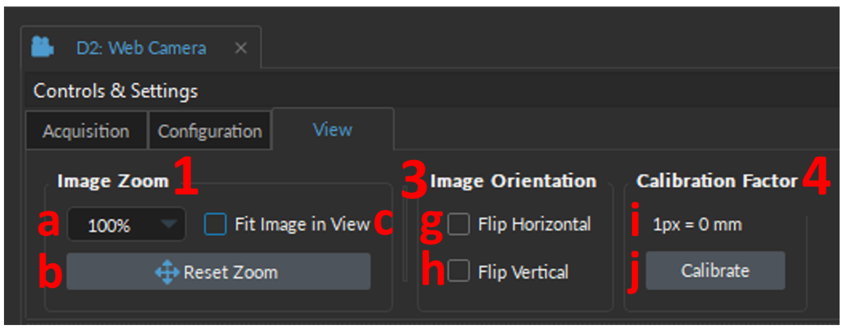
7. The **IMU Configuration** button (Fig. 7.5, 7) can be used to add an Inertial Measurement Unit channel to measure changes in acceleration, head movements and other parameters linked to subject displacement.

8. The **Save Configuration** button (Fig. 7.5, 8) stores the current settings (from Acquisition, Configuration, and View tabs) in a *.doric* file for future use.

9. The **Load Configuration** button (Fig. 7.5, 9) allows users to open a previously saved *.doric* configuration file and will automatically preset all the parameters of the **Web Camera** module.

7.1.3 View Tab

The **View** tab (Fig. 7.6) 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 7.6: *Camera Module Comparison, View Tab*

1. The **Image Zoom** (Fig. 7.6, 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. 7.6, a) specifies the zoom factor for the image display, which ranges between 10%-500%.

   b) The **Reset Zoom** (Fig. 7.6, b) button returns the zoom factor to 100%.

   c) The **Fit Image** checkbox (Fig. 7.6, c) automatically adjusts the image to fit the entire Acquisition View.

2. The **Image Offset** (Fig. 7.6b, 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 of the **Image Zoom**.

   d) The **Auto-center** checkbox (Fig. 7.6b, 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. 7.6b, e) allows users to move the camera image horizontally by the selected number of pixels.

f) The **Y Offset** slider (Fig. 7.6b, f) allows users to move the camera image's vertical axis by the selected number of pixels.

3. The **Image Orientation** (Fig. 7.6, 3) contains parameters that control the direction of the image displayed in the **Acquisition View**:

g) The **Flip Horizontal** checkbox (Fig. 7.6, g) displays a mirrored image where the left side becomes the right, and vice versa.

h) The **Flip Vertical** checkbox (Fig. 7.6, h) displays a mirrored image where the top becomes the bottom and vice versa.

4. The **Calibration Factor** box (Fig. 7.6, 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. 7.7, 5) which are required to calculate a new **Calibration Factor**. The image calibration can only be done once the **Live** / **Record** mode is started and stopped. Note that once the **Calibrate** button is selected, it turns into the **Apply** button (Fig. 7.7, j).

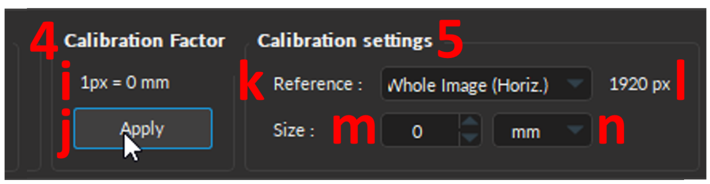


Figure 7.7: *Camera Module - Calibration*

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. 7.7, i).

k) The **Reference** drop-down list (Fig. 7.7, 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. 7.8). 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. 7.7, l - 1920px).

m) The **Size** text-box (Fig. 7.7, m & n) specify the real dimensions of the reference and its unit (mm, cm, or inches). Select the **Apply** button (Fig. 7.7, j) to recalculate the **Calibration Factor** using the new **Size** .

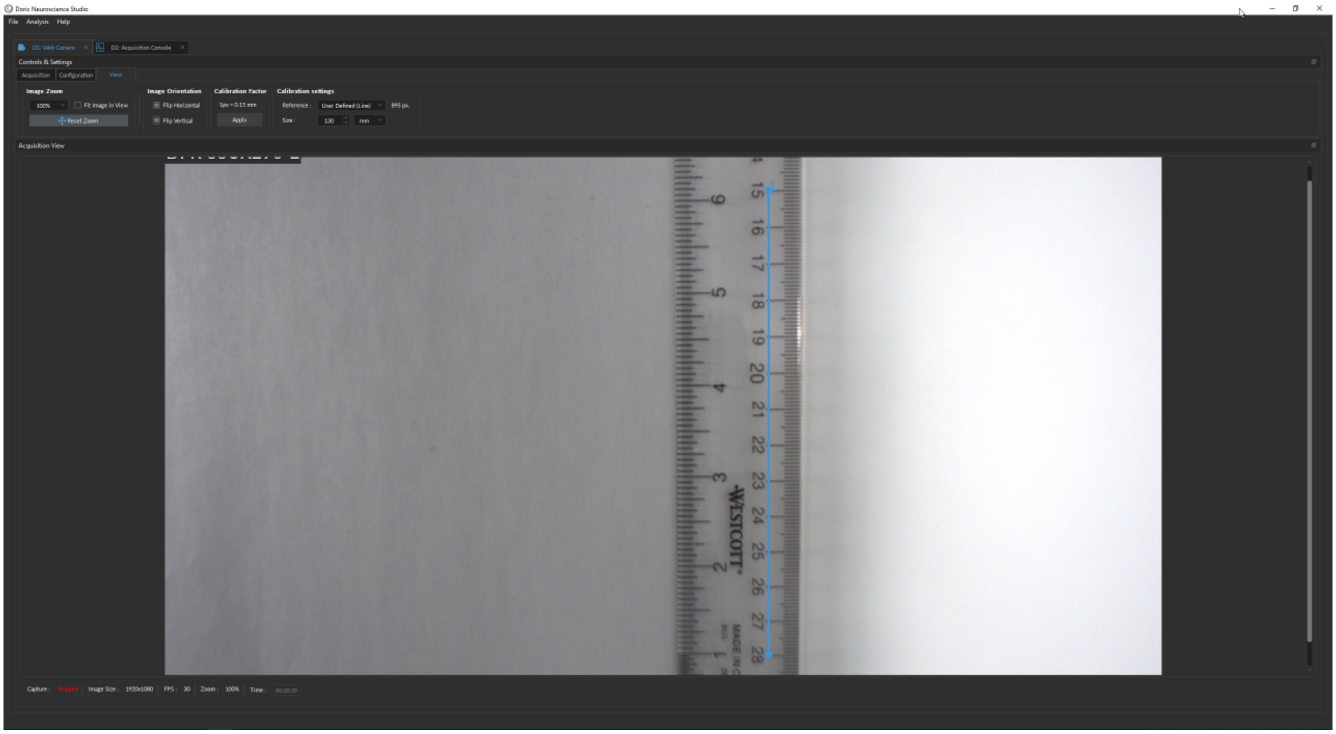


Figure 7.8: *Camera Module - Calibration User-defined line*

7.1.4 Live feed monitoring bar

The constant live feed allows the user to quickly track the status of the camera feed.

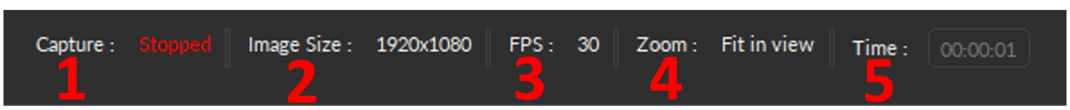


Figure 7.9: *Camera Module - Live Feed Monitoring bar*

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 7.1.2 - no. 2.
3. The **FSP** displays the value selected in Section 7.1.2 - no. 3.
4. The **Zoom** displays the value selected in Section 7.1.3 - no. 1a, or 1c.
5. The **Time** displays the time since the camera was turned on.

7.1.5 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, door opening, construction noise, etc.
- Records 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.

7.1.5.1 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the bottom of the window (Fig. 7.10, left). To remove a KeyPress, use the - button (Fig. 7.10, 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.7.10).

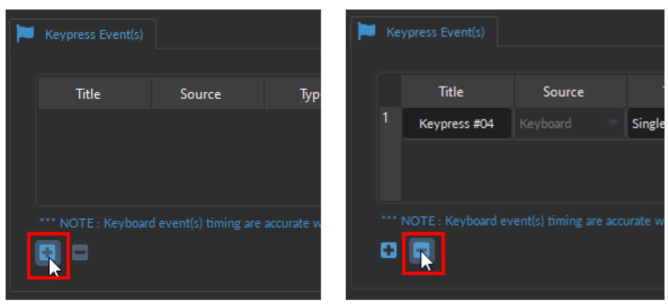


Figure 7.10: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 7.11) in the **Acquisition View**.

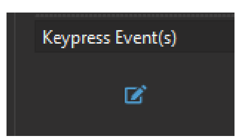
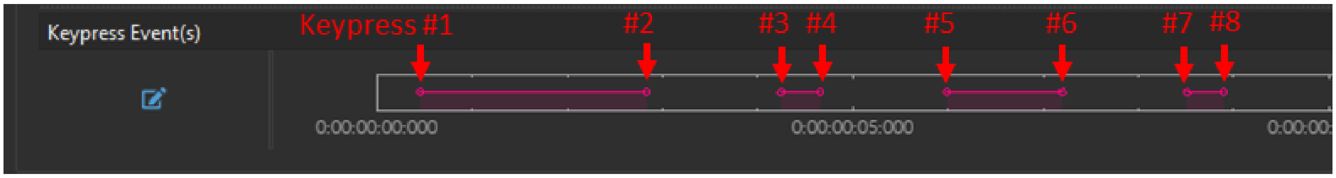


Figure 7.11: *Edit Keypress Event(s) Channel*

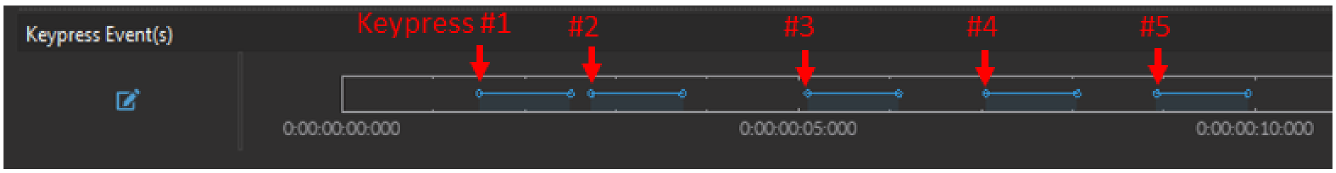
The following are the configurable parameters of a **Keypress Event,** per Fig. 7.13:

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. 7.12a).
   - **Toggled** - Records the start and end of an event using the same key. The first press denotes the start of the event while the second press denotes the end of it (Fig. 7.12b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 7.12c). Every keypress is a new event, with the start of the event occurring when the key is depressed.

(a) *Single*



(b) *Toggled*



(c) *Timed*

Figure 7.12: *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 is properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 7.13, column 6).

7. The **Information** column provides space to make notes or write a short description of the Keypress Event.

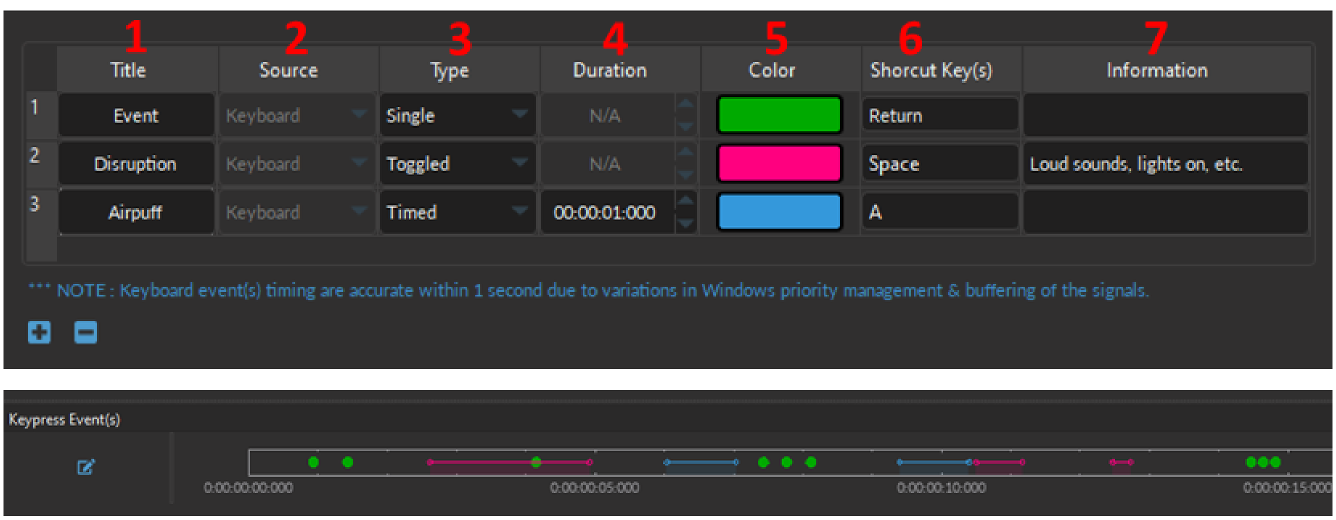


Figure 7.13: *Channel(s) configuration window, KeyPress Event(s)*

7.2 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. 7.14, 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 7.2.1, 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 7.2.1, no. 5) with proper configurations, simplifying set-up.

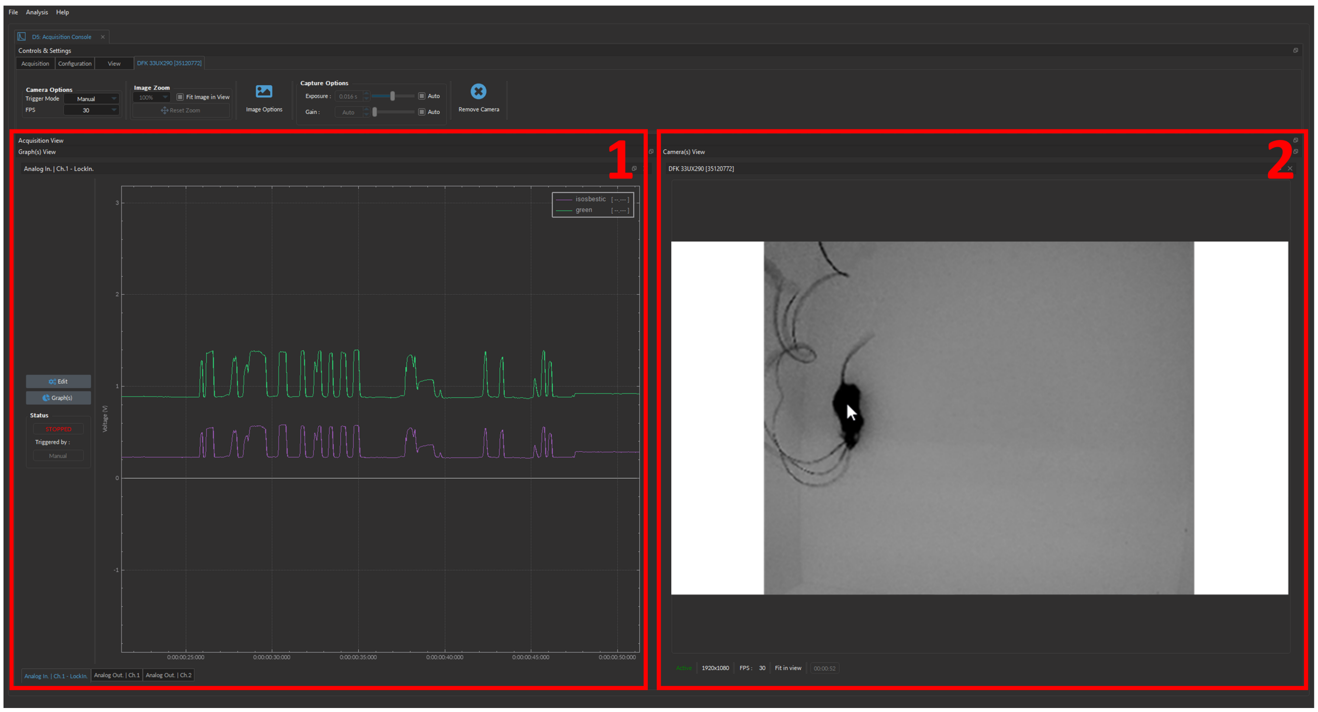


Figure 7.14: *Integrating Camera into Acquisition Console module*

7.2.1 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. 7.15). This will open the *Channel(s) Configuration* window (Fig. 7.16).

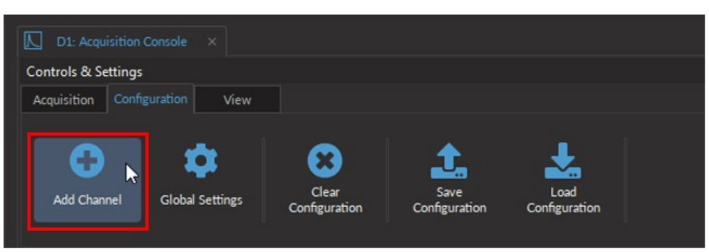


Figure 7.15: *Add Channel button opens the Channel Configuration window*

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

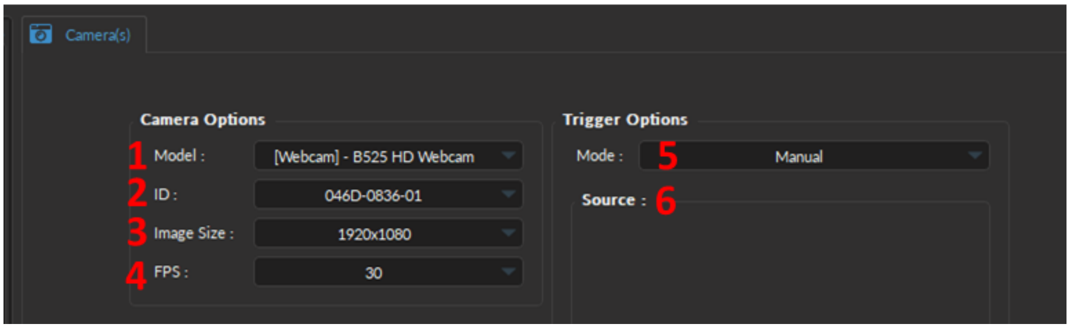


Figure 7.16: *Channel(s) configuration window, Camera Channel*

Camera Options:

1. The **Model** (Fig. 7.16, 1) allows you to select the camera of choice based on the type of camera.
2. **ID** drop-down list (Fig. 7.16, 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. 7.16, 3) sets the resolution of the image. The larger 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. 7.16, 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. 7.16, 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. 7.17). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

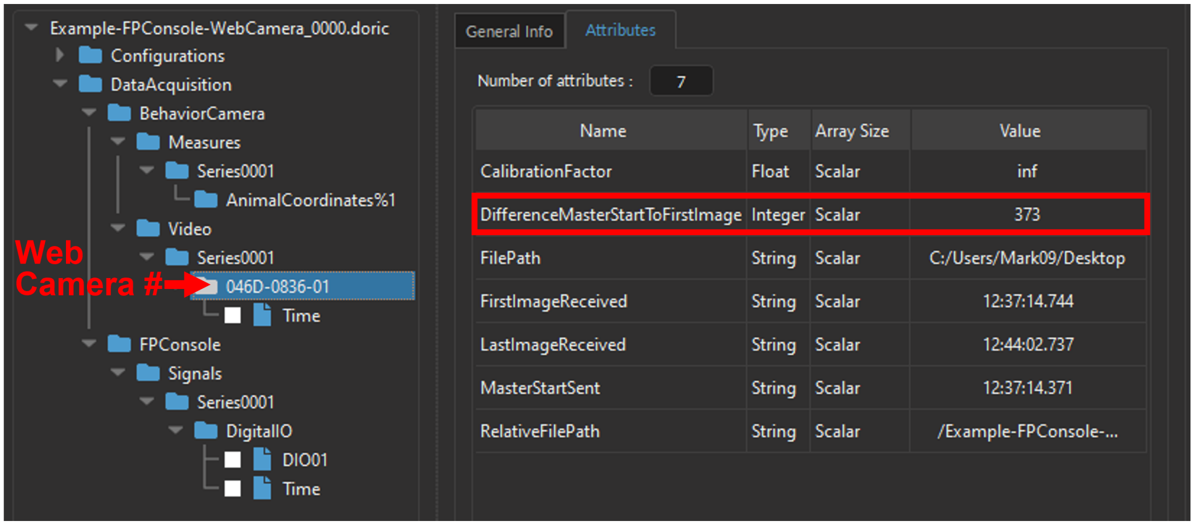


Figure 7.17: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **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. 7.16, 6 & Fig. 7.18) 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. 7.18). For a detailed description of each Digital I/O parameter see the corresponding section in the Fiber Photometry System Manuel (Section 6.4.3). Briefly, key parameters include:

   a) The **Channel** (Fig. 7.18, 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. 7.18, 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. 7.18, 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. 7.18, d) is by default 50%. The frame will be taken at the start of each square pulse.

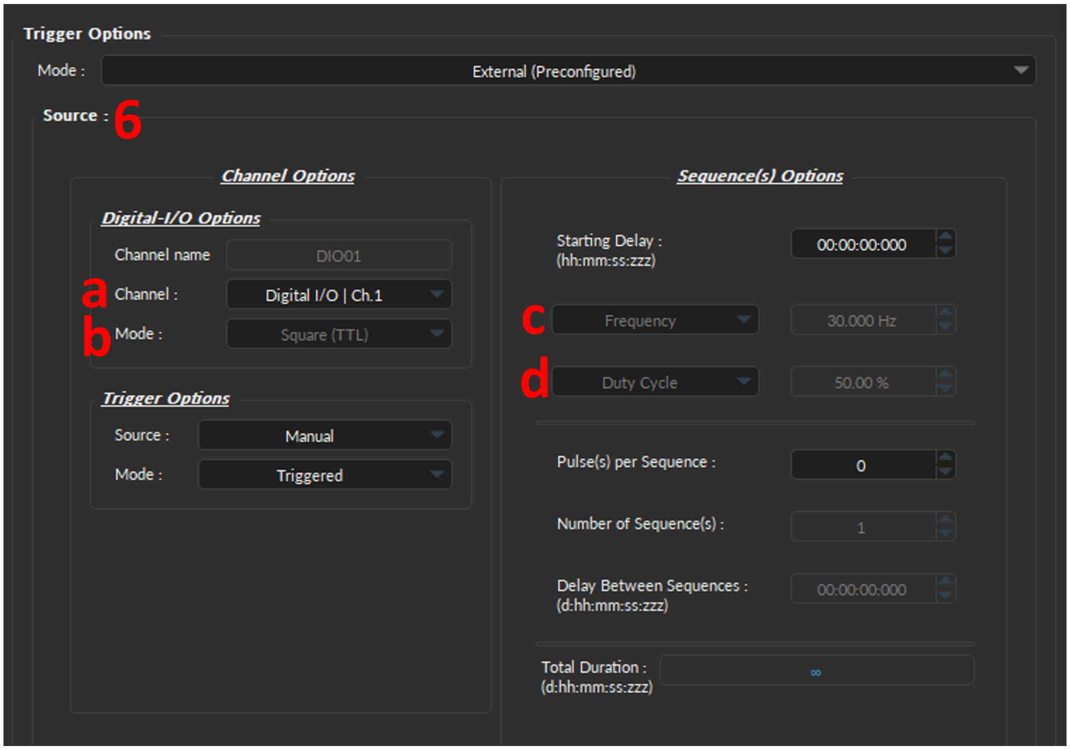
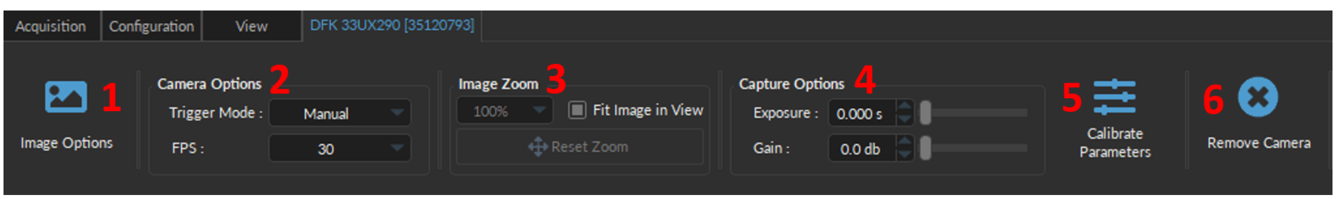


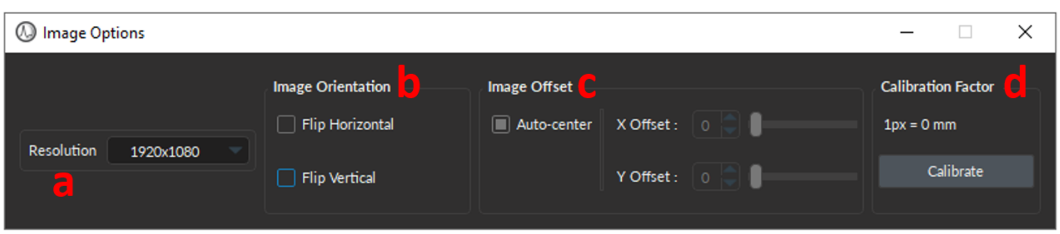
Figure 7.18: *Camera Channel - External (Preconfigured) mode*

7.2.2 Camera Control & Settings within Acquisition Console

Most of the Camera **Control & Settings** parameters (from Section 7.1) 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 7.19: *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. 7.19a). 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 **Image Options** (Fig. 7.19a, 1) - Opens the **Image Options** window (Fig. 7.19b), which contains the following:
    a) The **Resolution** (Fig. 7.19b, a) - Section 7.1.2, no. 2.
    b) The **Image Orientation** (Fig. 7.19b, b) - Section 7.1.3, no. 3.
    c) The **Image Offset** (Fig. 7.19b, c) - Section 7.1.3, no. 3.
    d) The **White Balance** (Fig. 7.19b, d) - Section 7.1.2, no. 6
    e) The **Calibration Factor** (Fig. 7.19b, e) - Section 7.1.3, no. 4.
2. The **Camera Options** (Fig. 7.19a, 2) - Section 7.1.2, no. 3 & 4.
3. The **Image Zoom** (Fig. 7.19a, 3) - Section 7.1.3, no. 1.
4. The **Capture Options** (Fig. 7.19a, 4) - Section 7.1.2, no. 5.
5. The **Calibrate Parameters** (Fig. 7.19a, 5) - Section 7.1.2 no. 6
6. The **Remove Camera** button (Fig. 7.19a, 6) 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.

8

Microscope

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

(a) *Snap-In* (b) *2-Color* (c) *Gen 3.0 - efocus*

Figure 8.1: *Fluorescence Microscope Drivers*

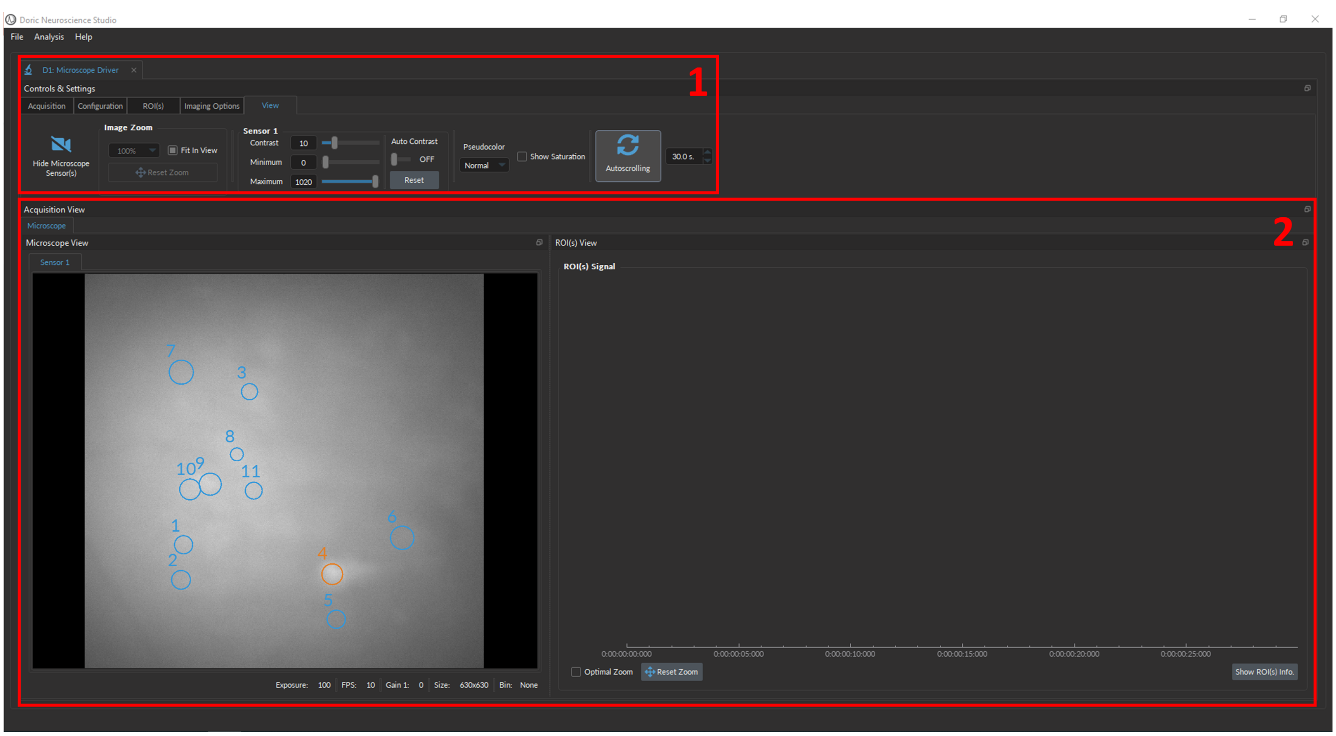


Figure 8.2: *Miscroscope Driver interface*

8.1 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 a USB port (as in Fig. 8.3).

To add a device to the *Doric Neuroscience Studios*, select the device of choice in the *Available device(s)* sections (bottom half of window), then click **Connect Devices**. 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. 8.3).

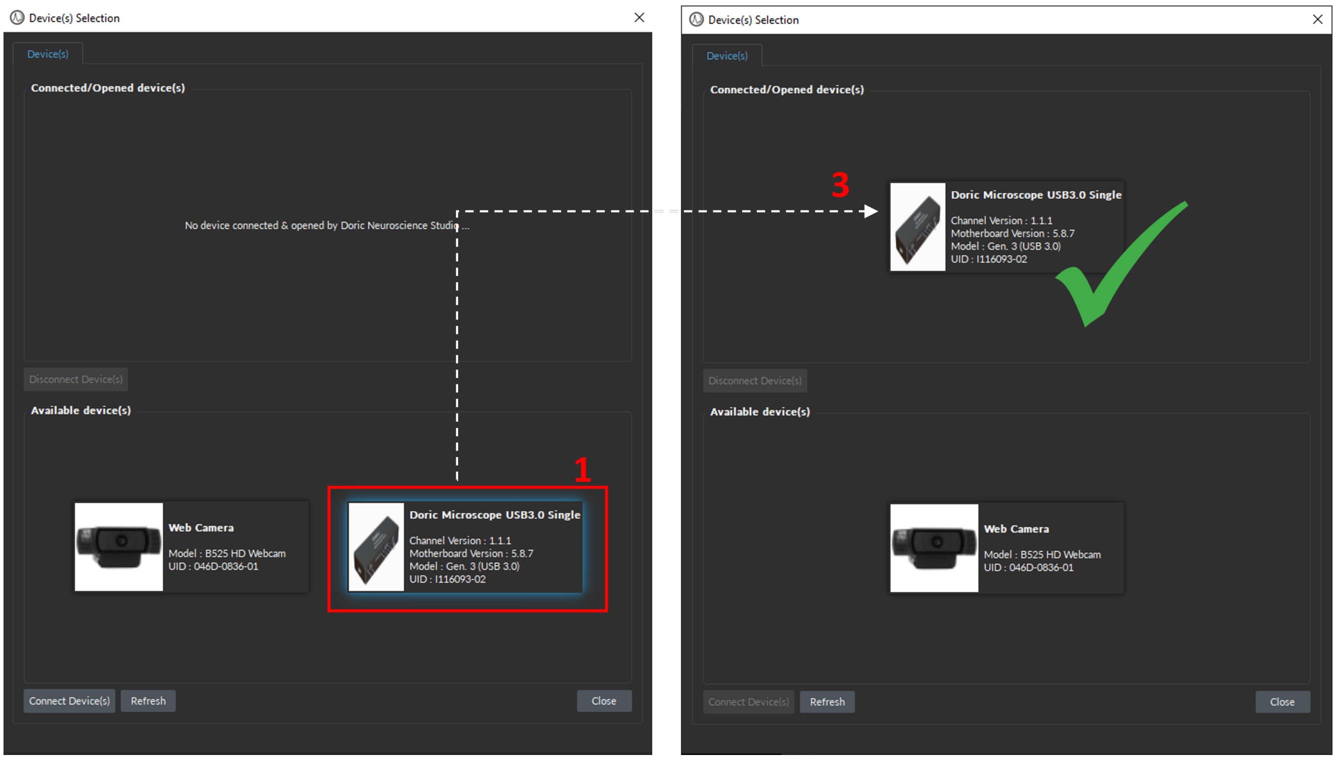


Figure 8.3: *Double click on the device of choice to connect it to Doric Neuroscience Studio*

NOTE: If you have switched to Doric Neuroscience Studio 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. 8.4) or use the hot key: *Ctrl+N*.

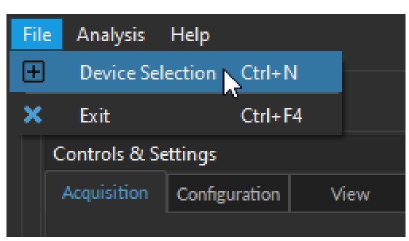


Figure 8.4: *Open Device Selection Window*

Mask Required Warning: If the following warning message pops up (as in Fig. 8.5), see Section 8.6. Note that only the *2-color fluorescence microscope* and the *eFocus fluorescence microscope* require manual Mask loading.

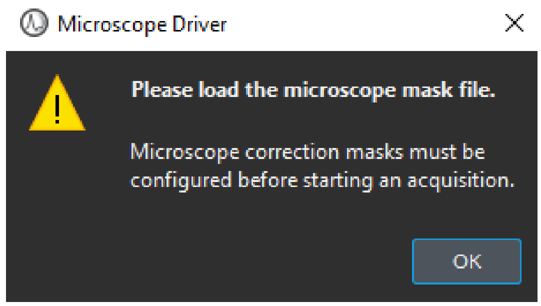


Figure 8.5: *Install Mask warning message*

8.2 Overview

The **Microscope Driver** interface (Fig. 8.6) of *Doric Neuroscience Studio* software is split into two sections: **(1) Controls and Settings tabs** (Section 8.3) are used to manage different elements of the software (Acquisition, Configuration, and View); and (2) the **Acquisition view** (Section 8.5) allows simultaneous visualization of both the **Microscope View** (Section 8.5.2) and the **ROIs View** (Section 8.5.3).

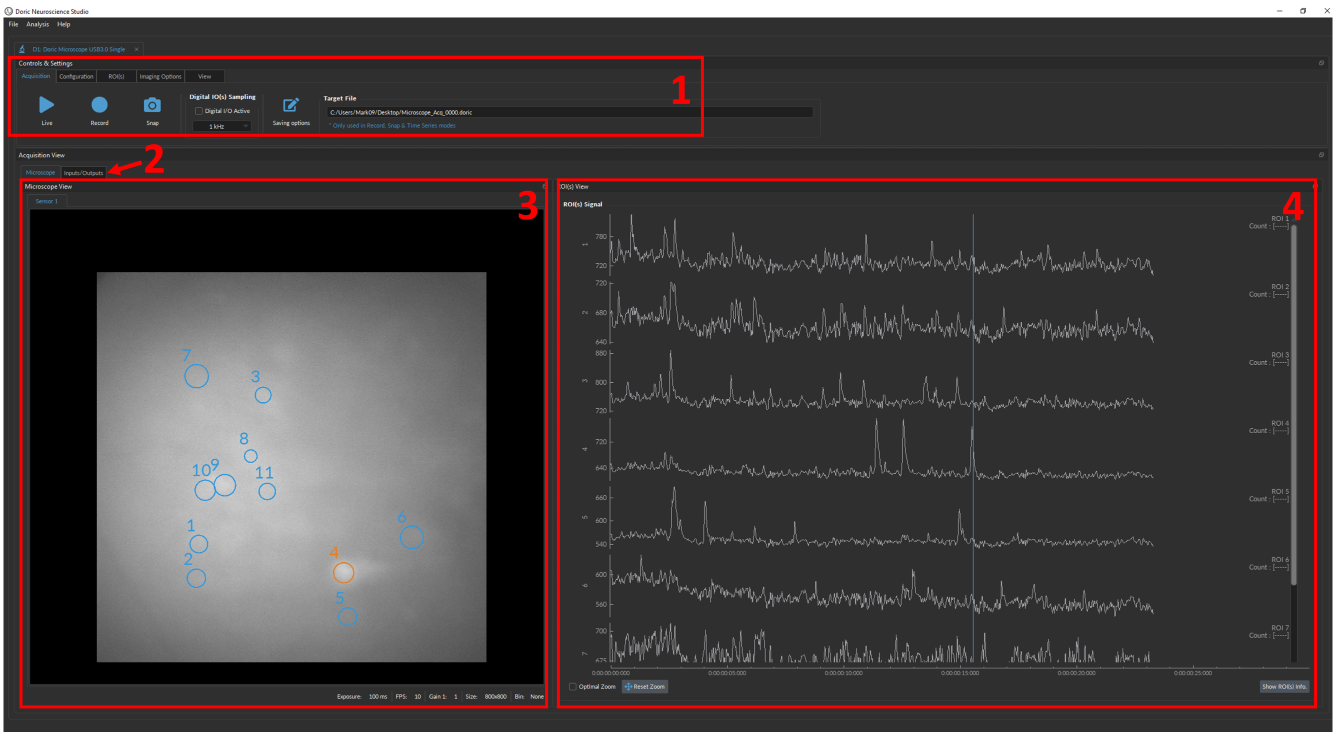


Figure 8.6: *Doric Neuroscience Studio user interface*

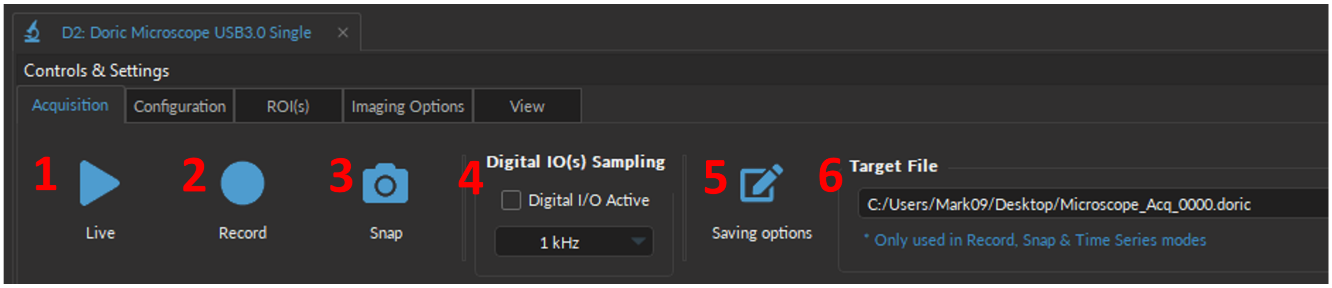
8.3 Controls and Settings tabs

The **Controls and Settings** are used to manage the different parts of the software and are split into five tabs and are treated in the following sections:

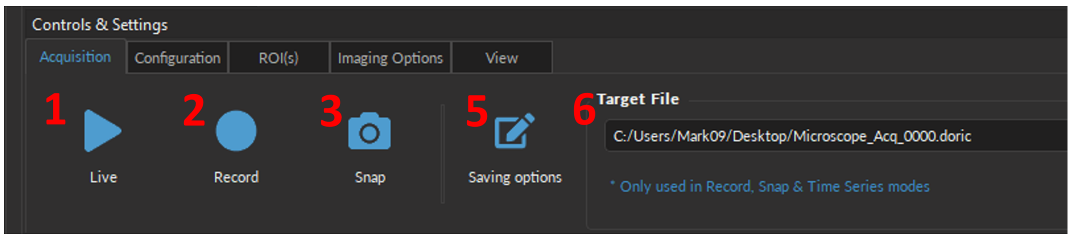
1. *Acquisition* - Section 8.3.1
2. *Configuration* - Section 8.3.2
3. *ROIs* - Section 8.3.3
4. *Imaging Options* - Section 8.3.4
5. *View* - Section 8.3.5

8.3.1 Acquisition Tab

The **Acquisition** tab (Fig. 8.7a) is used to start a live/recording session and sets the **Saving Options**.



(a) *eFocus*



(b) *Snap-In & 2-color*

Figure 8.7: *Microscope, Acquisition Tab*

1. The **Live** button (Fig. 8.7, 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 test the recording software quickly and to ensure that the parameters were properly set and that the recording is working as intended.

2. The **Record** button (Fig. 8.7, 2) activates all prepared channels while periodically saving recorded data to the disk. This mode is recommended for long measurement sequences.

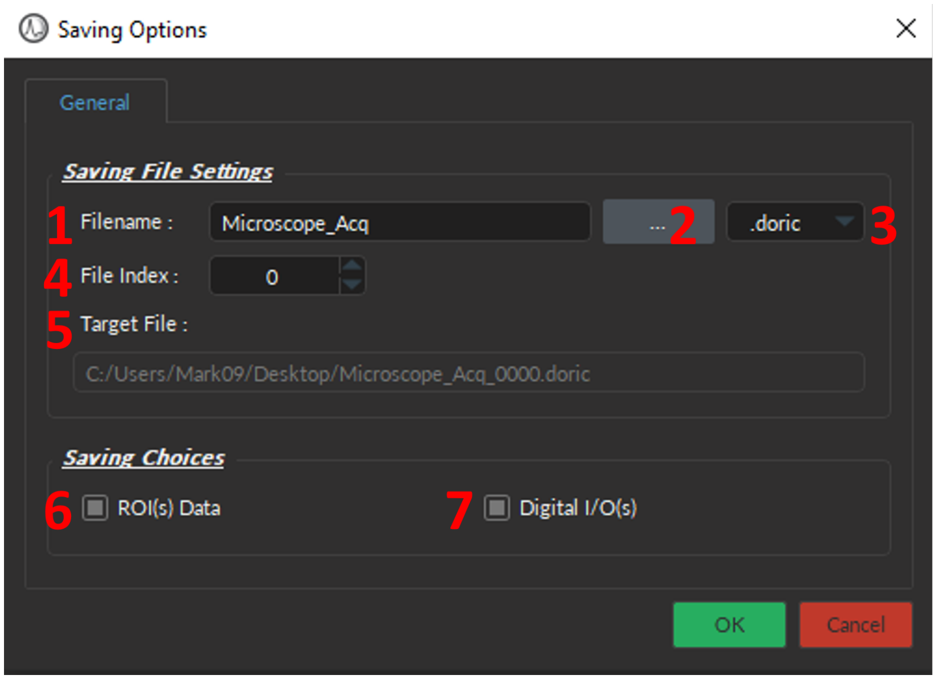
3. The **Snap** button (Fig. 8.7, 3) saves an image per channel in a .doric file. The saved file does not contain ROI or DIO data.

4. The **Digital IO(s) Sampling** (Fig. 8.7a, 4) are available for *efocus Microscope Drivers* only, and include:

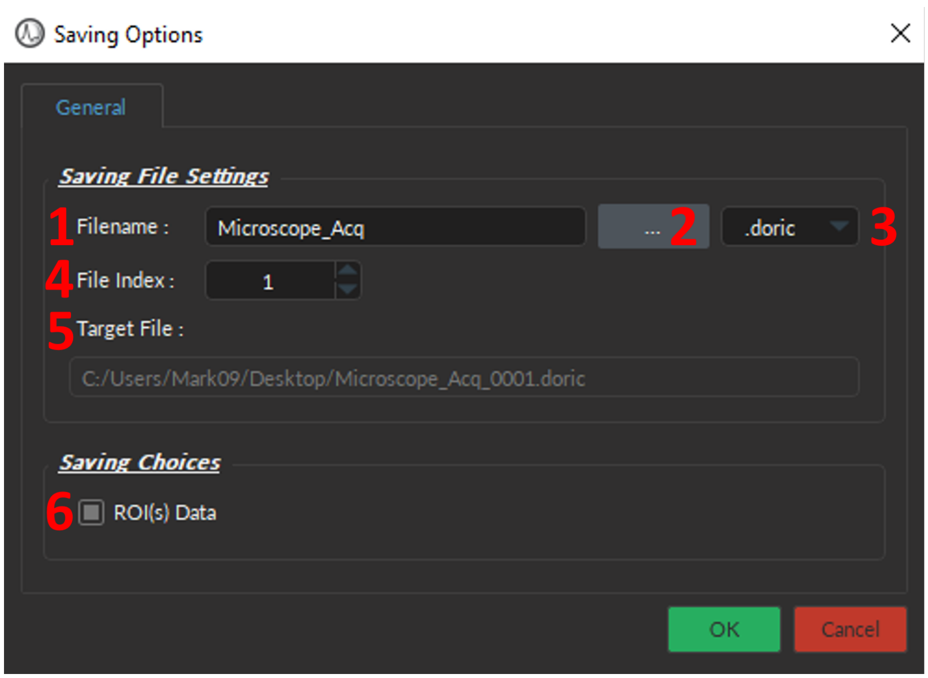
   - The **Digital IO(s) Active** checkbox activates the DI/O channels (displayed in **Inputs/Output View**, Section 8.28). If disabled, even if **Live**/**Record** buttons are selected, the Digital I/O will not run.
   - The **Sampling rate** drop-down list specifies the frequency at which digital inputs and outputs will be collected (in kHz). Three options are available: 1, 5, 10 kHz.

5. The **Saving Options** (Fig. 8.7, 5) button opens the **Saving Options** window (Fig. 8.8a). See section 8.3.1.1 for greater details.

6. The **Target File** (Fig. 8.7, 6) 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.



(a) *eFocus*



(b) *Snap-In & 2-color*

Figure 8.8: *Saving Options Window*

8.3.1.1 Saving Options

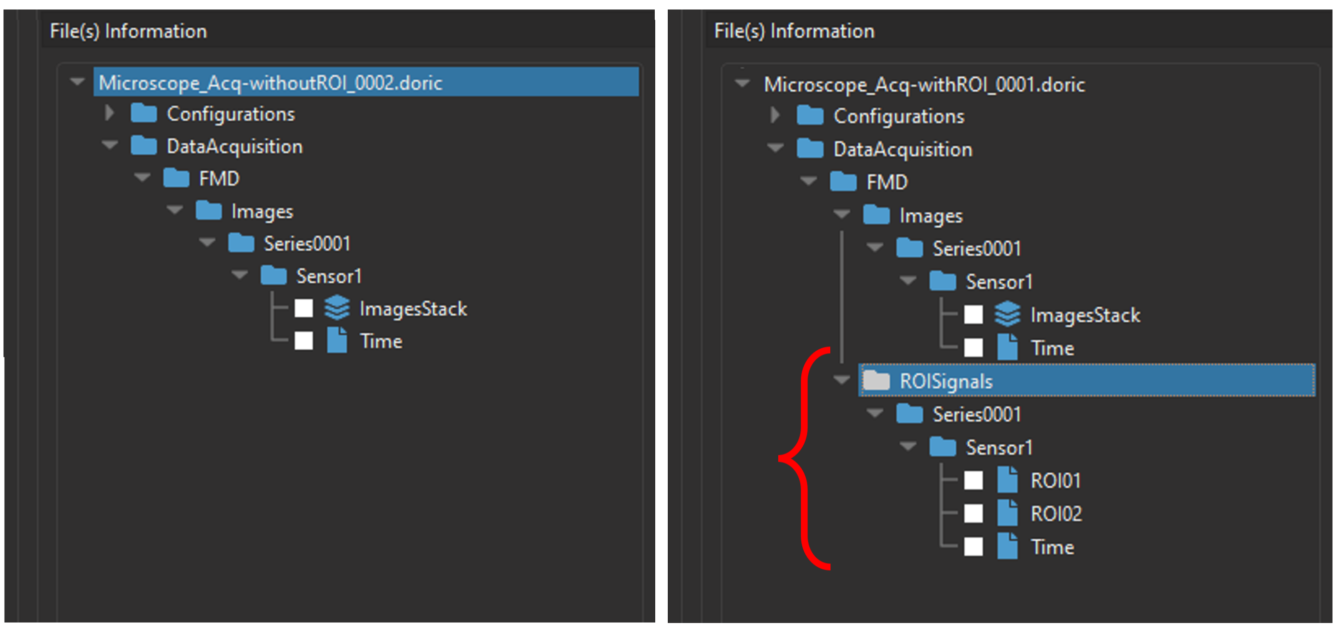
The **Saving Parameter** window defines how and where the file is saved. This window is opened by selecting the **Saving Options** button in the Acquisition Tab (Fig. 8.7, 5).

Saving File Settings:

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 8.8, 1).
2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 8.8, 2).
3. The **File format** (Fig. 8.8, 3) is **.doric**, an HDF5-based format that supports metadata (signal, video, images, tables, parameters, etc.). Alternatively, microscope files can be saved using a .tiff format. However the file size may not exceed 4 Gb. Version 6 of *Doric Neuroscience Studio* is no longer compatible with other file formats (.csv, .xlsx). 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 Editor** module.
4. The **File Index** (Fig. 8.8, 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. 8.8, 5) displays the absolute path and filename where the data will be saved.

Saving Choices:

6. The **ROI(s) Data** check-box (Fig. 8.8, 6), when enabled, will save the ROIs fluorescent fluctuations within the *.doric* file in addition to the raw ImageStack, as per Fig. 8.9. By default, the ROI(s) Data will be included.



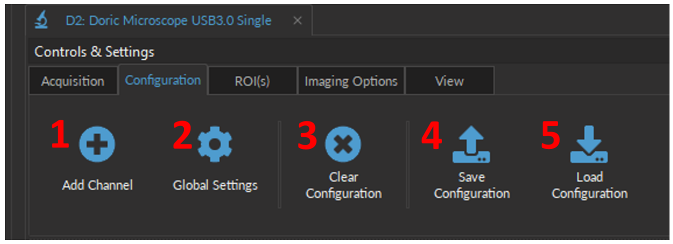
(a) *Without ROI(s) Data* (b) *Including ROI(s) Data*

Figure 8.9: *Saving Choices can include or exclude ROI(s) Data within data file*

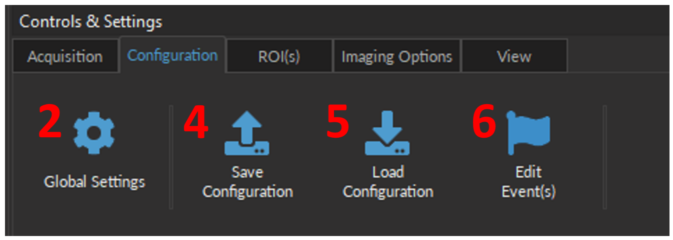
7. The **Digital I/O(s)** check-box (Fig. 8.8a, 7,) when enabled, will save the digital inputs and outputs within the *.doric* file. **Only available for efocus Microscope Drivers.**

8.3.2 Configuration Tab

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



(a) *eFocus*



(b) *Snap-In & 2-color*

Figure 8.10: *Microscope, Configuration Tab*

1. The **Add Channel** button (Fig. 8.10a, 1) opens the **Microscope Configuration** window in Fig. 8.10a, where either **Digital I/O** or **Keypress Event(s)** channels can be created. These channels are useful to record stimuli or behavior-related inputs and to synchronize external devices. See Sections 8.4 for more details.
2. The **Global Settings** (Fig. 8.10, 2) opens the **Global Options** window in Fig. 8.11, where user can specify the master start options and Trigger Out options. See Sections 8.3.2.1 for more details.
3. The **Clear configuration** button (Fig. 8.10a, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.
4. The **Save configuration** button (Fig. 8.10, 4) allows a microscope 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.
5. The **Load configuration** button (Fig. 8.10, 5) imports a previously configured .*doric* file into the module.
6. The **Edit Event(s)** button (Fig. 8.10a, 6) opens the **Keypress Event(s)** window (Fig. 8.26), which allows users to flag behavior events or experimental disruption at the press of a keyboard key. See Section 8.4.2. **Only for snap-in Microscope Drivers.**

8.3.2.1 Global Settings

The **Global Settings** is used to specify the element that will start the entire recordings, including both software and external hardware options.

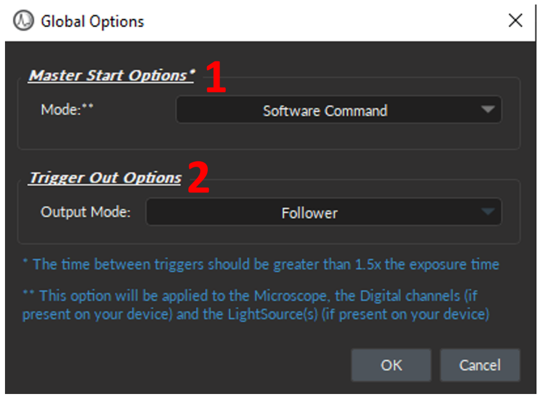
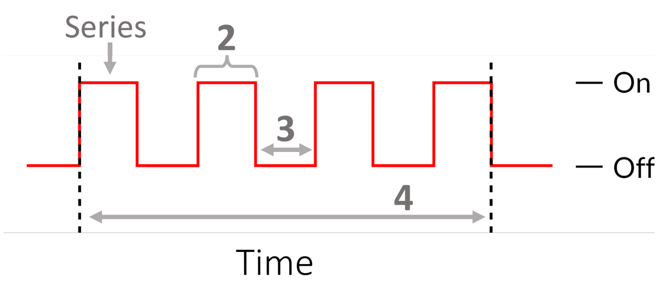


Figure 8.11: *Global Options Window*

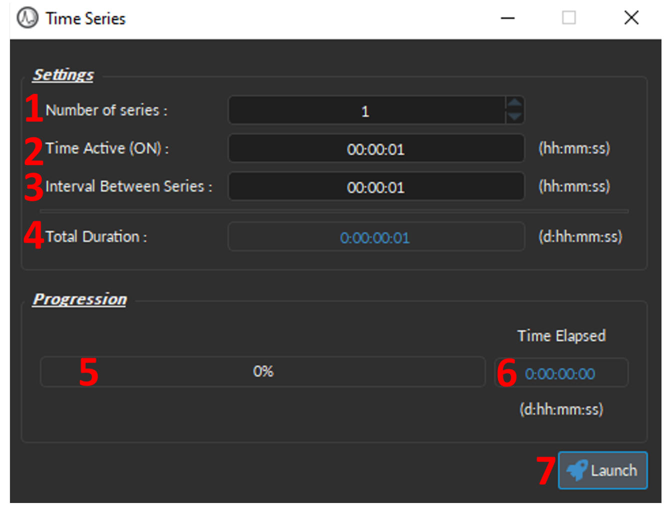
1. The **Master Start Options** (Fig. 8.11, 1) sets the origin (internal, external or time-series) of the trigger that will start the recording session and synchronize all the external and internal devices. Four options are available for different use cases:
   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 8.7, 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 8.12a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 8.12b) pops up. See section 8.3.2.2 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 an image is captured.

8.3.2.2 Time Series

The **Time Series** Window (Fig 8.12b) can be opened by clicking on the **Record** button (Fig. 8.7, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 8.11, 1). 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 8.12: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 8.12) sets the following parameters:

1. The **Number of series** (Fig. 8.12, 1) defines the amount of times the serie is repeated.
2. The **Time Active (ON)** (Fig. 8.12, 2) defines the duration of a serie.
3. The **Interval Between Series** (Fig. 8.12, 3) defines the amount of time between each series if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 8.12, 4) displays the total amount of time that the time series recording will take place.
5. The **Progression bar** (Fig. 8.12, 5) indicates the progression of the time series (in %).
6. The **Time Elapsed** (Fig. 8.12, 6) counter indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** (Fig. 8.12, 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 ROIs Tab

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

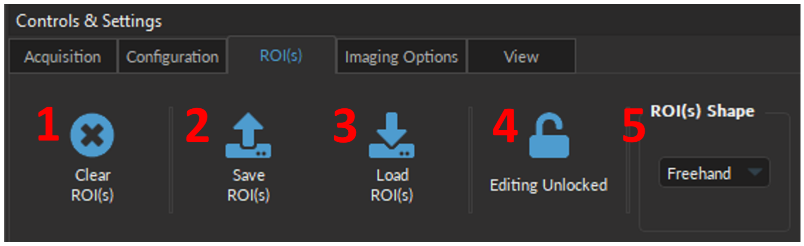


Figure 8.13: *ROIs Tab*

1. The **Clear ROI(s)** button (Fig. 8.13, 1) will delete all drawn regions of 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. 8.13, 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. 8.13, 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. 8.13, 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. 8.13, 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. 8.14, 1-4). Multiple different shapes can be used within a single **Sensor View**.

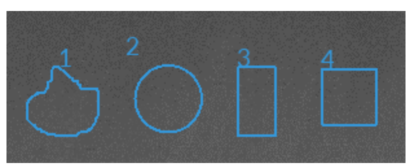


Figure 8.14: *ROI(s) Shape*

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. 8.7, 1-3).

8.3.4 Imaging Options Tab

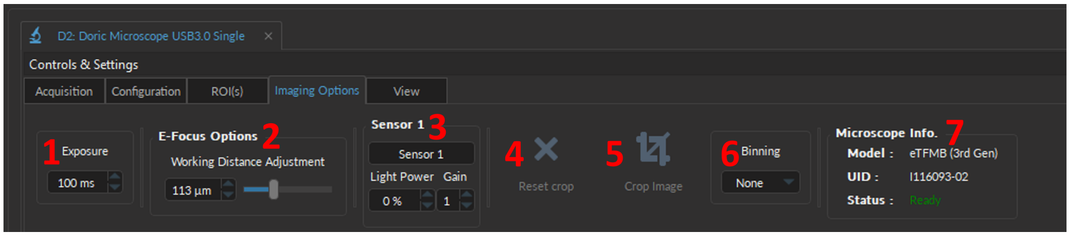


Figure 8.15: *Imaging Options Tab*

1. The **Exposure (ms)** textbox (Fig. 8.15, 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 **E-Focus Options** (Fig. 8.15, 2) allows users to adjust the **Working Distance** of the microscope focus using a slide bar from -45 to 45 um for *snap-in efocus fluorescence microscope* and from 0 to 350 um for *Twist-on efocus fluorescence microscope*.

3. The **Sensor #** (Fig. 8.15, 3) sets parameters that is specific for the specified sensor. When a microscope used has multiple sensors, multiple SENSOR sections will be displayed, one for each sensor.

   - *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.
   - *Light Source* drop-down list - ONLY AVAILABLE for *2-color fluorescence microscope* (Fig. 8.42, Sensor 1 & 2). This allows users to set the excitation source, which can be either **LED** or **LISER**.

4. The **Reset Crop** button (Fig. 8.15, 4) 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 **Record** sequence is activated.

5. The **Crop Image** button (Fig. 8.15, 5) 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.

6. The **Binning** drop-down (Fig. 8.15, 6) averages the pixels values based on the binning value selected: either none, 2x2, or 4x4 pixel squares will be averaged 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.

7. The **Microscope Info.** (Fig. 8.15, 7) includes the following details:

   - *Model* - displays the type of microscope currently connected to the software.
   - *UID* - displays the connected microscope's unique serial number.
   - *Status* - displays whether the microscope is *Stopped*, *Active* or, *Waiting for image*.

8. The **Mask Info.** (Fig. 8.42, 2) displays the file which is used to calibrate the microscope image. For SFMB/OSFM microscopes, the masks are automatically loaded when the **Microscope Driver** module is opened. For eSMFB, eTMFB and 2CFM microscopes, the masks needs to be loaded manually when first connected to the computer. eTFMB Gen 3.0 microscope doesn't requires masks to be loaded.

8.3.5 View Tab

The **View Tab** (Fig. 8.16) is used to modify the presentation of microscope image and the ROI(s) / Digital IO graphs in the **Acquisition view**.

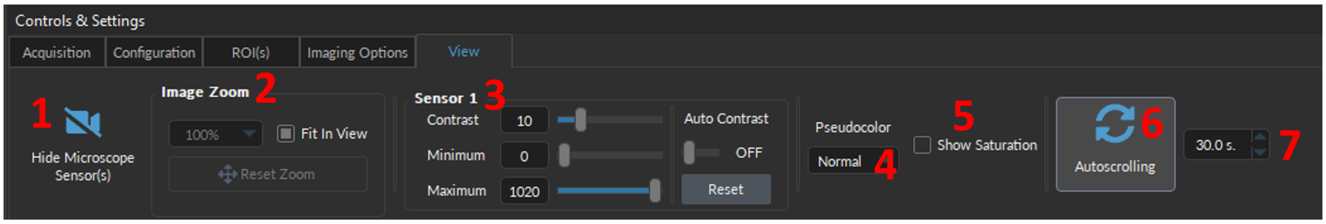


Figure 8.16: *View Tab*

The **View** parameters are as follows:

1. The **Hide Microscope Sensor(s)** button (Fig. 8.16, 1) removes the **Microscope View** from the **Acquisition View**, automatically enlarging the **ROIs / Signal View**. Disabling **Hide Microscope Sensor(s)** (renamed **Show Camera Feed(s)**), re-displays the **Microscope View**.

2. The **Image Zoom** (Fig. 8.16, 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 **Microscope View**.
   - The **Rest Zoom** button returns the zoom factor to 100%.

3. The **Sensor 1** (Fig. 8.16, 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 the maximal pixel count, all pixels with a higher count will appear saturated.
   - The **Auto Contrast** (Fig. 8.16, 4) will activate 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. 8.16, 4) maps the pixels values to a pseudocolor range (of 13 possibles options) to facilitate viewing.

5. The **Show Saturation** checkbox (Fig. 8.16, 5) is only available when the **Auto Contrast** setting is disabled.. When enabled, saturation pixels will turn red. This function is only available if no pseudocolor is selected.

6. The **Autoscrolling** button (Fig. 8.16, 6), when selected, automatically set the graphs to scroll as new data appears.

7. The **Zoom range** text-box (Fig. 8.16, 7) sets the graph zoom to the value of choice, specified in the text-box.

8.4 Microscope Configuration

Two additional channels-types can be created when using the *efocus Microscope Driver* by opening the **Microscope Configuration** window (Fig. 8.10a, 1):

- *Digital I/O* - Section 8.4.1
- *KeyPress Events* - Sections 8.4.2

For all other microscopes drivers, **KeyPress Events** can be created using **Edit Event(s)** button (Fig. 8.10b, 6), and are detailed in Section 8.4.2.

8.4.1 Digital I/O Channels

Each **Digital I/O** channel are ONLY available when using the *eFocus Microscope Driver* and 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. 8.17): (1) the **Channel Options** (Section 8.4.1.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 8.4.1.2).

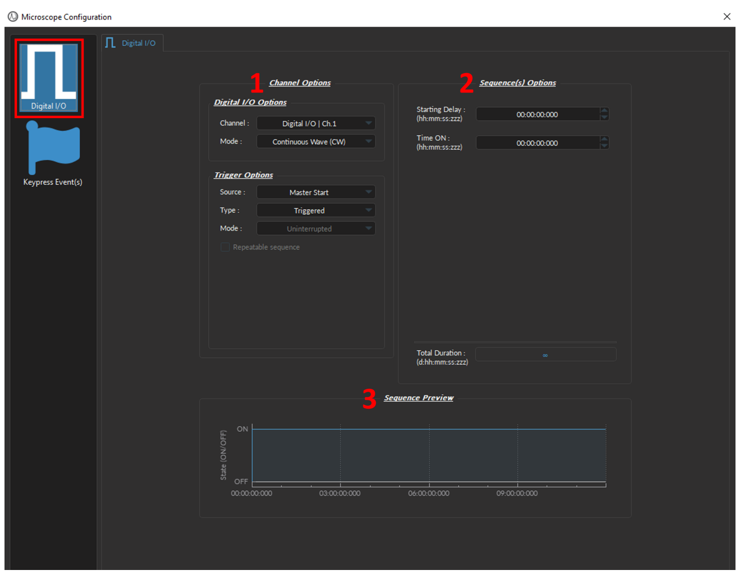


Figure 8.17: *Channel(s) configuration window, Digital I/O*

8.4.1.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.

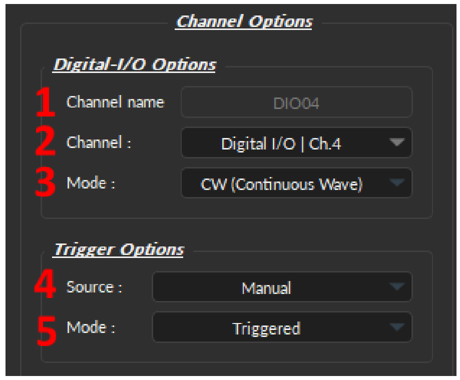


Figure 8.18: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

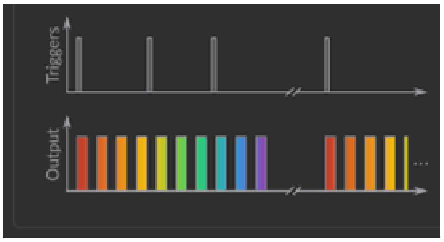
1. The **Channel name** (Fig 8.18, 1) allows user to specify a label for each channel.
2. The **Channel** (Fig 8.18, 2) identifies the channels available to create a Digital I/O. The channel can be changed by selecting a new one from the drop-down list. Each numbered channel on the physical microscope corresponds to the same number of the digital channel within the software.
3. The **Mode** (Fig 8.18, 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. 8.19a);
   - The **Square (TTL)** Mode (Fig. 8.19b);
   - 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 microscope (See Section 8.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.

(a) *Continuous* (b) *Square*

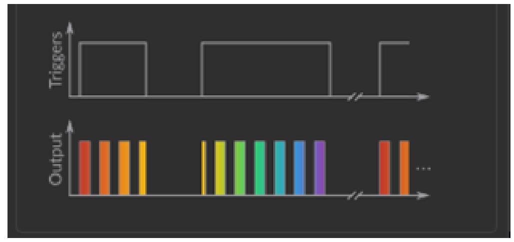
Figure 8.19: *Channel Options - Output Modes*

Trigger Options:

1. The **Source** trigger option (Fig 8.18, 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 8.18, 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. 8.20a), 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. 8.20b), 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 8.20: *Trigger Options Modes*

8.4.1.2 Sequence Options & Preview

The **Sequence options** section (Fig. 8.21a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 8.21b) 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**.

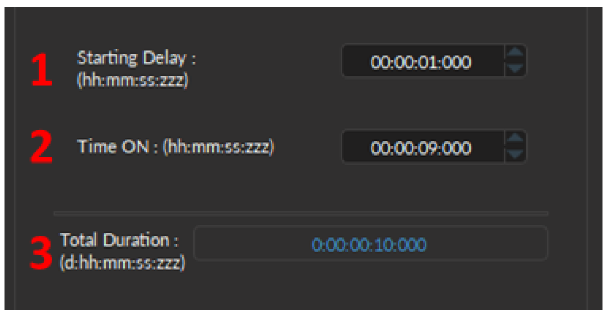
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 8.18), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 8.21) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

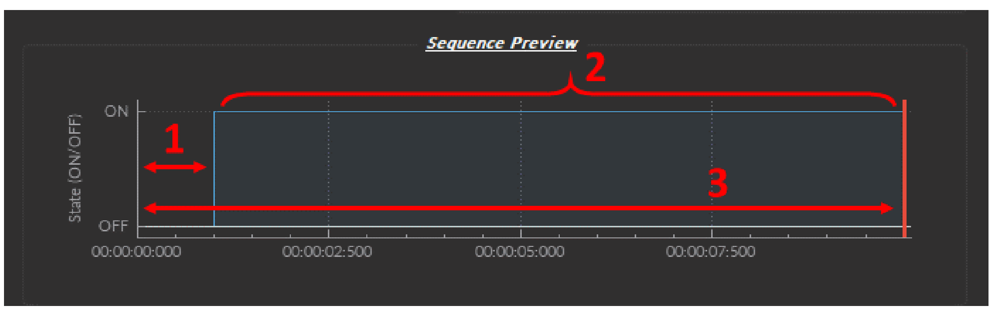
  1. The **Starting Delay** (Fig 8.21, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Time ON** (Fig 8.21, 2) defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse sequence is stopped manually.
  3. The **Total Duration** (Fig 8.21, 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**.

- The **Square** channel mode (Fig. 8.22) allows the creation of a square TTL pulse sequence. This includes the following parameters:

  1. The **Starting Delay** (Fig 8.22, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Frequency** (Fig. 8.22a, 2) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period** (Fig. 8.22a, 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. 8.22, 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 **Pulse(s) per sequence** (Fig. 8.22, 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. 8.22, 5) sets the number of times that the sequence will be repeated.
  6. The **Delay between sequences** (Fig. 8.22, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).
  7. The **Total Duration** (Fig 8.22, 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**.

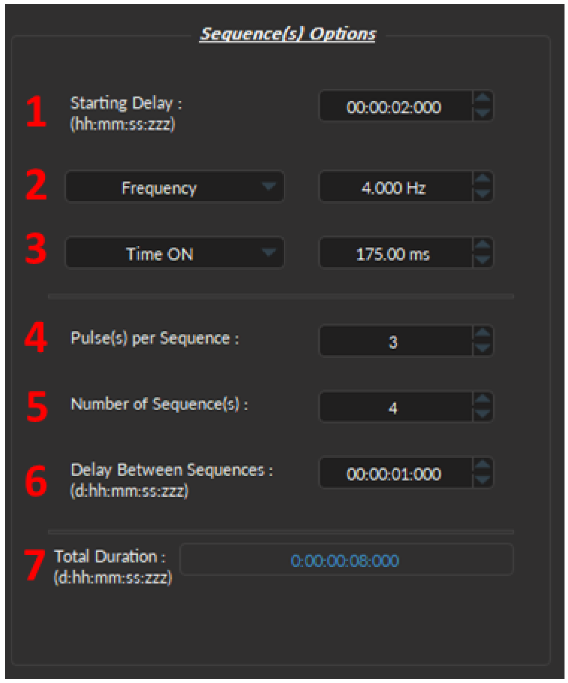


(a) *Sequence Options*

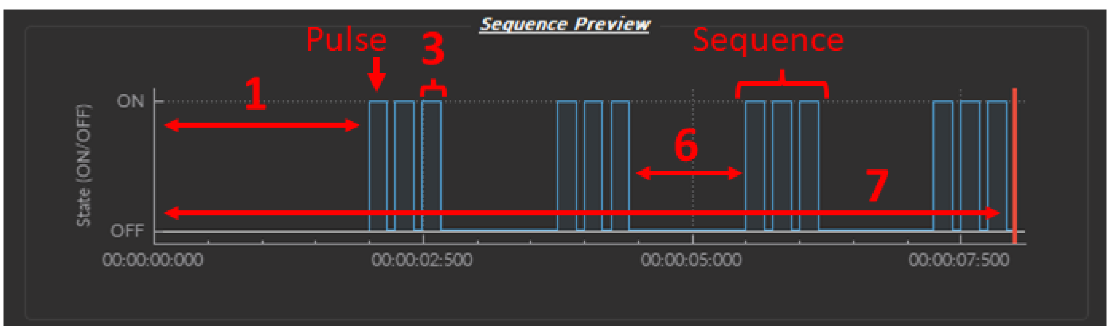


(b) *Sequence Preview*

Figure 8.21: *Channel(s) configuration window, Digital I/O - CW Mode*



(a) *Sequence Options*



(b) *Sequence Preview*

Figure 8.22: *Channel(s) configuration window, Digital I/O - Square Mode*

8.4.2 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.

Adding/Removing KeyPress Event(s)
To add a new **Keypress Event**, select the + sign at the botton of the window (Fig. 8.23, left). To remove a KeyPress, use the - button (Fig. 8.23, 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 the video feed (Fig.8.23).

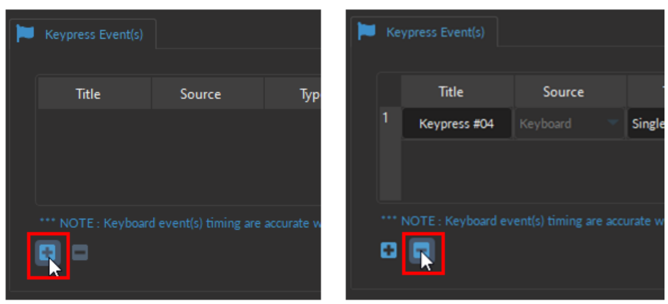


Figure 8.23: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 8.24) in the **Acquisition View**.

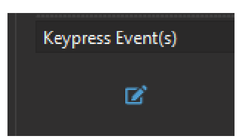
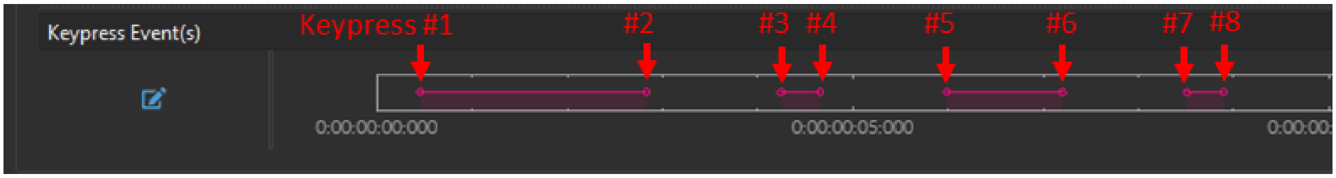


Figure 8.24: *Edit Keypress Event(s) Channel*

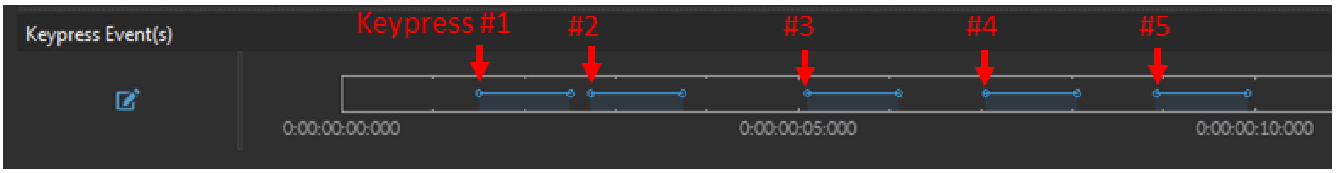
The following are the configurable parameters of a **Keypress Event,** per Fig. 8.26:

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. 8.25a).
   - **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. 8.25b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 8.25c). 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 8.25: *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 that 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. 8.26, column 6).
7. The **Information** column provides space to make notes or write a short description of the Keypress Event.

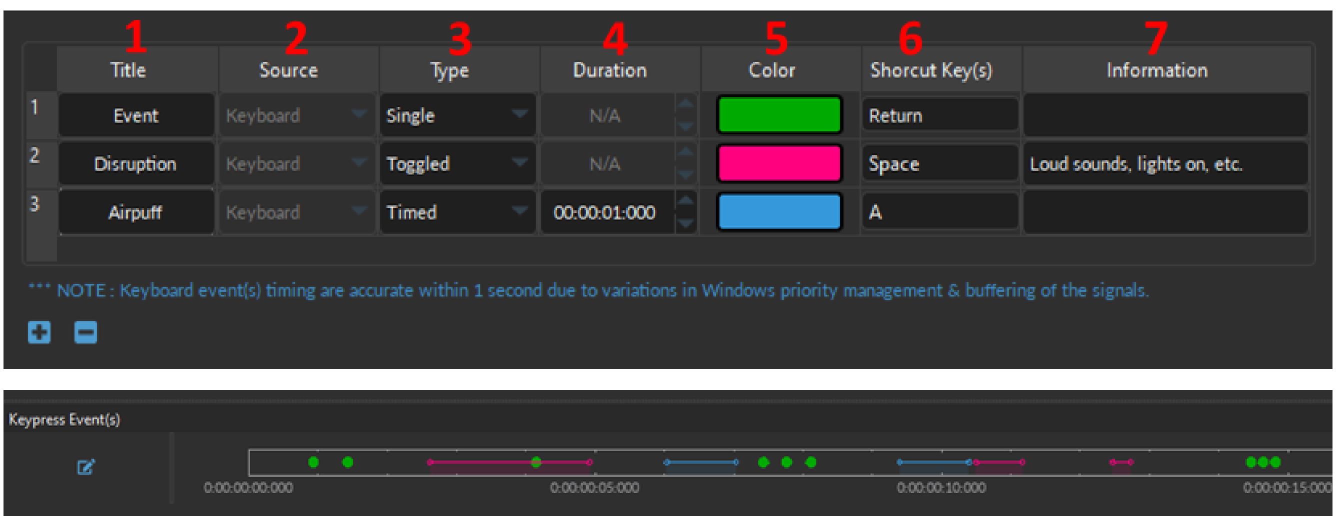


Figure 8.26: *Channel(s) configuration window, KeyPress Event(s)*

8.5 Acquisition View

The **Acquisition View** is split into three sections (Fig. 8.27):

1. The **Input / Output View** (Fig. 8.27, 1) - Section 8.5.1
2. The **Microscope View** (Fig. 8.27, 2) - Section 8.5.2.
3. The **ROI(s) View** (Fig. 8.27, 3) - Section 8.5.3.

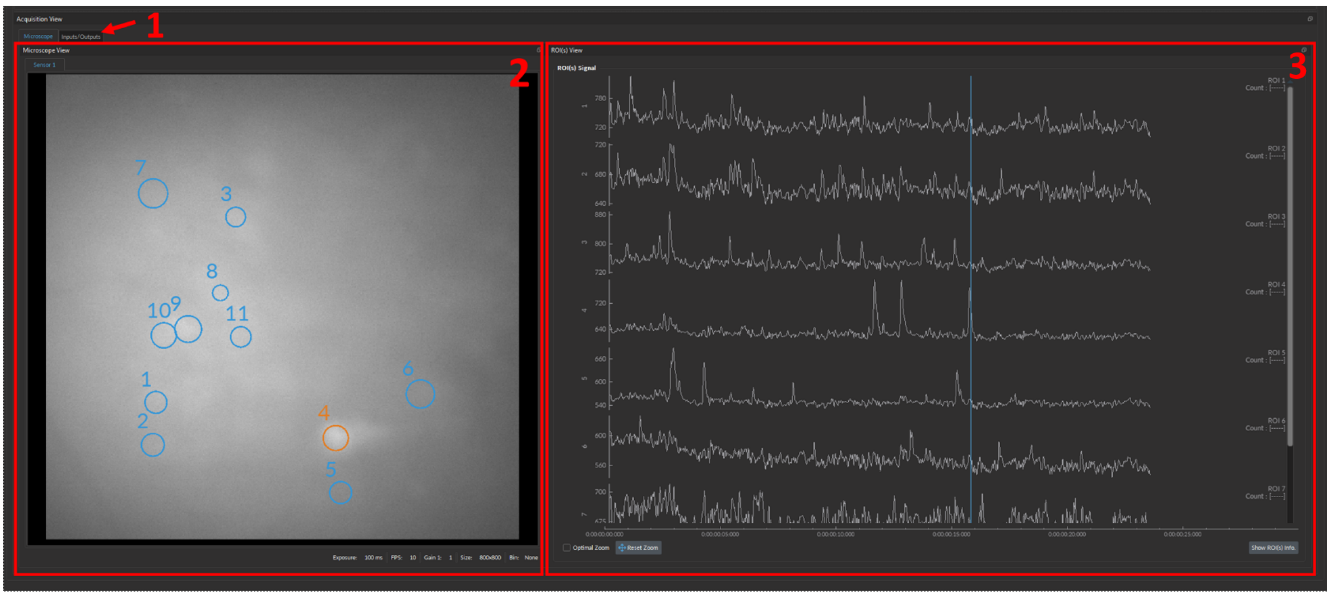


Figure 8.27: *Acquisition View*

8.5.1 Inputs/Outputs View

The **Inputs/Outputs View** displays the active Digital channels. Each Digital I/O channel includes: a **Control Box** (Fig. 8.28, 1), and a **Graph(s)** (Fig. 8.28, 2).

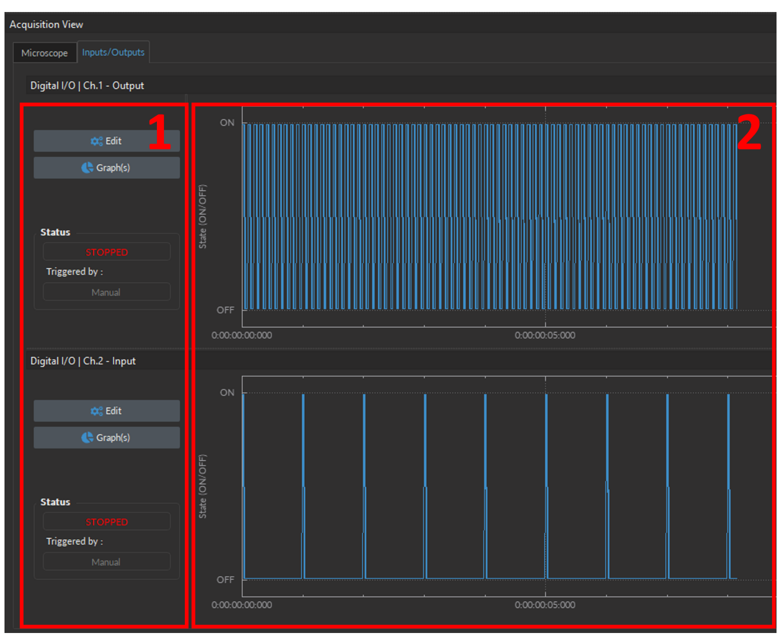


Figure 8.28: *Digital I/O(s) View*

8.5.1.1 DIO Control Box

The **Control box** of each channel allows users to track the status and edit the graph trace or the channel parameters.

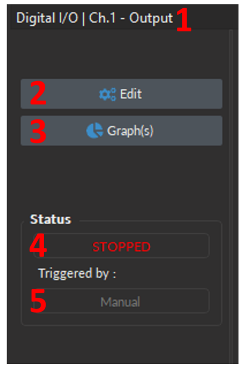


Figure 8.29: *Digital I/O View, Control box*

The following elements are contained within the **Control Box** of every Digitial channel (Fig. 8.29):

1. The **Channel name** (Fig. 8.29, 1) is located on the upper left of the **Control box**, identifying the type of channel and its number, corresponding to that on the *Microscope Driver*.
2. The **Edit** button (Fig. 8.29, 2) opens the **Channel Configuration** window, where the preset digital outputs can be modified (Fig. 8.30). For details on individual parameters, see Section 8.4.1.

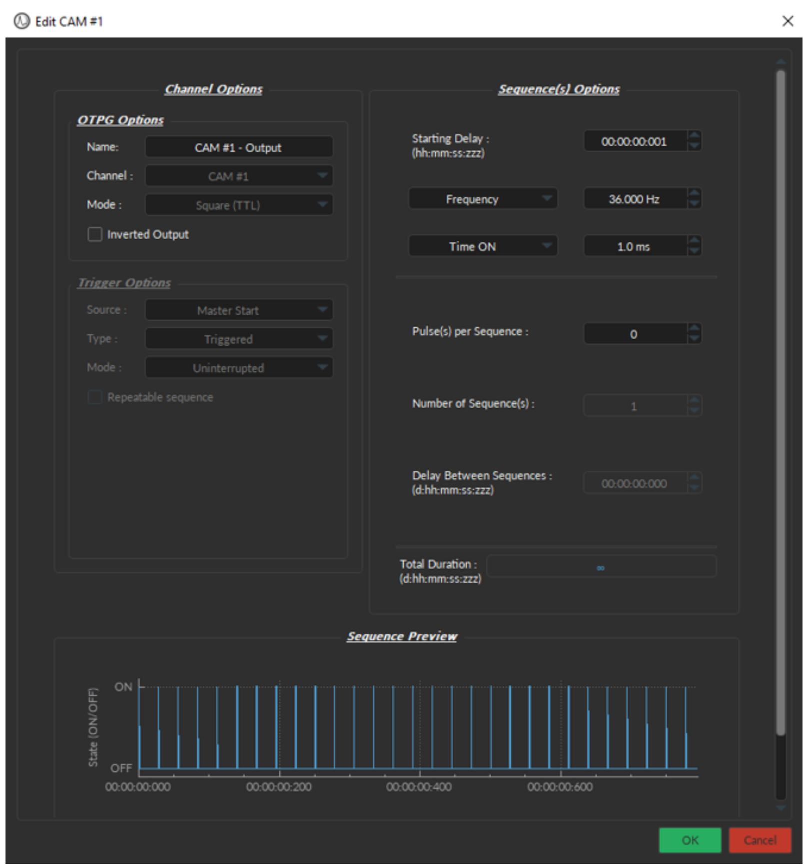


Figure 8.30: *Edit Digital I/O parameters]*

3. The **Graph(s)** (Fig. 8.29, 3) button opens the **Graph Options** window (Fig. 8.31) 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:

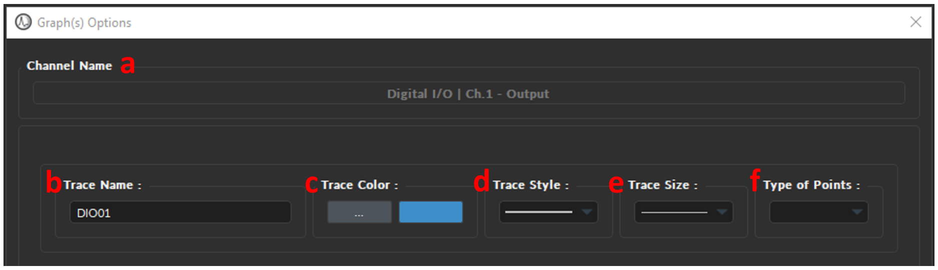


Figure 8.31: *Graph(s) Options Window*

   a) The **Channel Name** (Fig. 8.31, a) 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. 8.31, b) allows users to specify a name for the trace, instead of the default name generated by the software.
   c) The **Trace Color** button (**...**) (Fig. 8.31, c) opens the **Color Select** window (Fig. 8.32), 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. 8.31, d) 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. 8.31, e) 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. 8.31, f) 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.

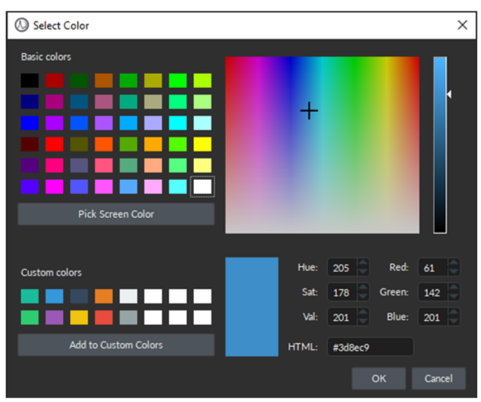


Figure 8.32: *Select Color Window*

4. The **Status** bar (Fig. 8.29, 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 8.10a, no. 2).

5. The **Triggered by:** (Fig. 8.29, 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.

8.5.1.2 DIO Graph

The **Digital I/O** traces are displayed in the **Graph** box (Fig. 8.28, 2). Each channel graph includes the following components:

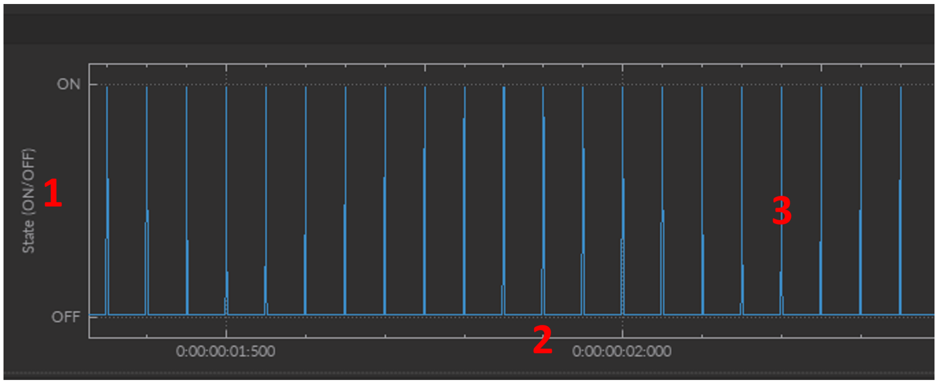


Figure 8.33: *Digital I/O(s) View - Graph*

1. The **Y-axis** (Fig. 8.33, 1) displays the Digital **State** of the channel, which can be either ON (1) or OFF (0).
2. The **X-axis** (Fig. 8.33, 2) displays the time in d:hh:mm:ss:zzz.
3. The **Trace** (Fig. 8.33, 3) can be edited by selecting the **Graph** button in Section 8.5.1.1, no. 3.

While Section 8.5.1.1, no. 3 allow users to control the trace display, there are other features of **Graph** view that can be directly manipulated by selecting elements of the **Graph** itself, such as:

- *Axis Options* - Each **Graph** (Fig. 8.34) 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. 8.34) 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.

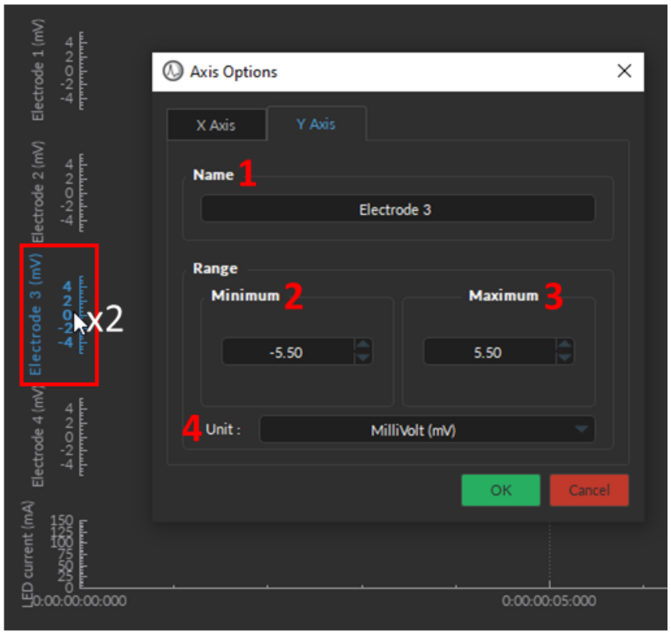


Figure 8.34: *Double click on any axis to open its Axis Options window*

- 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. 8.35). 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.

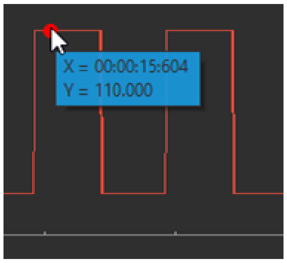


Figure 8.35: *Acquisition View - Instant values*

8.5.2 Microscope View

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

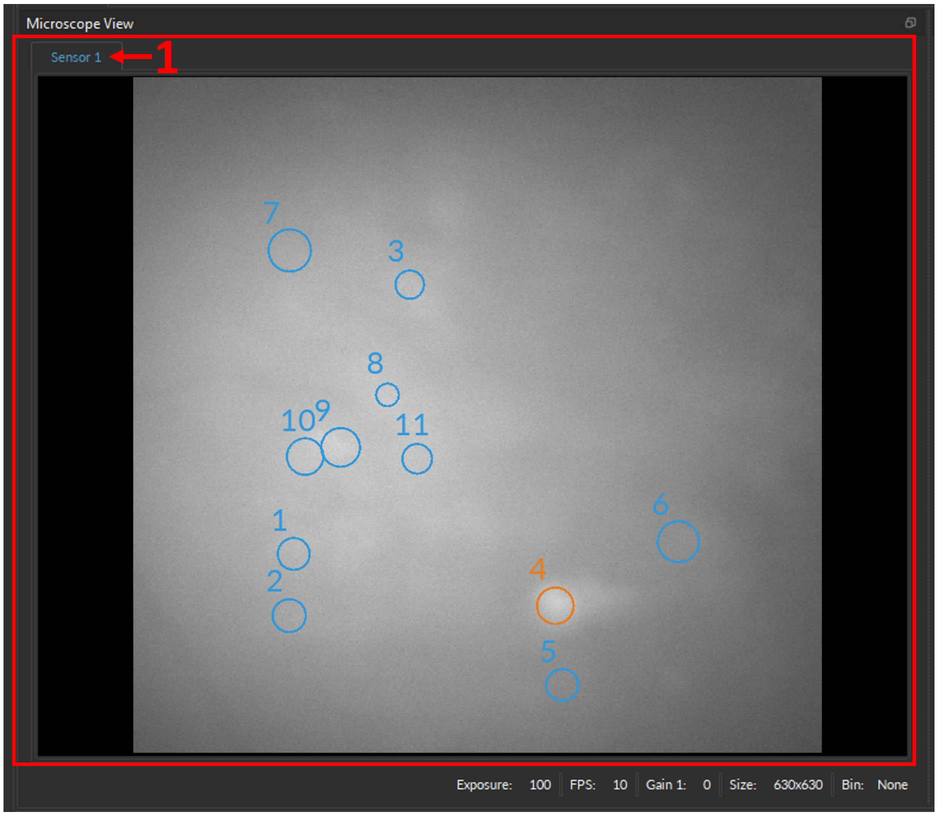


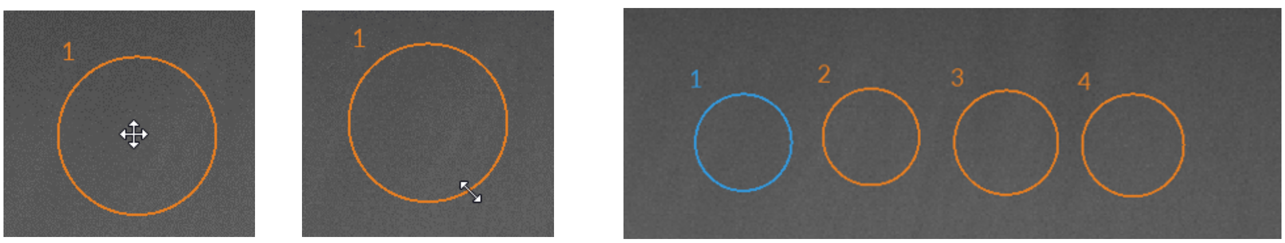
Figure 8.36: *Microscope View*

1. The **Sensor(s)** tab (Fig. 8.36, 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 (Figures 8.13, 8.15, 8.16, respectively).

Within the **Sensor** video feed, users can:

- **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 8.3.3, 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. 8.41).
- **Delete individual ROI** - Select a ROI (as detailed above) and press the **Delete** key on the Keyboard. To delete all ROIs, see Section 8.3.3, no. 1.
- **Displace ROI** - Select the ROI and hove above the center of the ROI until a *Move* icon (Fig. 8.37a) 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. 8.37b) 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. 8.37c). This selection allows multi-ROI deletion or displacement.



(a) *Move ROI* (b) *Resize ROI* (c) *Multi-select*

Figure 8.37: *Edit ROI(s)*

2. The **Microscope Monitoring Bar** (Fig. 8.38) displays the parameters currently being used during the recording:

Figure 8.38: *Microscope Monitoring Bar*

a) The **Exposure** (Fig. 8.38, a) displays the value specified in Section 8.3.4, no. 1.
b) The **FPS** (Fig. 8.38, b) displays 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 8.3.4, no. 1 will change the *FPS*.
c) The **Gain** (Fig. 8.38, c) displays the value specified in Section 8.3.4, no. 3.
d) The **Size** (Fig. 8.38, d) displays the image resolution. If the image is **Cropped** (Section 8.3.4, no. 5), this value will be automatically updated.
e) The **Bin** (Fig. 8.38, e) displays the value (none, 2x2 or 4x4) specified in Section 8.3.4, no. 6.

8.5.3 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 8.39: *ROI(s) View*

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

1. The **ROI(s) signal graph** (Fig. 8.39, 1) displays the raw signal trace for each ROI(s).
    a) The **ROI(s) ID** (Fig. 8.40, a) specifies which ROI the signal graph belongs to. The graphs are displayed in order of ROI created.
    b) The **y-axis** (Fig. 8.40, b) represents the mean signal intensity of the ROI, which is unit-less.
    c) The **x-axis** (Fig. 8.40, c) represents the time in d:hh:mm:ss:zzz.
    d) The **Trace** (Fig. 8.40, 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. 8.40, e)
        - *ROI label* - displays the ROI **Name** (specified within the **Name** column of **ROI(s) Infromation** tab; Fig., 8.41, b), followed by the **Sensor Name** in parenthesis (which can be specified in Fig. 8.15, 2).
        - *Counts* - displays the value of the last data point of the ROI trace (in average pixel intensity value).

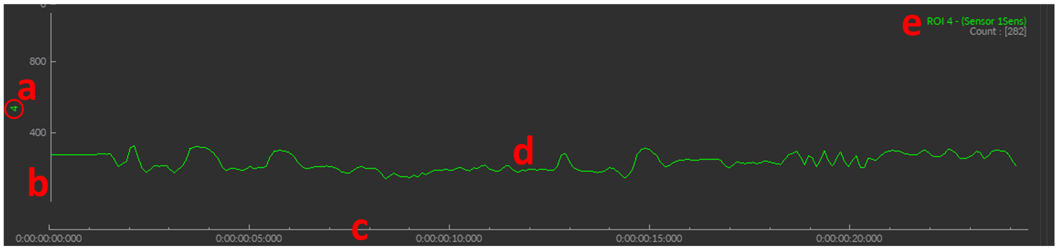


Figure 8.40: *ROI(s) View, graphs*

2. The **Optimal Zoom** checkbox (Fig. 8.39, 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. 8.39, 3) readjusts the graph zoom to the value specified in the zoom range text-box.

4. The **Show ROI(s) Info.** button (Fig. 8.39, 4) opens or closes the **ROI(s) Information** Tab in Fig. 8.39, 5.

5. **ROI(s) Information** Tab (Fig. 8.39, 5) displays a table with ROI basic data, including:

   a) *ID* (Fig. 8.41, a) displays the number associated with ROI.

   b) *Name* (Fig. 8.41, b) displays the label associated with the ROI. Double-click on the text-box to rename the ROI.

   c) *Area* (Fig. 8.41, c) displays the number of pixels that fill the perimeter of the ROI.

   d) *Edit* button (Fig. 8.41, d) will highlight in orange the corresponding ROI in the **Microscope View**. To edit or delete the selected ROI, see section 8.5.2, no. 1.

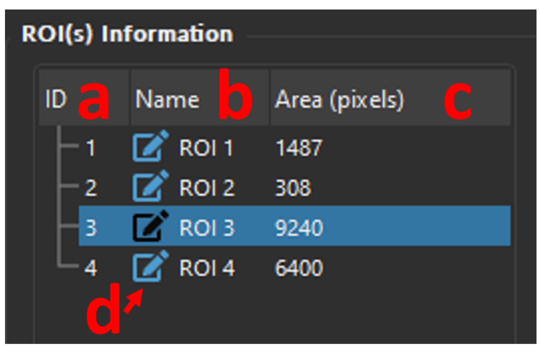


Figure 8.41: *ROI(s) View, ROI(s) information*

8.6 Mask Installation

For the *2-color fluorescence microscope* and the older generations of the *eFocus fluorescence microscope* (before Gen3.0) 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. 8.42). This opens a file selection window. Travel to the location of the mask file, select it and click **OK**.

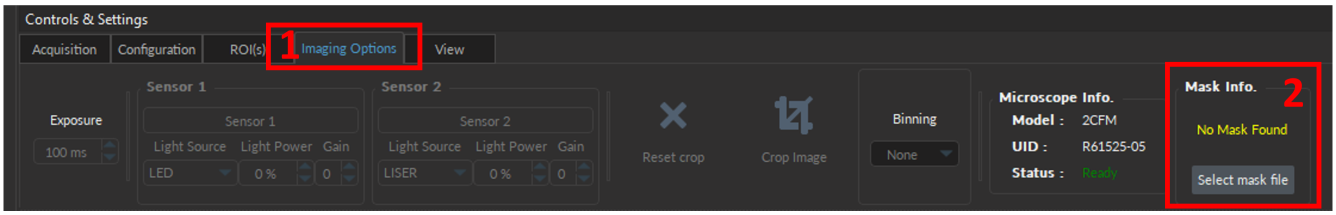


Figure 8.42: *Add Mask in the Imaging Options tab*

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. 8.43). This is replaced by **Masks Loaded** once loading is complete.

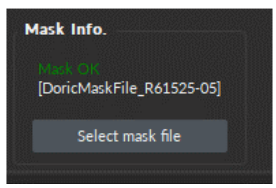


Figure 8.43: *Mask correctly added*

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

9

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.

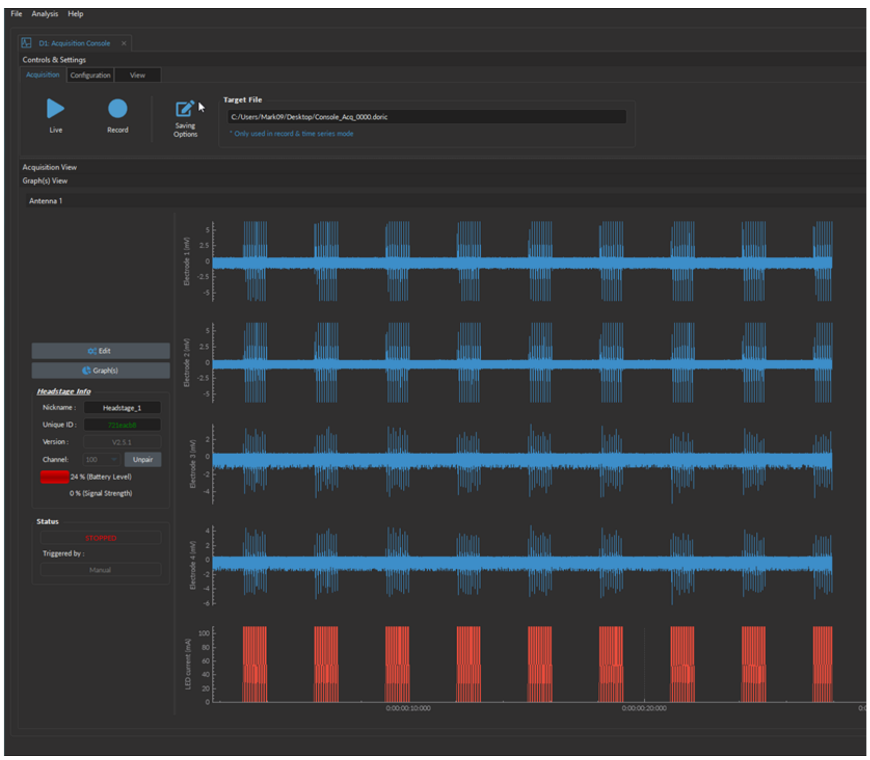


Figure 9.1: *Electrophysiology console user interface*

9.1 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. 9.2).

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. 9.2).

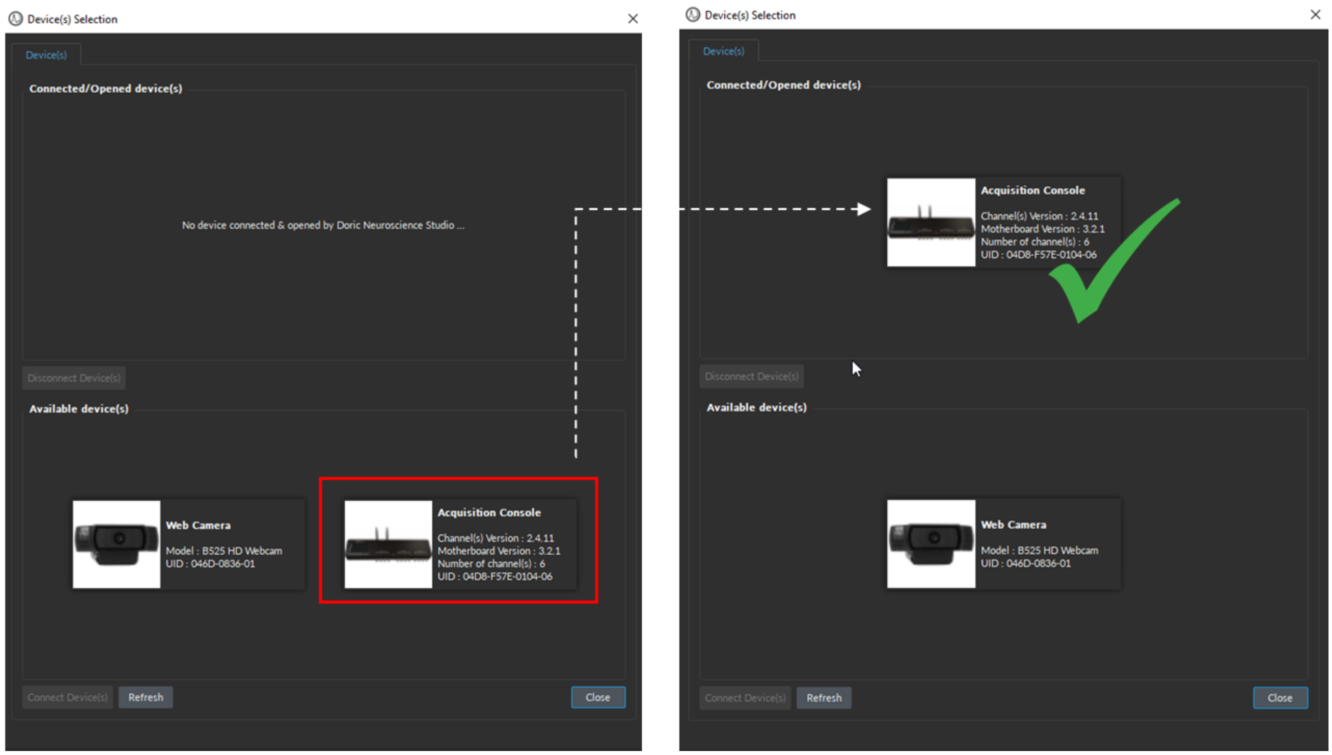


Figure 9.2: *Double click on device of choice to connect it to DNS*

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. 9.3) or use the hot key: *Ctrl+N*.

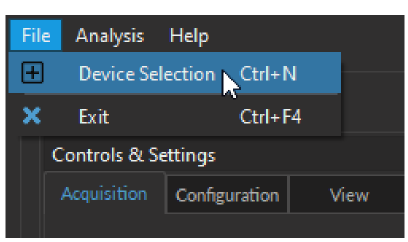


Figure 9.3: *Open Device Selection Window*

9.2 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 9.3) are used to manage different elements of the software (Acquisition, Configuration and View); **(2) Headstage Info** (Section 9.5) is used to pair the Headstage to the console; **(3) Acquisition view** (Section 9.5) displays the input and output traces for visualization.

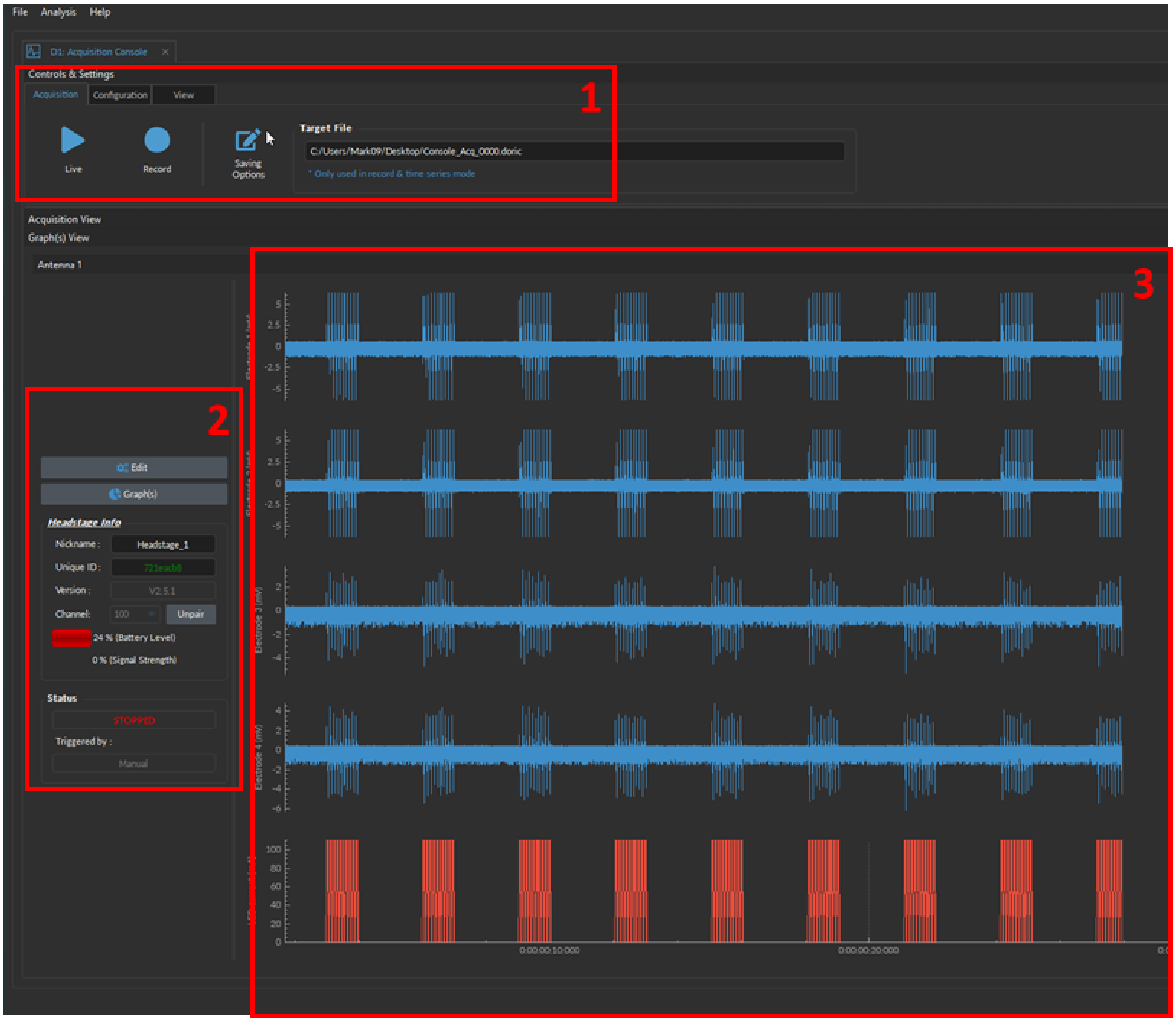


Figure 9.4: *DNS interface*

9.3 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 9.3.1), **Configuration** (Section 9.3.2), and **View** (Section 9.3.3).

9.3.1 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 9.4.1 to configure channels for recording.

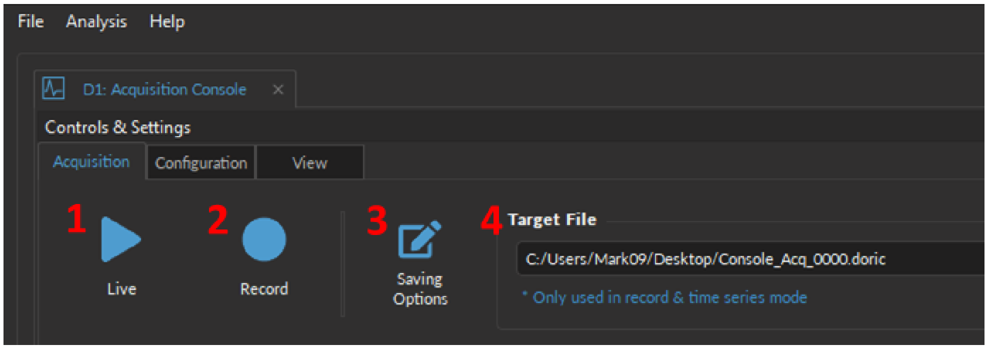


Figure 9.5: *Acquisition Tab*

1. The **Live** button (Fig. 9.5, 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. 9.5, 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. 9.5, 3) button opens the **Saving Parameters** window (Fig. 9.6). See section 9.3.1.1 for greater details.
4. The **Target File** (Fig. 9.5, 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.

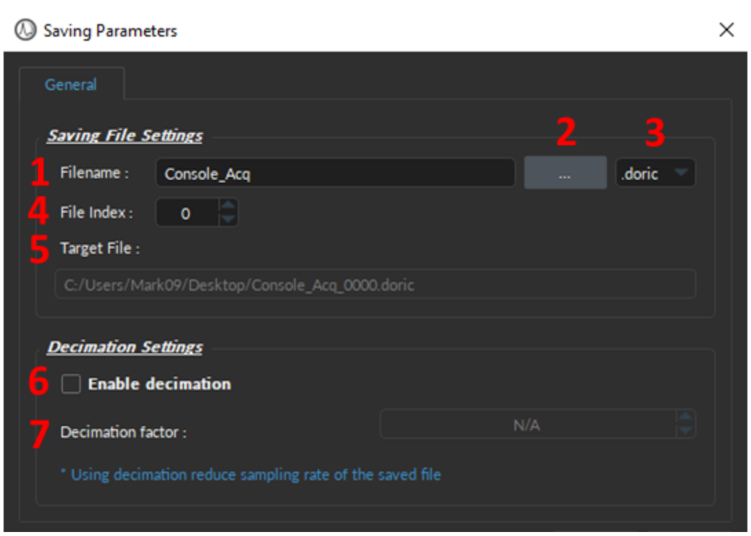


Figure 9.6: *Saving Menu Window*

9.3.1.1 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. 9.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 9.6, 1).
2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 9.6, 2).
3. The **File format** (Fig. 9.6, 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. 9.6, 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. 9.6, 5) displays the absolute path and filename where the data will be saved.
6. The **Enable decimation** checkbox (Fig. 9.6, 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. 9.6, 7) is used to define the number of points saved.[1]

9.3.2 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.

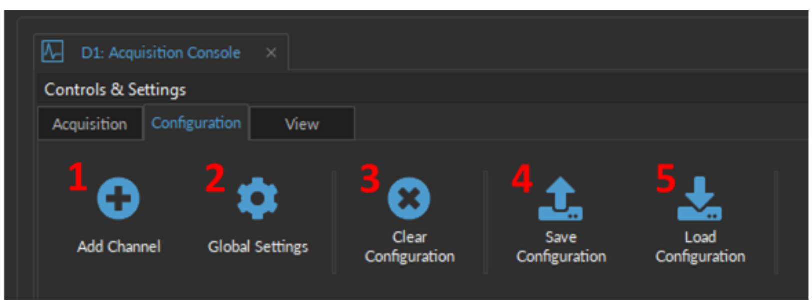


Figure 9.7: *Configuration Tab*

1. The **Add Channel** button (Fig. 9.7, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 9.4. Table 9.1 describes different types of channels available, their use cases and their individual sections.
2. The **Global Settings** (Fig. 9.7, 2) opens the **Global Options** window in Fig. 9.8, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 9.3.2.1 for more details.
3. The **Clear configuration** button (Fig. 9.7, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.
4. The **Save configuration** button (Fig. 9.7, 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. 9.7, 5) imports a pre-configured **.doric** file into the module.

[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.

9.3.2.1 Global Settings

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

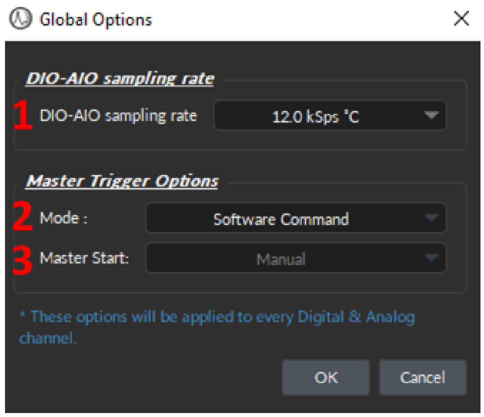


Figure 9.8: *Global Options Window*

1. The **DIO-AIO sampling rate** (Fig. 9.8-1) 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 9.3.1.1 to enable the *Decimation* and effectively reduce the saving sampling rate and restrict the data file size.

2. The **Mode** (Fig. 9.8, 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:

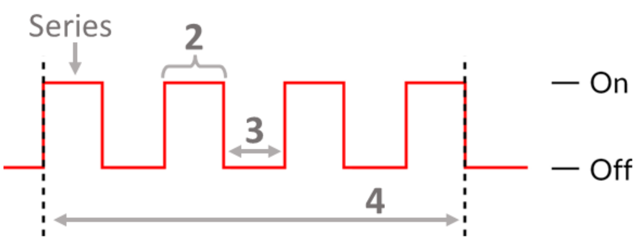
   - *Software Command* - The recording will start when the **Record** button is selected in the **Acquisition Tab** (Fig. 9.5, 2). The **Master Start** is, by definition, always **Manual**.
   - *Triggered* - The recording session starts when a trigger signal is received (from the **Master Start**, either 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 15.10a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 9.9b) pops up. See section 9.3.2.2 for more details.

3. The **Master Start** (Fig. 9.8, 3) defines the source that will automatically start the recording. This source can either be:

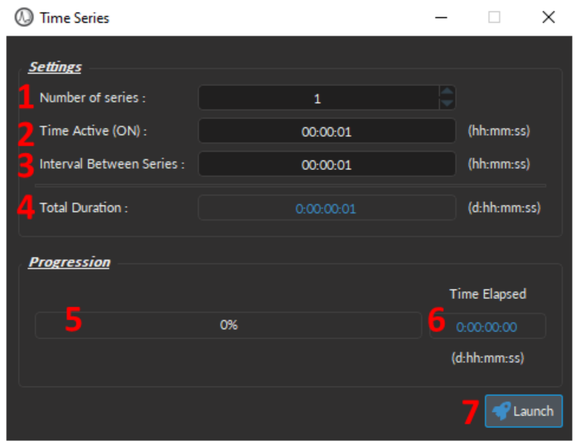
   - *Manuel* - the user ultimately starts the recording session by clicking **Record** within *Doric Neuroscience Studio*;
   - *Digital I/O Channel (1-4)* - The specified channel will automatically begin the recording session when it receives a digital trigger pulse from an external device. ***However,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

9.3.2.2 Time Series

The **Time Series** Window (Fig 9.9b) can be opened by clicking on the **Record** button (Fig. 9.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 9.8, 2). Every **Time series** sequence is automatically saved to the *.doric* file defined in **Saving Options** (Section 9.3.1.1).



(a) *Time Series Acquisition Timing Diagram*



(b) *Time Series Window*

Figure 9.9: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 9.9b) sets the following parameters:

1. The **Number of series** (Fig. 9.9b, 1) defines the amount of times the series is repeated.
2. The **Time Active (ON)** (Fig. 9.9b, 2) defines the duration of the series.
3. The **Interval Between Series** (Fig. 9.9b-3) defines the amount of time between each series, if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 9.9b, 4) displays the total amount of time that the timeseries recording will take place.
5. The **Progression bar** (Fig. 9.9b, 5) indicates the progression of the timeseries (in %).
6. The **Time Elapsed** (Fig. 9.9b-6) counter indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** (Fig. 9.9b, 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.

9.3.3 View Tab

The **View Tab** (Fig. 9.10) is used to modify the presentation of graphs in the **Acquisition view**.

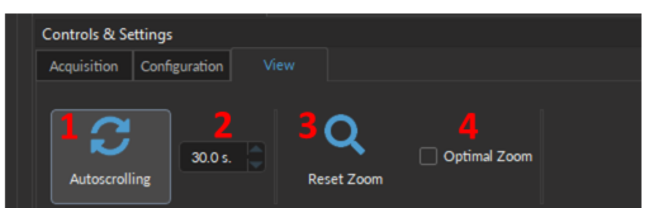


Figure 9.10: *View Tab*

The **View** parameters are as follows:

1. The **Autoscrolling** button (Fig. 9.10, 1), when selected, automatically set the graphs to scroll as new data appears.
2. the **Zoom range** (Fig. 9.10, 2) sets the graph zoom to the value of choice, specified in the text-box.
3. The **Reset Zoom** button (Fig. 9.10, 3) readjusts the graph zoom to the value specified in the zoom range text-box.
4. The **Optimal Zoom** check-box (Fig. 9.10, 4) automatically adjusts the graph range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

9.4 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.

9.4.1 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. 9.11). This will open the **Channel(s) Configuration** window (Fig. 9.13).

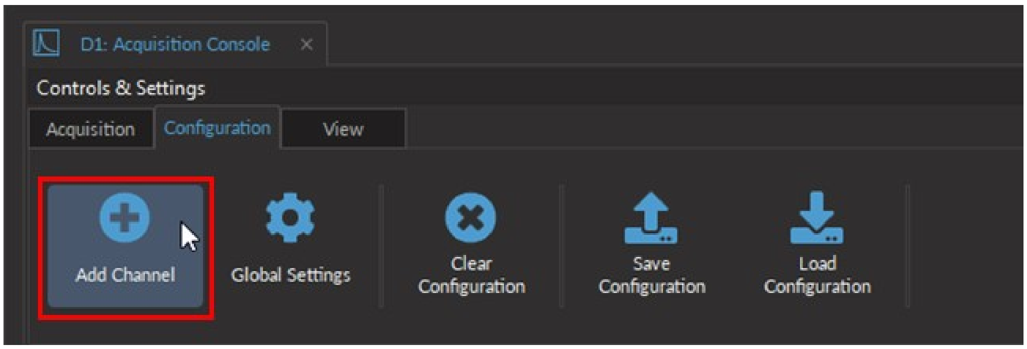


Figure 9.11: *Add Channel button opens the Channel Configuration window*

To generate a new **Channel** using the **Channel(s) configuration** window (Fig. 9.12):

1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 9.12). Table 9.1 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 9.1 for hyperlinks to the relevant sections.
3. Select the **Add** button (Fig. 9.12) 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.

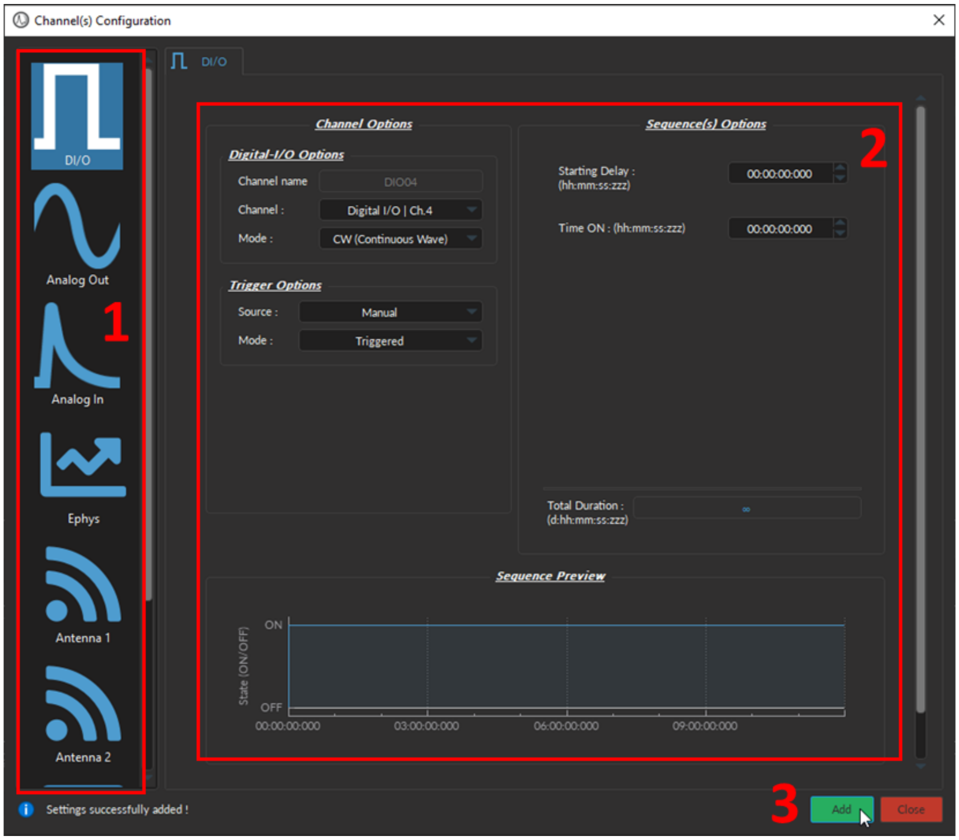


Figure 9.12: *Channel(s) configuration window, Digital I/O input*

9.4.2 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 9.11). Table 9.1 details the types of inputs and output the console and the software can handle and gives a quick access to their sections.

Table 9.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	Antenna	Pair and record electrophysiological signal from the FiWi Headstage	9.4.3
	Digital I/O	For input and output of TTL signals	9.4.4
	Analog Output	For the output of analog signals, such as sine, stair or customized	9.4.5
	Analog Input	To collect the fluorescent signal (such as GCamp, RCamp, Isosbestic or FRET)	9.4.6
	Camera(s)	To collect video for behaviour experiments	9.4.7
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	9.4.8

9.4.3 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. 9.13): (1) the **Channel Options** (Section 9.4.3.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 9.4.3.3), and (4) the **LED Trigger Options** (Section 9.4.3.2).

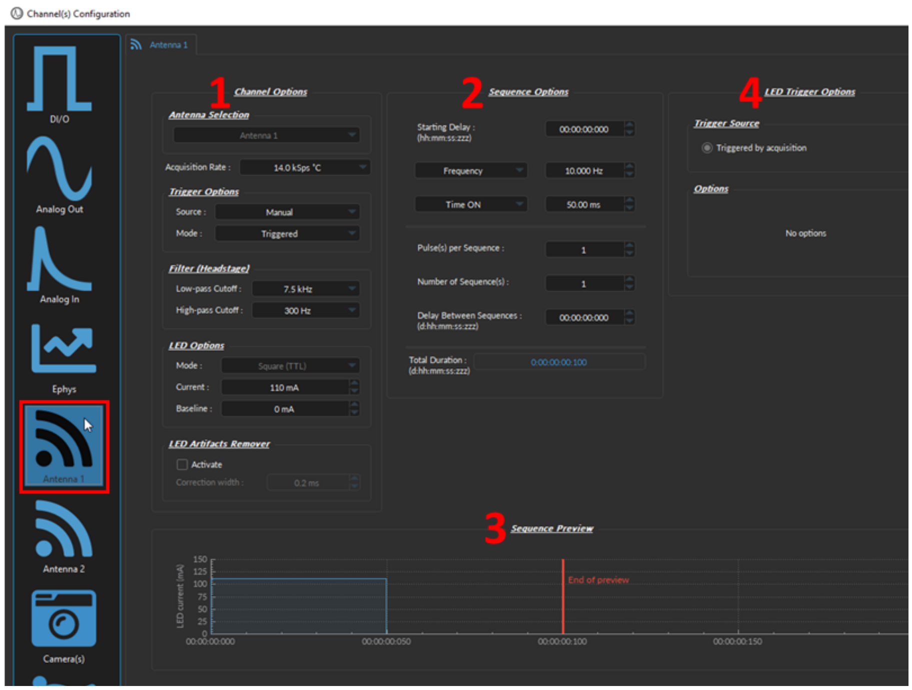


Figure 9.13: *Electrophysiology channel configuration window*

9.4.3.1 Channel Options

The **Channel Options** (Fig. 9.14) of the Antenna *Channel Configuration* window sets the trigger and recording parameters, as follows:

1. The **Antenna selection** (Fig. 9.14, 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. 9.14, 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. 9.14, 3) filters define the high and low-pass frequency cutoff for electrical signal received by the headstage.
   - The **Low-pass Cutoff** (Fig. 9.14, 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. 9.14, 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. 9.14, 4) are all parameters used to control the light source of the cannula connected to the *Fi-Wi headstage*, such as:
   - The **Mode** (Fig. 9.14, 4) allows the selection of the pulse sequence mode.
   - The **Maximum current** (Fig. 9.14, 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. 9.14, 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.

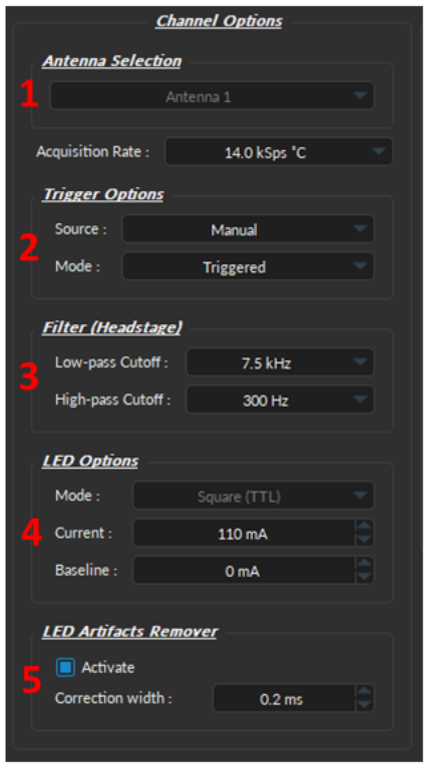


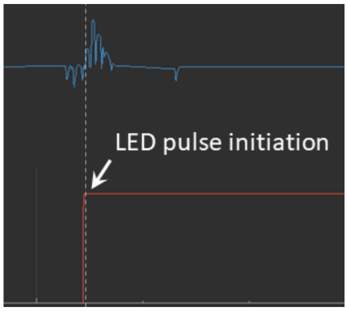
Figure 9.14: *Antenna Channel Options*

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. 9.15a) and termination (Fig. 9.15b) of the LED.

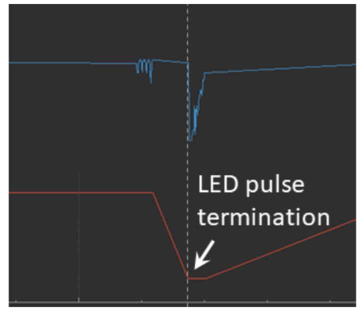
   To remove the artifacts, an algorithm detects the first spike occurring after the initiation or termination of the LED (Fig. 9.15). All the value between the time of the first spike until the specified *Correction width* are set to 0V.

   To turn on the **LED Artifact Remover**:
   - Check the **Active** box (Fig. 9.14, 5);
   - Specify the size of the **Correction width** (Fig. 9.14). 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!

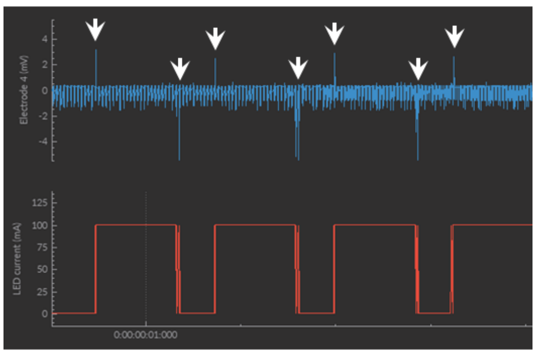


(a) *Initiation artifact*

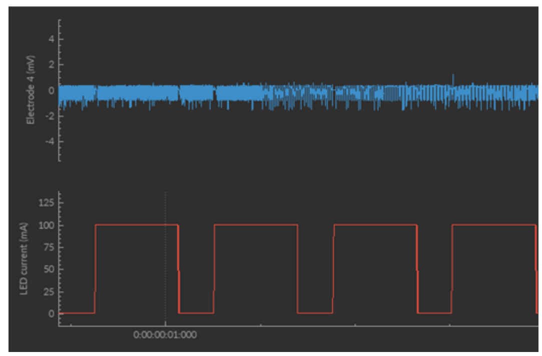


(b) *Termination artifact*

Figure 9.15: *Electrical artifacts caused by LED pulses*



(a) *LED artifacts during recording*



(b) *LED artifacts removed during recording*

Figure 9.16: *LED Artifact Remover Option Example*

9.4.3.2 LED Trigger Options

The **LED Trigger Options** (Fig. 9.17), allow users to select what device triggers the LED source. By default the **Trigger Source** is the DNS acquisition system.

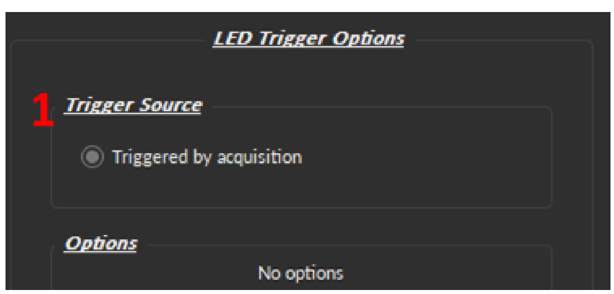
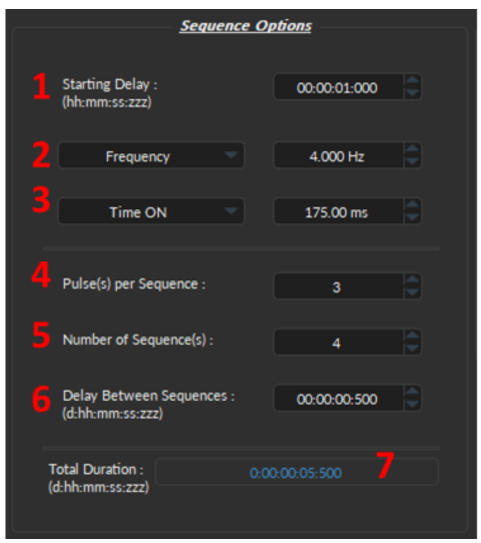


Figure 9.17: *Antenna LED Trigger Options*

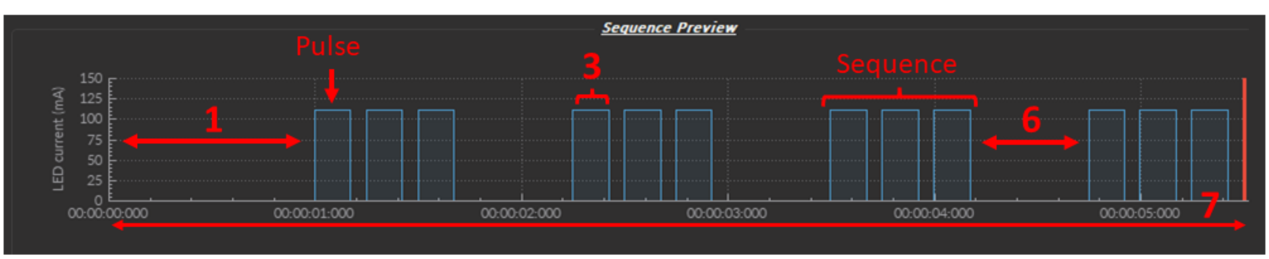
9.4.3.3 Sequence Options & Preview

The **Sequence options** section (Fig. 9.18a) contains the LED pulse sequence parameters, while the **Sequence Preview** section (Fig. 9.18b) displays the corresponding shape and timing of the sequence.

1. The **Starting Delay** (Fig. 9.18) sets the delay (in hh:mm:ss:zzz format) before the first pulse.
2. The **Frequency/Period** (Fig. 9.18a) 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. 9.18) 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. 9.18a) 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. 9.18a) 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. 9.18) 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. 9.18) 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 9.18: *Channel(s) configuration window, Antenna - Sequence Options and Preview*

9.4.4 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. 9.13): (1) the **Channel Options** (Section 9.4.4.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 9.4.4.2).

Figure 9.19: *Channel(s) configuration window, Digital I/O - CW mode*

9.4.4.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.

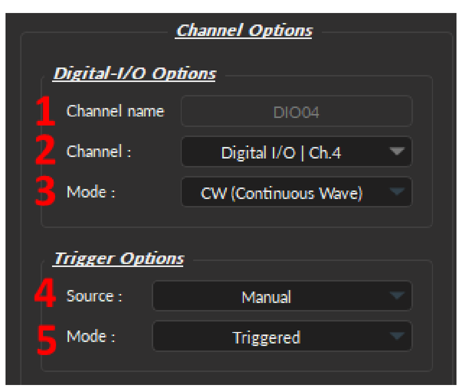
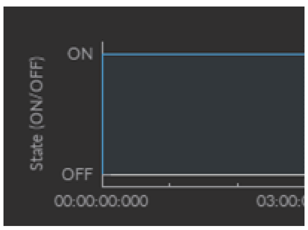


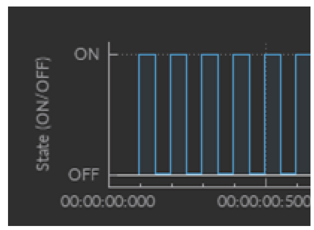
Figure 9.20: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel name** (Fig 9.20, 1) allows user to specify a label for each channel.
2. The **Channel** (Fig 9.20, 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 9.20, 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. 9.21a);
    - The **Square (TTL)** Mode (Fig. 9.21b);
    - 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 9.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.



(a) *Continuous*

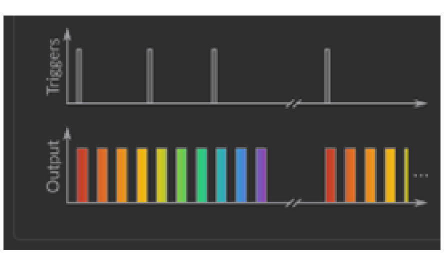


(b) *Square*

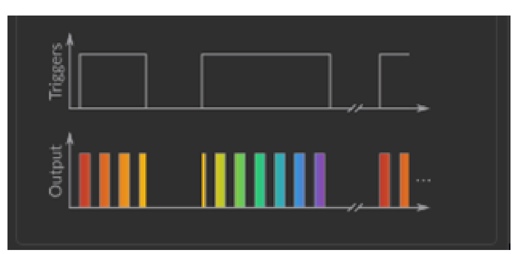
Figure 9.21: *Channel Options - Output Modes*

Trigger Options:

1. The **Source** trigger option (Fig 9.20, 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 9.20, 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. 9.22a), 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. 9.22b), 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 9.22: *Trigger Options Modes*

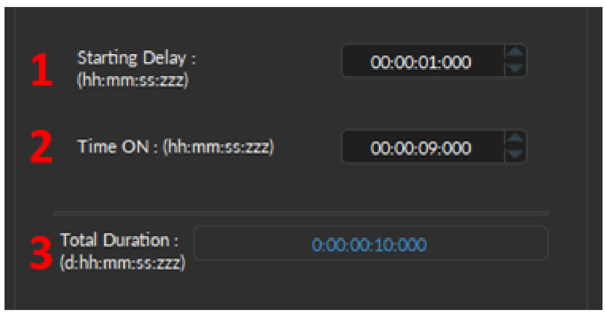
9.4.4.2 Sequence Options & Preview

The **Sequence options** section (Fig. 9.23a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 9.23b) 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**.

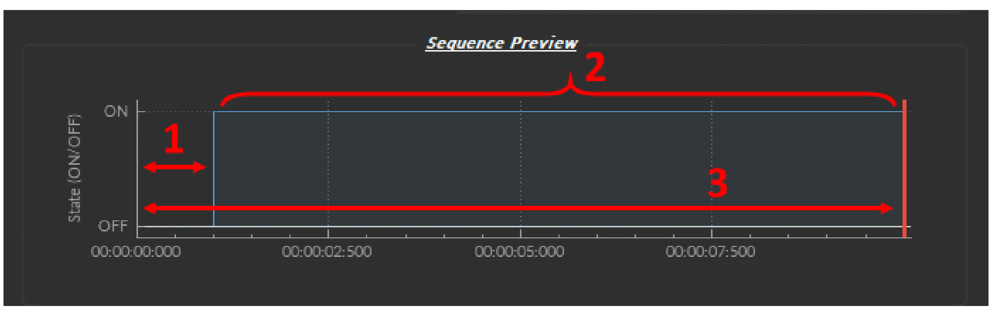
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 9.20), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 9.19) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

  1. The **Starting Delay** (Fig 9.23, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Time ON** (Fig 9.23, 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 9.23, 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*



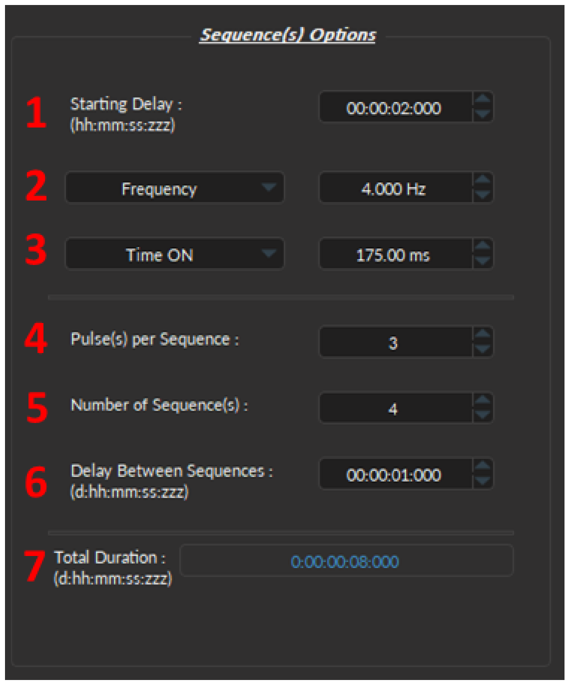
(b) *Sequence Preview*

Figure 9.23: *Channel(s) configuration window, Digital I/O - CW Mode*

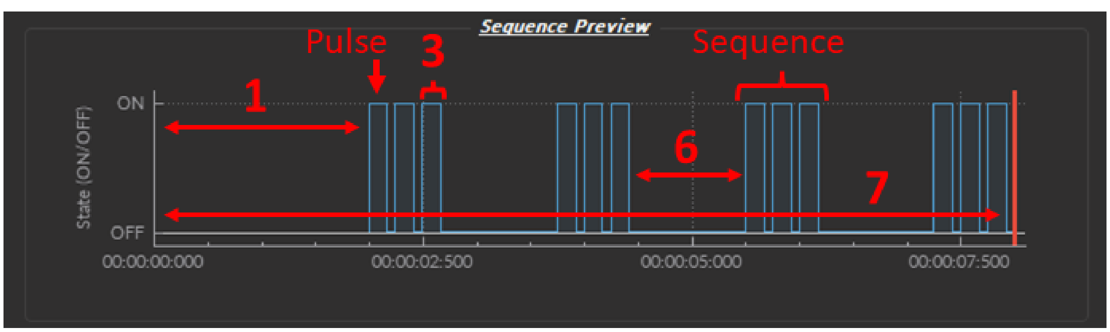
- The **Square** channel mode (Fig. 9.20) allows the creation of a square TTL pulse sequence. This includes all sequence options as the **CW** mode (Fig. 9.23, 1-3), with the following additions:

  1. The **Starting Delay** (Fig 9.23, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Frequency** (Fig. 9.24a, 2) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period** (Fig. 9.24a, 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. 9.24, 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. 9.24, 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. 9.24, 5) sets the number of times that the sequence will be repeated.
6. The **Delay between sequences** (Fig. 9.24, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).
7. The **Total Duration** (Fig 9.23, 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*



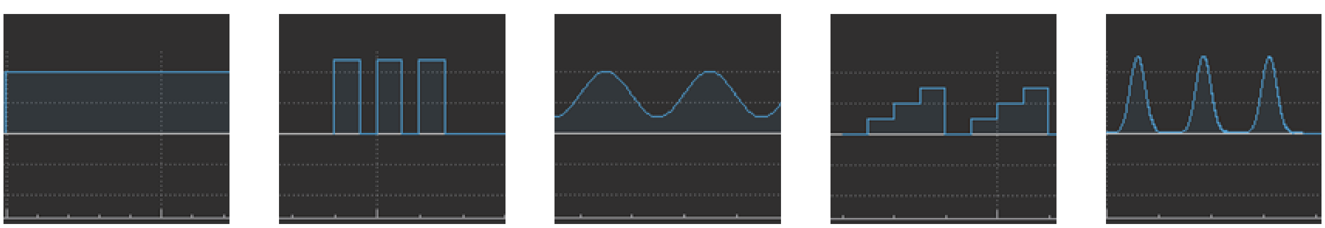
(b) *Sequence Preview*

Figure 9.24: *Channel(s) configuration window, Digital I/O - Square Mode*

9.4.5 Analog Output

Figure 9.25: *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. 9.26).



(a) *Continuous* (b) *Square* (c) *Sine* (d) *Stair* (e) *Custom*

Figure 9.26: *Analog Output Modes*

9.4.5.1 Continuous Wave (CW) Mode

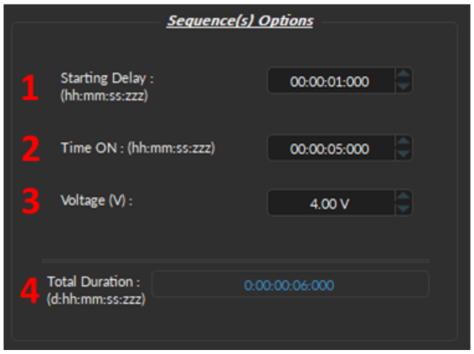
The **CW (Continuous wave)** channel mode (Fig. 9.27) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box (Fig. 9.27a).

1. The **Starting Delay** (Fig. 9.27) defines the time between the activation of the pulse sequence and the beginning of the signal.

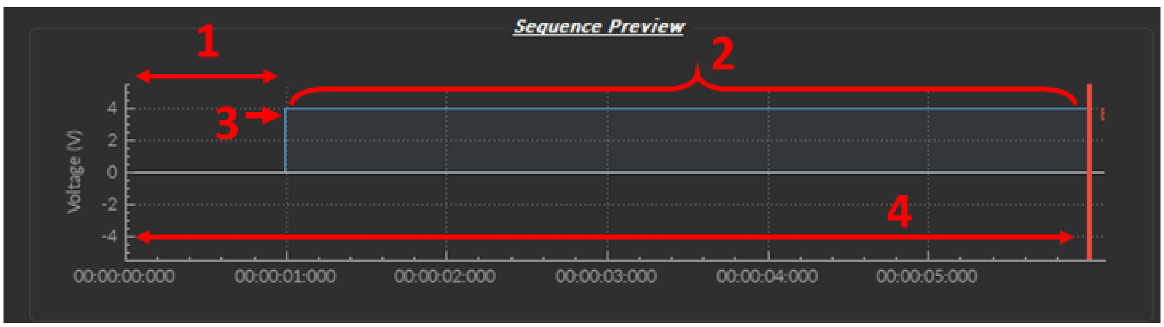
2. The **Time ON** (Fig. 9.27) defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse sequence is stopped manually.

3. The **Voltage** (Fig. 9.27) defines the voltage of the continuous signal, in volts. The signal cannot go beyond ±4.75 V.

4. The **Total Duration** (Fig. 9.27) 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 9.27: *Channel(s) configuration window, Analog Output CW*

9.4.5.2 Square Mode

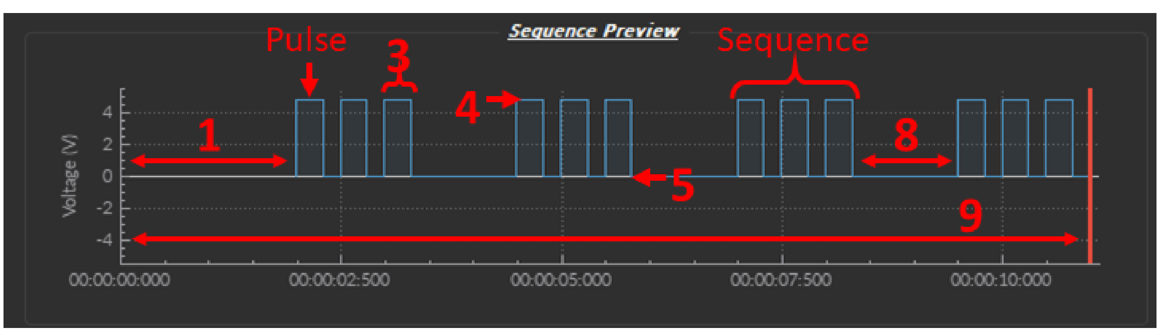
The **Square** channel mode (Fig. 9.28) creates a sequence of pulses with the minimum of the pulses at **V-** and the maximum of each pulse at **V+**.

1. The **Starting Delay** (Fig. 9.28, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Frequency** (Fig. 9.28, 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. 9.28, 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. 9.28, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.
5. The **Minimum Voltage (V-)** (Fig. 9.28, 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. 9.28, 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. 9.28, 7) sets the number of times that the sequence will be repeated.
8. The **Delay between sequences** (Fig. 9.28, 8) sets the delay between each sequence.
9. The **Total Duration** (Fig. 9.28, 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*

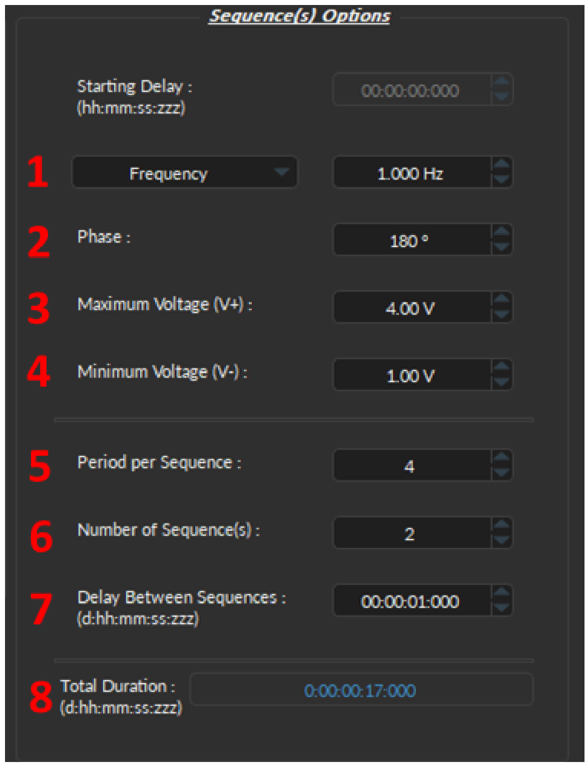
Figure 9.28: *Channel(s) configuration window, Analog Output Square*

9.4.5.3 Sine Mode

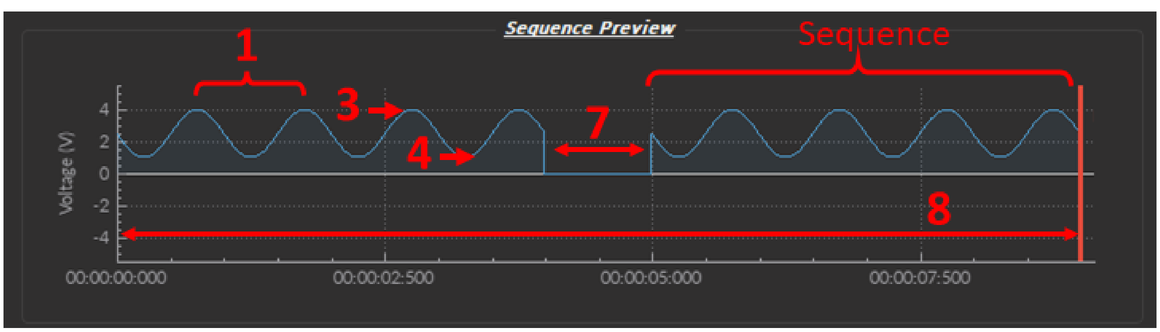
The **Sine** mode (Fig. 9.29) creates a sinusoidal pulse sequence with peaks at **V+** and **V-**.

Note: The **Starting Delay** is not available for this mode (Fig. 9.29a).

1. The **Frequency** (Fig. 9.29, 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. 9.29, 2) replaced **Time ON** (Fig. 9.28, 3). This allows the choice of the sine wave phase, in degrees.
3. The **Maximum Voltage (V+)** (Fig. 9.29, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +4.75 V.
4. The **Minimum Voltage (V-)** (Fig. 9.29, 5) defines the minimum voltage of each pulse, in volts. The signal cannot go below -4.75 V.
5. The **Period per Sequence** (Fig. 9.29, 5) is similar to the **Pulse per Sequence** parameter in Square mode (Section 9.4.5.2, Square), but where the period (a single sine wave from peak to peak, Fig. 9.29b, 1) replaces the pulse.
6. The **Number of sequence(s)** (Fig. 9.29, 7) sets the number of times that the sequence will be repeated.
7. The **Delay between sequences** (Fig. 9.29, 8) sets the delay between each sequence.
8. The **Total Duration** (Fig. 9.29, 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*

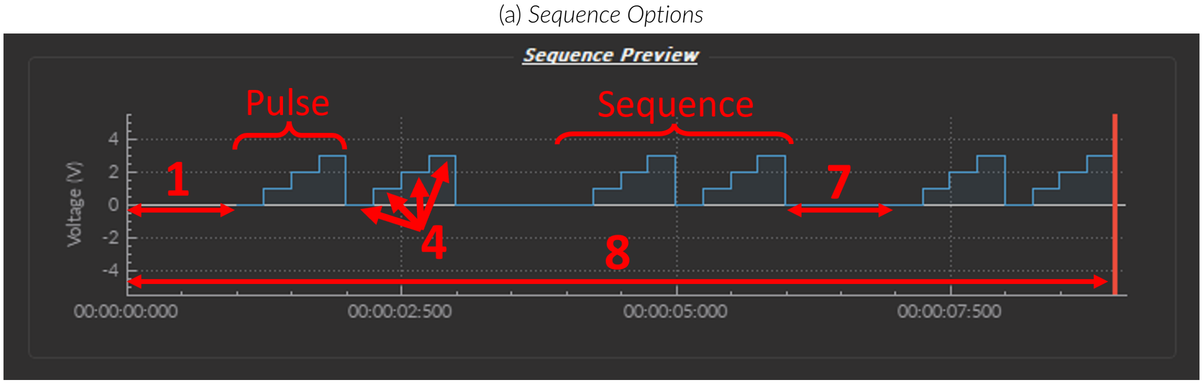
Figure 9.29: *Channel(s) configuration window, Analog Output Sine*

9.4.5.4 Stairs Mode

The **Stairs** mode (Fig. 9.30) creates a stepwise pulse sequence with peaks at several different Voltage levels **V+**.

1. The **Starting Delay** (Fig. 9.30, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
2. The **Frequency** (Fig. 9.30b, 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. 9.30b, 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. 9.30, 4).
5. The **Pulse(s) per sequence** (Fig. 9.30, 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. 9.30, 6) sets the number of times that the sequence will be repeated.
7. The **Delay between sequences** (Fig. 9.30, 7) sets the delay between each sequence.
8. The **Total Duration** (Fig. 9.30, 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 9.30: *Channel(s) configuration window, Analog Output Stairs*

9.4.5.5 Custom Mode

The **Custom** mode (Fig. 9.31) provides users with the ability to design a pulse with non-standard shape to fit experimental needs.

1. The **Select File** button (Fig. 9.31a, 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. 9.31b. The values can be any value between **-4.75V** and **+4.75V**.

2. The **Starting Delay** (Fig. 9.31, 2) defines the time between the activation of the pulse sequence and the beginning of the signal.

3. The **Period** option (Fig. 9.31, 3) replaces the **Time ON** option (Fig. 9.28, 3). This option will stretch or shrink the 2500 value sequence to fit the specified amount of time.

4. The **Period per Sequence** (Fig. 9.31, 4) is similar to the **Pulse per Sequence** field found in **Square** modes (Fig. 9.4.5.2, 6), where the pulse is replaced by the period sequence (Fig. 9.31c, Sequence).

5. The **Number of sequence(s)** (Fig. 9.31a, 5) sets the number of times that the sequence will be repeated.

6. The **Delay between sequences** (Fig. 9.31, 6) sets the delay between each sequence.

7. The **Total Duration** (Fig. 9.31, 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**.

9.4.6 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. 9.32): (1) the **Channel Options** (Section 9.4.6.1) and (2) the **Mode-specific Options** (*Linear*, Section 9.4.6.2; *Interleaved*, Section 9.4.6.3; *Lock-In*, Section 9.4.6.4).

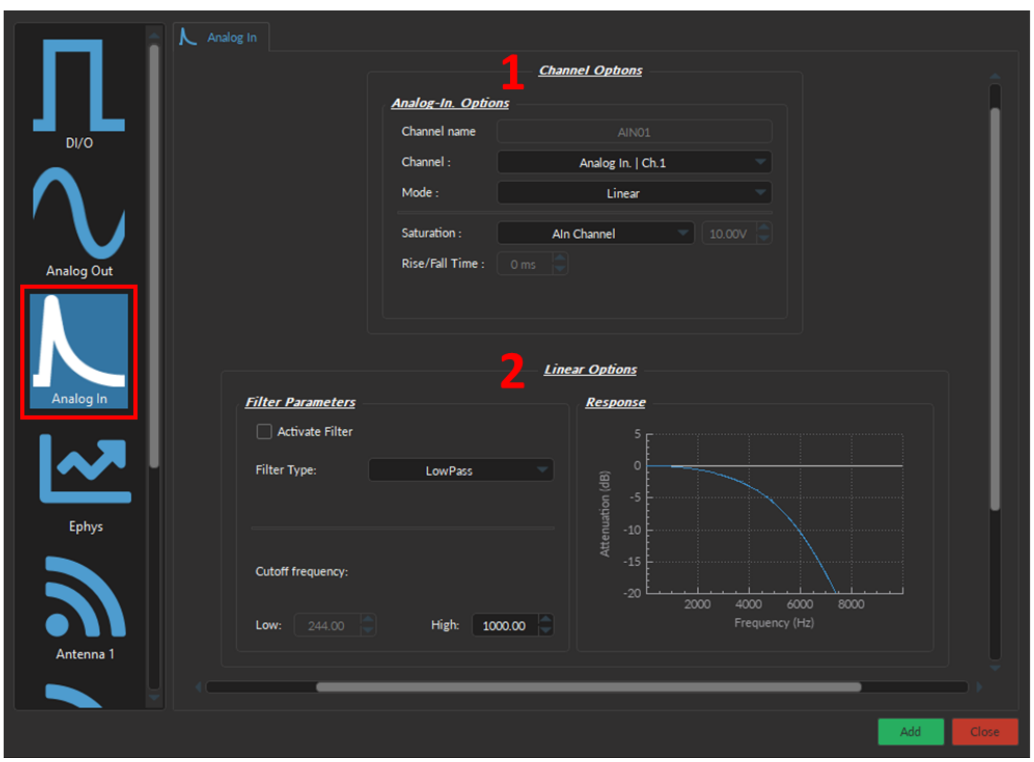
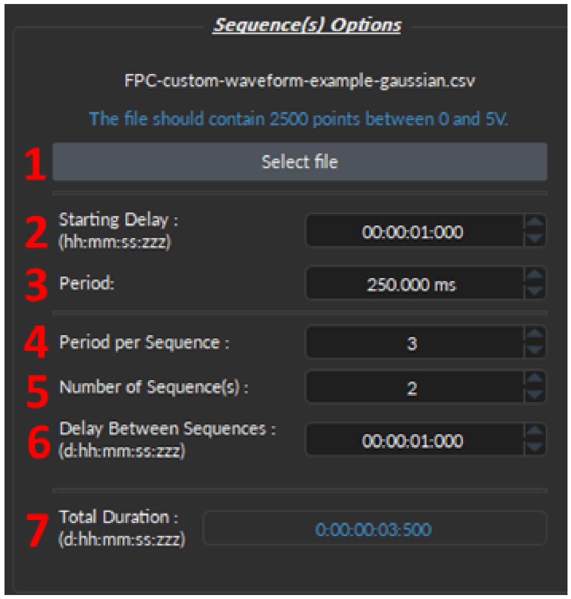
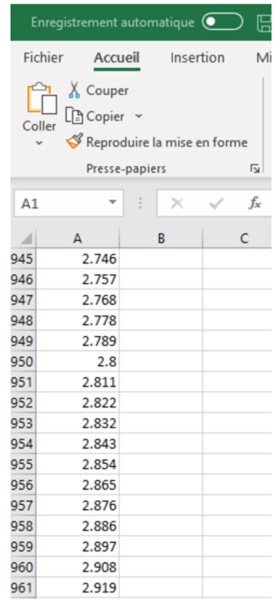


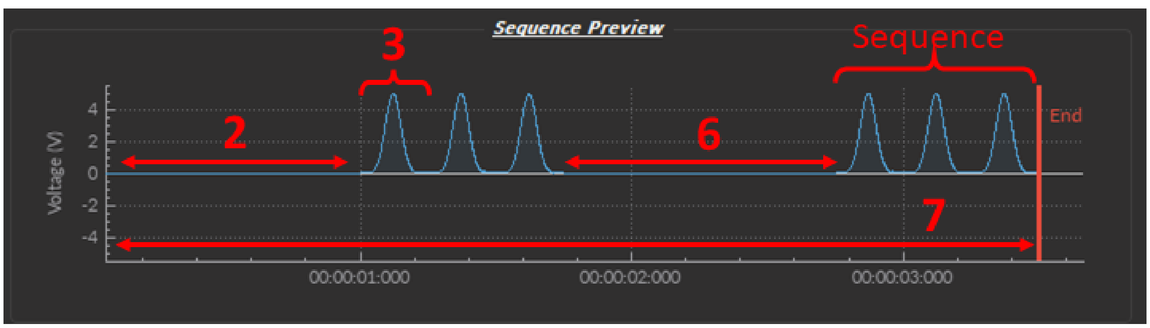
Figure 9.32: *Channel(s) configuration window, Analog Input*



(a) *Sequence Options*



(b) *Example .csv file*



(c) *Sequence Preview*

Figure 9.31: *Channel(s) configuration window, Analog Output Custom*

9.4.6.1 Channel Options

The **Channel Options** (Fig. 9.33) defines the channel, source and mode of the digital signal, as following:

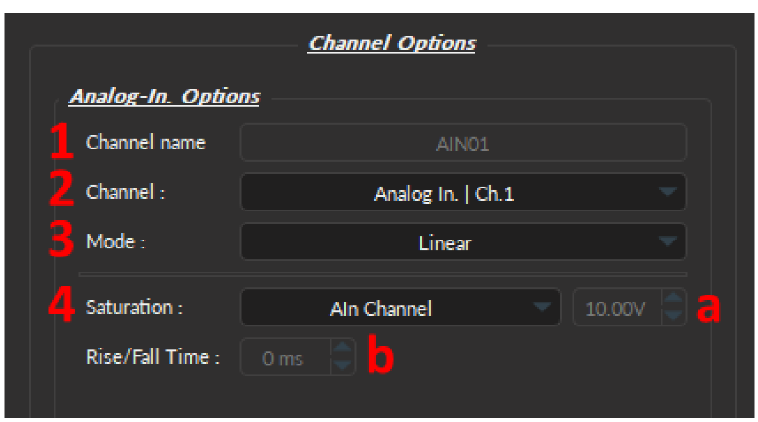


Figure 9.33: *Channel(s) configuration window, Analog Input - Channel Options*

1. The **Channel name** (Fig 9.33, 1) allows user to specify a label for each channel.

2. The **Channel** (Fig 9.33, 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. 9.32, 3):

   - *Linear* - Section 9.4.6.2;
   - *Interleaved* - Section 9.4.6.3;
   - *Lock-In* - Section 9.4.6.4.

4. The **Saturation** (Fig. 9.33, 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. 9.33, 4a)

   b) The **Rise/Fall Time** (Fig. 9.33, 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.

9.4.6.2 Linear

The **Linear** channel mode (Fig. 9.34) allows the direct measurement of signal received by a channel. The linear mode-specific parameters are as follows:

Figure 9.34: *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.

9.4.6.3 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. 9.35).

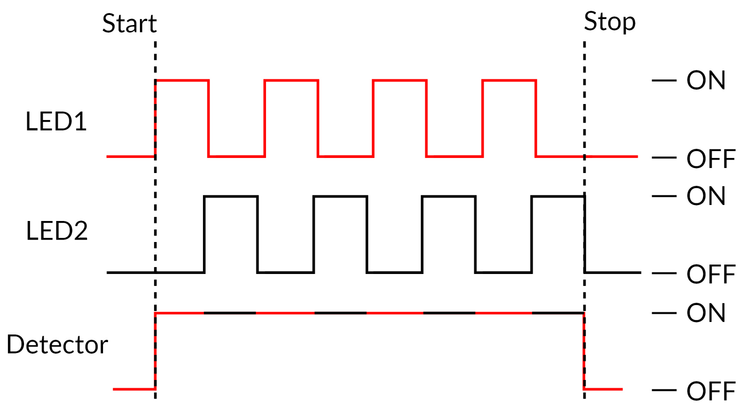


Figure 9.35: *Interleaved Acquisition Timing Diagram*

The interleave preset is using 50% duty cycle for each LED, without delay between them (Fig. 9.35). Thus, depending on the Rise/Fall time of the detector in use (Fig. 9.33, 4b, Detector Rise/Fall Time), there will be more or less crosstalk between the interleaved channels (Fig. 9.36).

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. 9.36). Figure 9.36 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. 9.36).

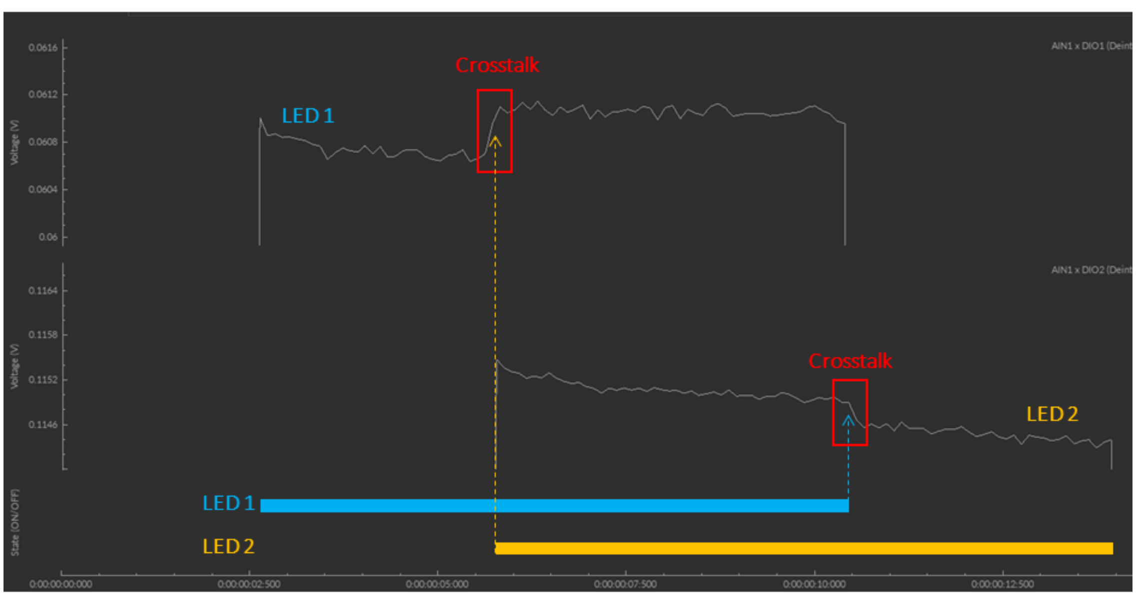


Figure 9.36: *Interleaved Cross-talk*

Strategies to Mitigate Crosstalk:

1. If the sampling rate of the triggered device(s) is high enough (>120Hz), use the **Lock-In mode** (Section 9.4.6.4) 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.**

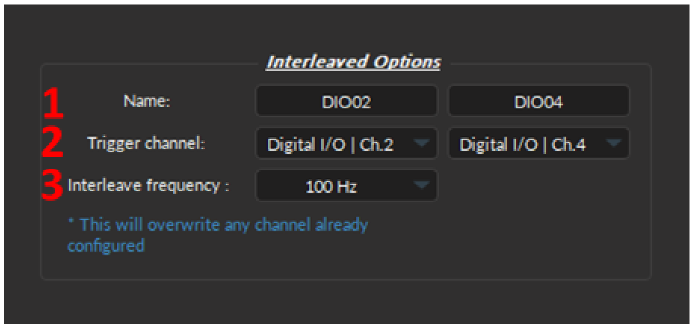


Figure 9.37: *Channel(s) configuration window, Analog Input Interleaved*

To use the **interleaved mode**, specify the parameters in the **Interleaved Options** section of the *Channel Configuration* window (Fig. 9.37):

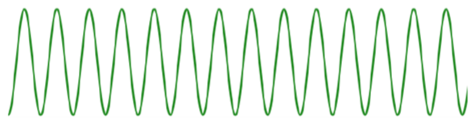
1. The **Name** (Fig. 9.37, 1) lets users customize the label of the channel to increase clarity of the acquisition system.
2. The **Trigger channel** (Fig. 9.37, 2) drop-down list allows the choice of interleaved outputs (can be either digital or analog). However, once the first channel is selected, 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. 9.37, 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.

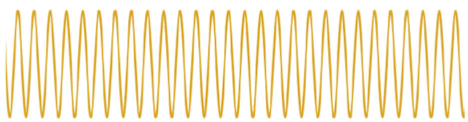
9.4.6.4 Lock-In

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.

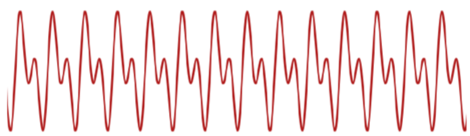
Each *LED light source* emits a sinusoidal illumination at a given frequency (Fig. 9.38a & 9.38b). The detector collects the fluorescent data at a frequency corresponding to the summation of the LED frequencies (Fig. 9.38c).



(a) *LED 1 frequency*



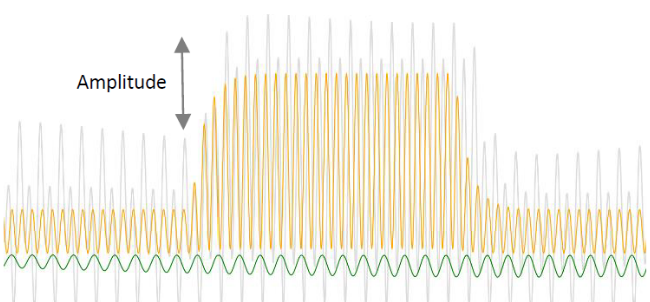
(b) *LED 2 frequency*



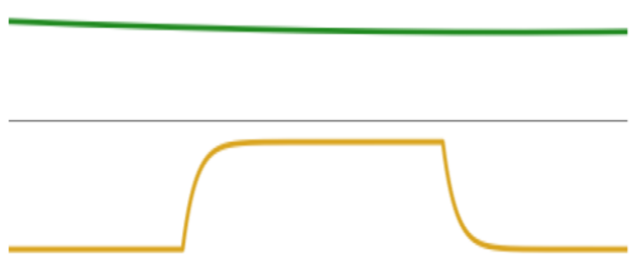
(c) *Raw signal*

Figure 9.38: *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. 9.39a). 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. 9.39). The result is separated from the ambient noise that occurred at different frequencies (Fig. 9.39b). The same principle can be applied to demodulate two fluorescent signals.



(a) *Amplitude changes (signal) are frequency dependent*



(b) *Separated signals*

Figure 9.39: *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. 9.40):

1. The **Enable** (Fig. 9.40, 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. 9.33, 2.

2. **Trace Name** (Fig. 9.40, 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. 9.33).
    - 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. 9.40), changing which port is enabled using the checkbox (Fig. 9.40) 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. 9.40, 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. 9.40, 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. 9.41).

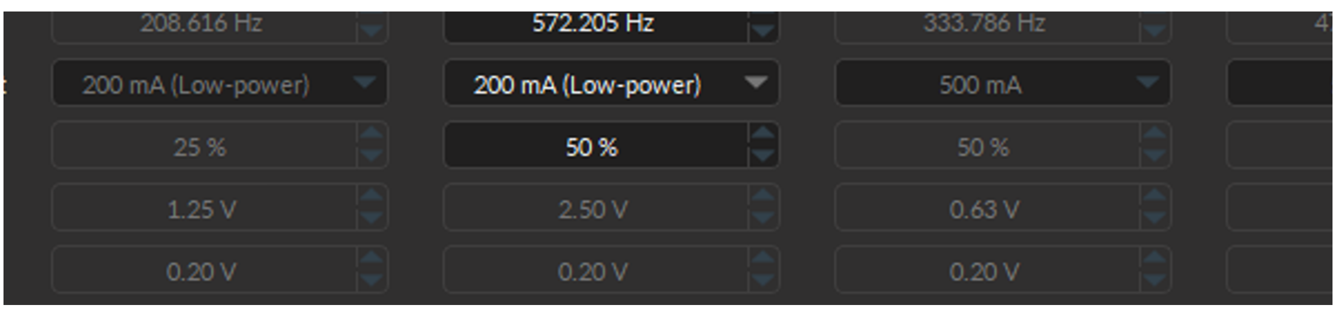


Figure 9.40: *Channel(s) configuration window, Analog Input Lock-In*

5. **LED power** (Fig. 9.40, 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. 9.40, 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.

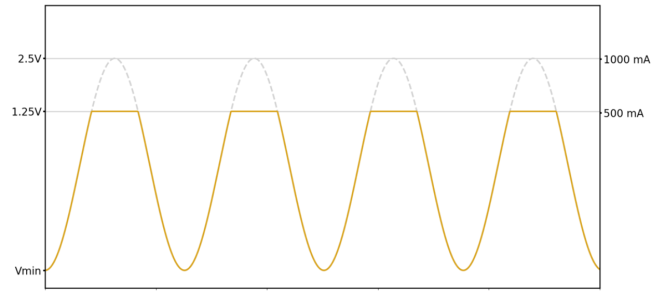


Figure 9.41: *Cropped LED excitation signal*

- **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. 9.40, 6).

7. **Vmin Preview** (Fig. 9.40, 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. 9.40, 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. 9.42).
   - **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.

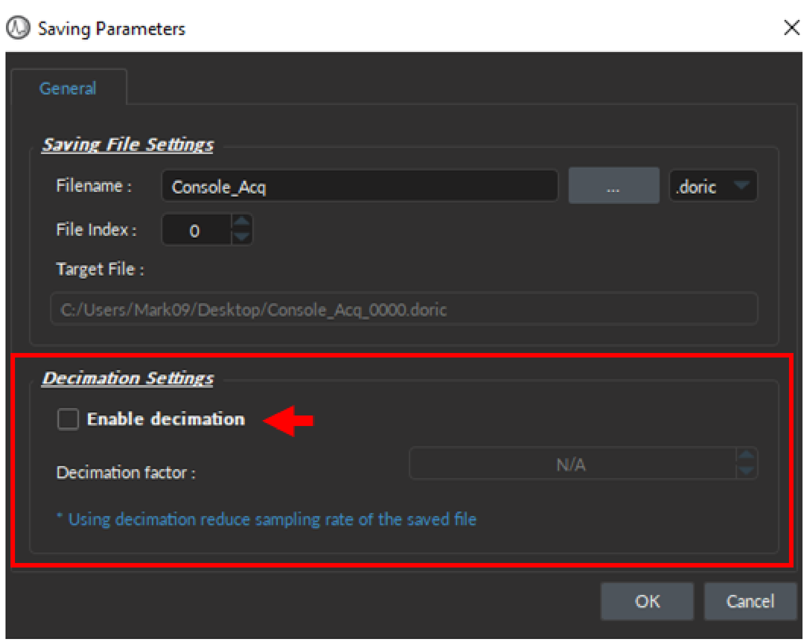


Figure 9.42: *Enable/Disable the decimation factor that reduces Sampling Rate of the saved files*

9.4.7 Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for its measurement.

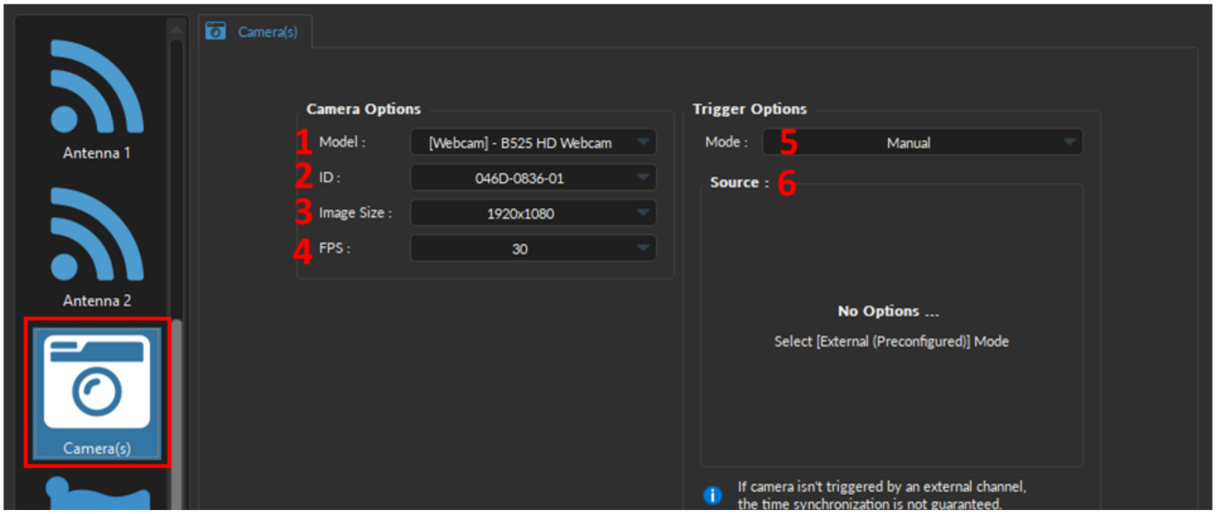


Figure 9.43: *Channel(s) configuration window, Camera*

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

Camera Options:

1. The **Model** (Fig. 9.43, 1) allows you to select the camera of choice based on the type of camera.
2. **ID** drop-down list (Fig. 9.43, 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. 9.43, 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. 9.43, 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. 9.43, 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. 9.44). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

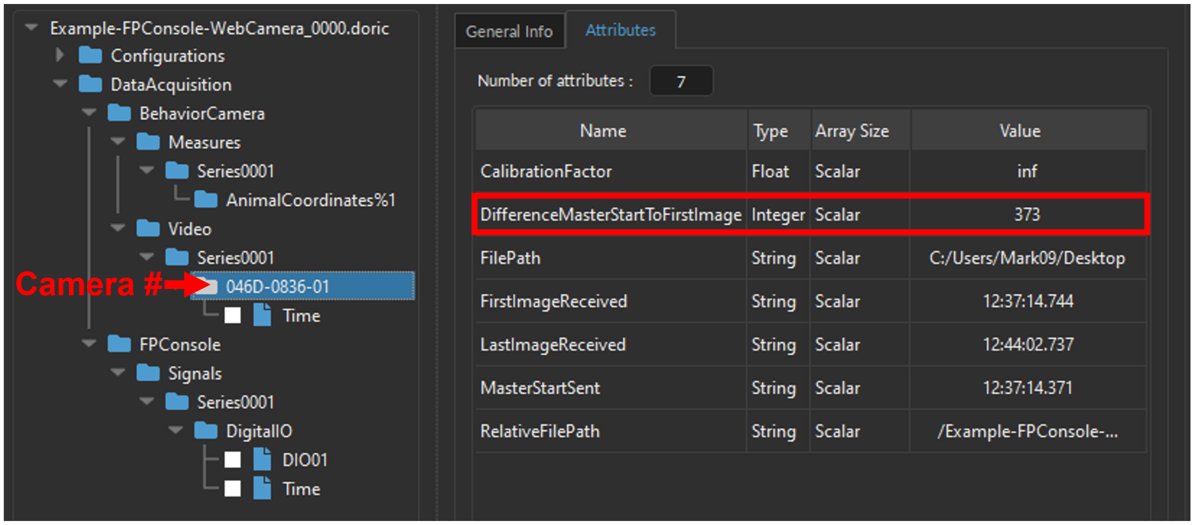


Figure 9.44: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **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. 9.43, 6 & Fig. 9.45) 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. 9.45). 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. 9.45, 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. 9.45, 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. 9.45, 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. 9.45, d) is by default 50%. The frame will be taken at the start of each square pulse.

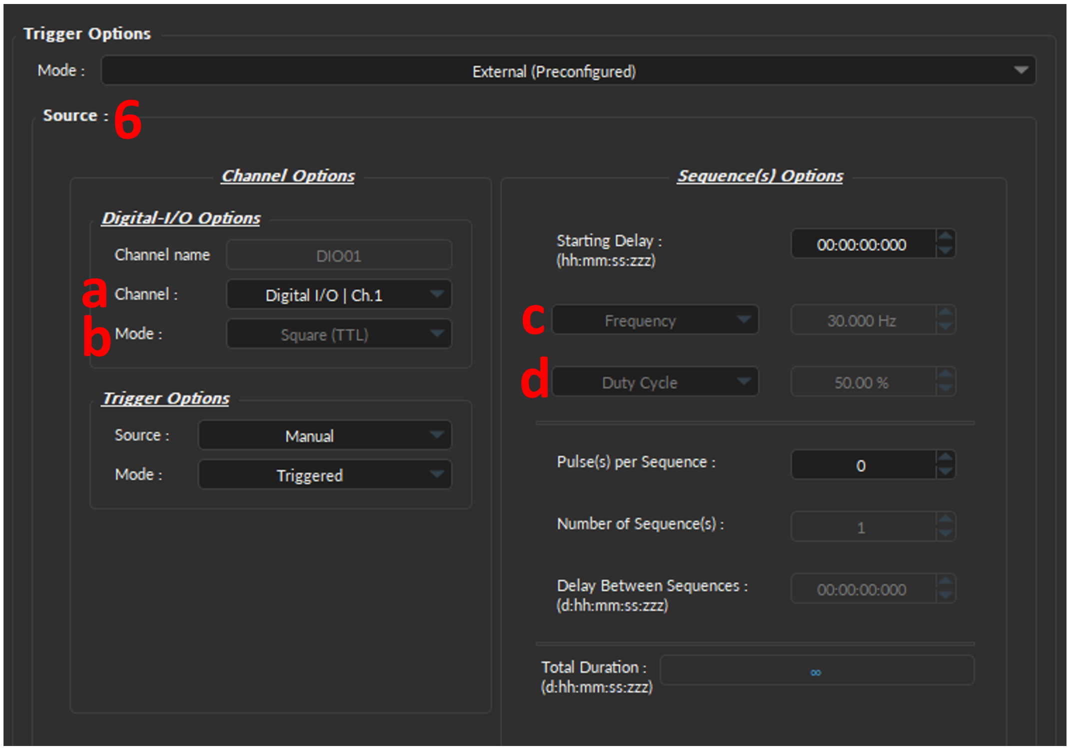


Figure 9.45: *Channel(s) configuration window, Camera - External (Preconfigured)*

9.4.8 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.

9.4.8.1 Adding/Removing KeyPress Event(s)

To add a new **Keypress Event**, select the + sign at the botton of the window (Fig. 9.46, left). To remove a KeyPress, use - button (Fig. 9.46, 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.9.46).

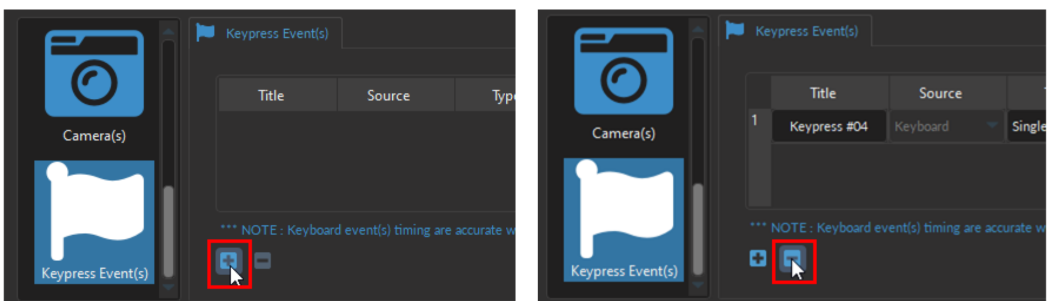


Figure 9.46: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 9.47) in the **Acquisition View**.

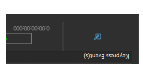


Figure 9.47: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 9.49:

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. 9.48a).

- **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. 9.48b).
- **Timed** - Records an event for a predetermined duration of time (Fig. 9.48c). 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 9.48: *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. 9.49, column 6).
7. The **Information** column provides space to make notes or write a short description of the Keypress Event.

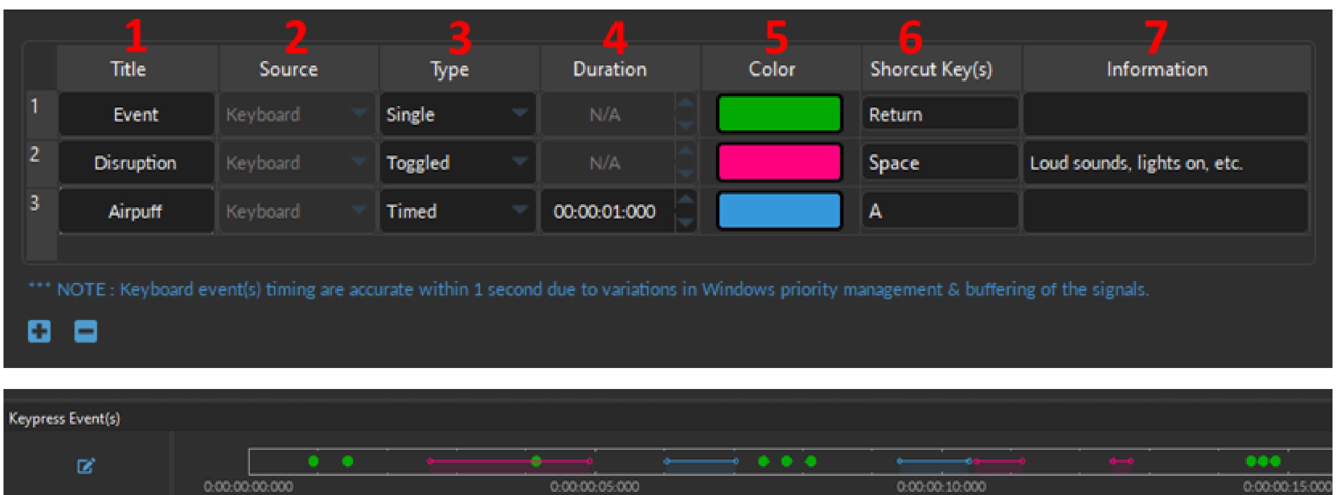
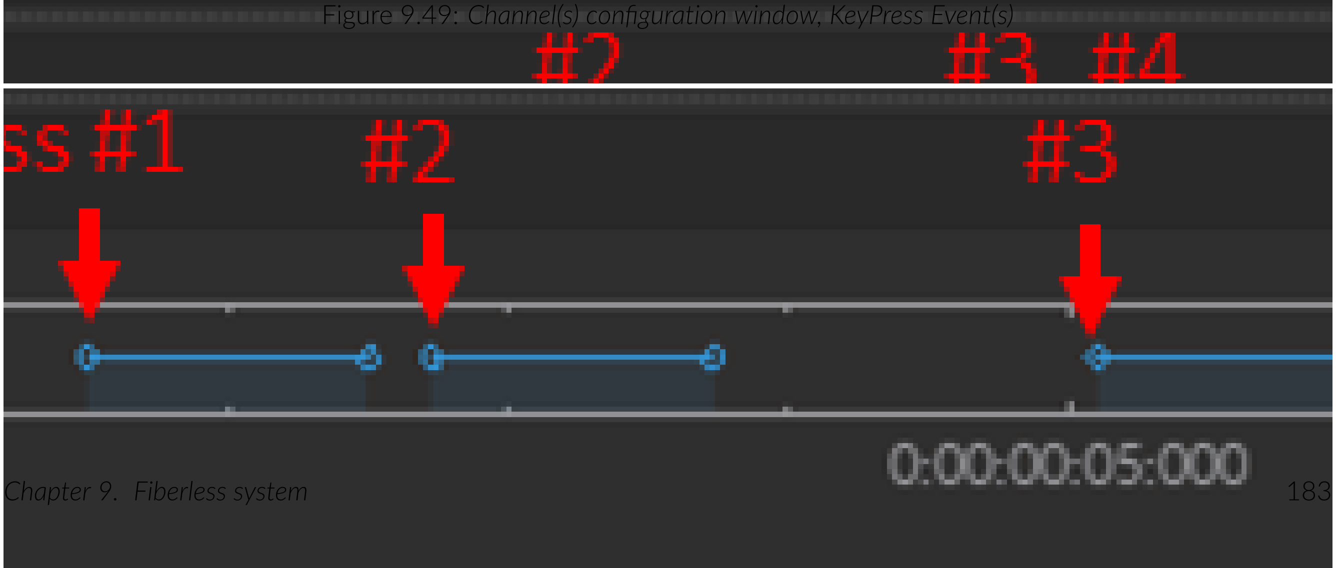


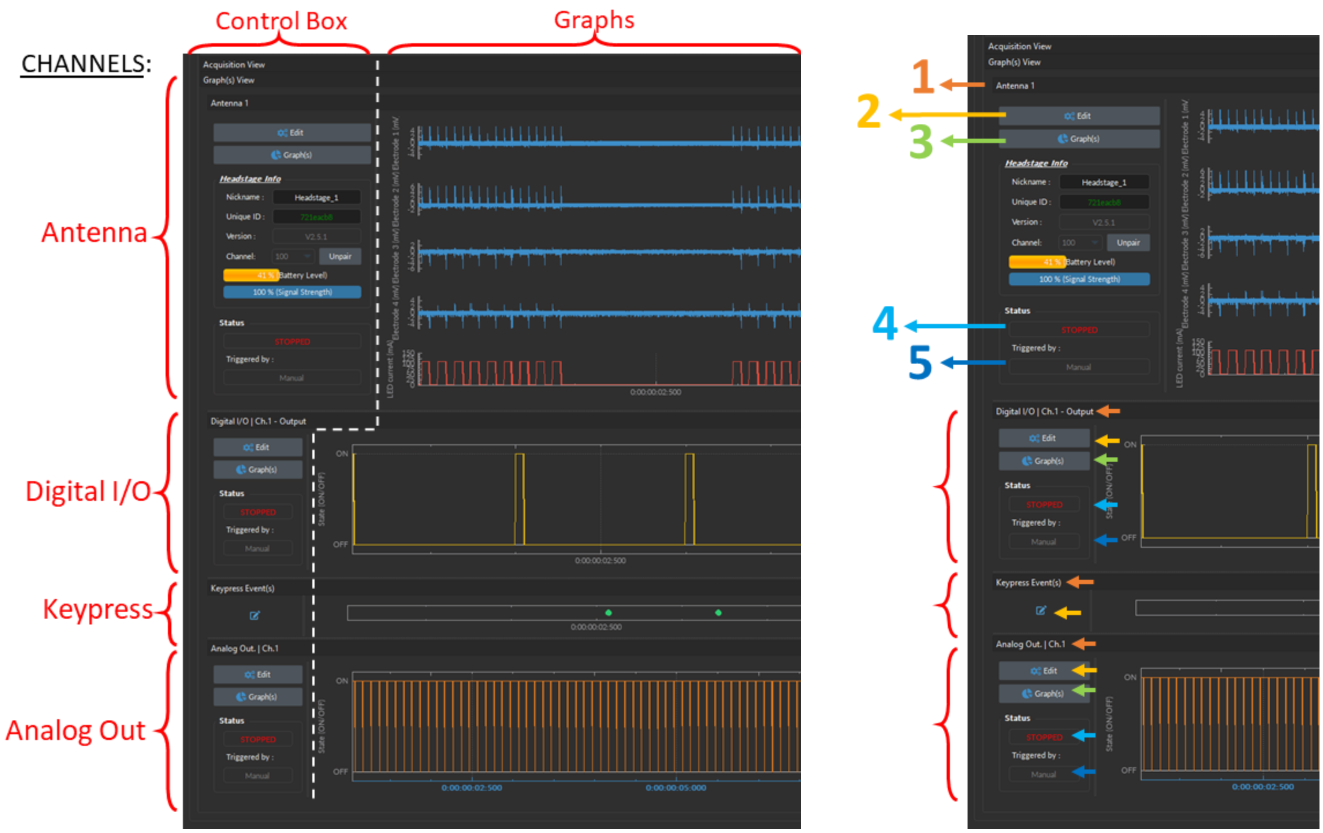
Figure 9.49: *Channel(s) configuration window, KeyPress Event(s)*



9.5 Acquisition View

The **Acquisition View** displays all the information concerning active channels: **Control box** (Fig.9.50a) and the **Graphs** (Fig.9.50b).

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 9.3.2) to load a *.doric* file with previously saved channel parameters, or user can manually add channels using the **Add Channel** button (see Section 9.3.2).



(a) *Channels*

(b) *Common parameters*

Figure 9.50: *Acquisition View*

9.5.1 Channel Control Box

Each channel **Control box** shows the following basic elements (Fig.9.50b), with additional elements available for specific channel types:

1. The **Channel name** (Fig. 9.50b, 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. 9.51).

2. The **Edit** button (Fig. 9.50b, 2) opens the **Channel Configuration** window, were channel parameters can be modified (See section 9.4.1).

3. The **Graph(s)** (Fig. 9.50b, 3) button opens the **Graph Options** window (Fig. 9.51) corresponding to the channel whose graph will be modified. This window allows users to configure visualization and naming parameters of each channel graph (Fig. 9.51). If a channel has multiple traces, parameters to configure each trace individually will appear automatically on different rows (Fig. 9.51). **Graph(s) Options** parameters (Fig. 9.51) are as follows:

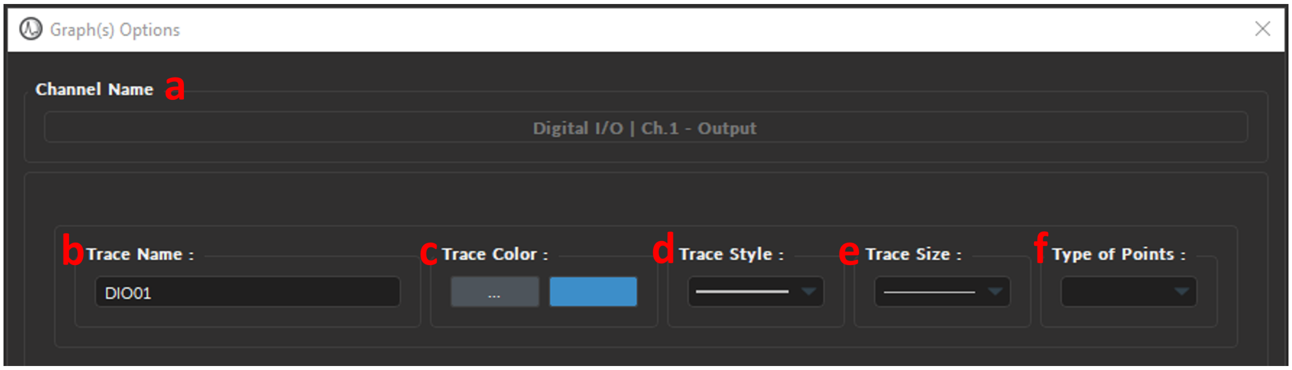


Figure 9.51: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 9.51, 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. 9.51, 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. 9.51, 3) opens the **Color Select** window (Fig. 9.52), 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. 9.51, 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. 9.51, 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. 9.51, 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.

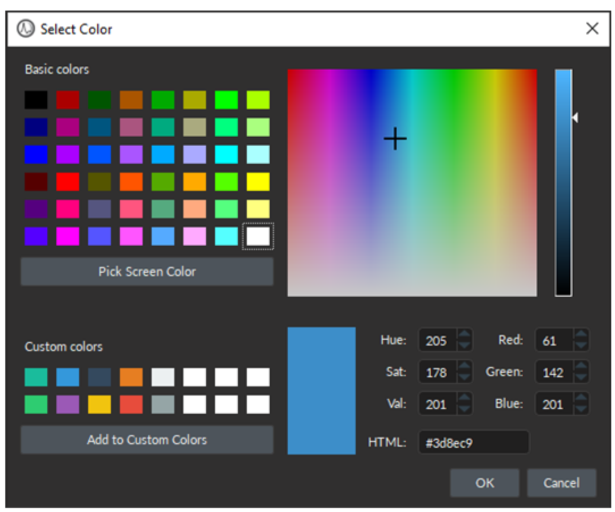


Figure 9.52: *Select Color Window*

4. The **Status** bar (Fig. 9.50b, 4) displays acquisition status. **STOPPED** is displayed when the acquisition is inactive, and **STARTED** when acquisition is active.

5. The **Triggered by:** (Fig. 9.50b, 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. 9.53) displays the *EP Console*-specific elements which are important to pair, control and monitor the wireless headstage. The following elements are included

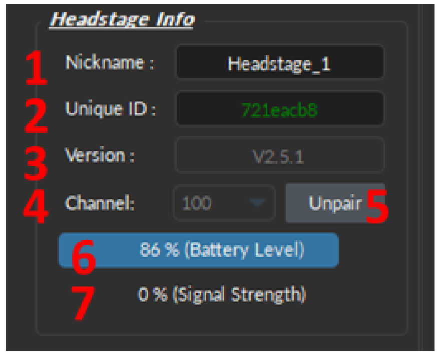


Figure 9.53: *Acquisition View, Controls*

1. The **Nickname** text-box (Fig. 9.53, 1) allows the user to change the name used for the connected headstage.
2. The **Unique ID** box (Fig. 9.53, 2) displays the unique ID sequence associated with the headstage currently in use.
3. The **Version** box (Fig. 9.53, 3) displays the headstage firmware version.
4. The **Channel** drop-down list (Fig. 9.53, 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. 9.53, 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. 9.53, 6) displays the headstage battery level, in %, at all times.
7. The **Signal strength** bar (Fig. 9.53, 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. 9.54), (1) click **Pair** and (2) unique ID in green will appear if correctly paired.

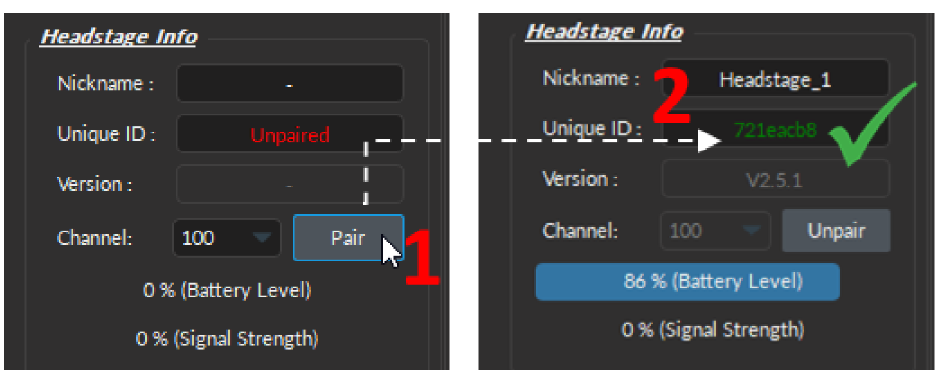


Figure 9.54: *Pairing Headstage*

9.5.2 Channel Graph Visualisation

Besides editing the trace of the channel **Graph**, which can be done through the **Edit** button of the **Control box** (section 9.5.1), 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.

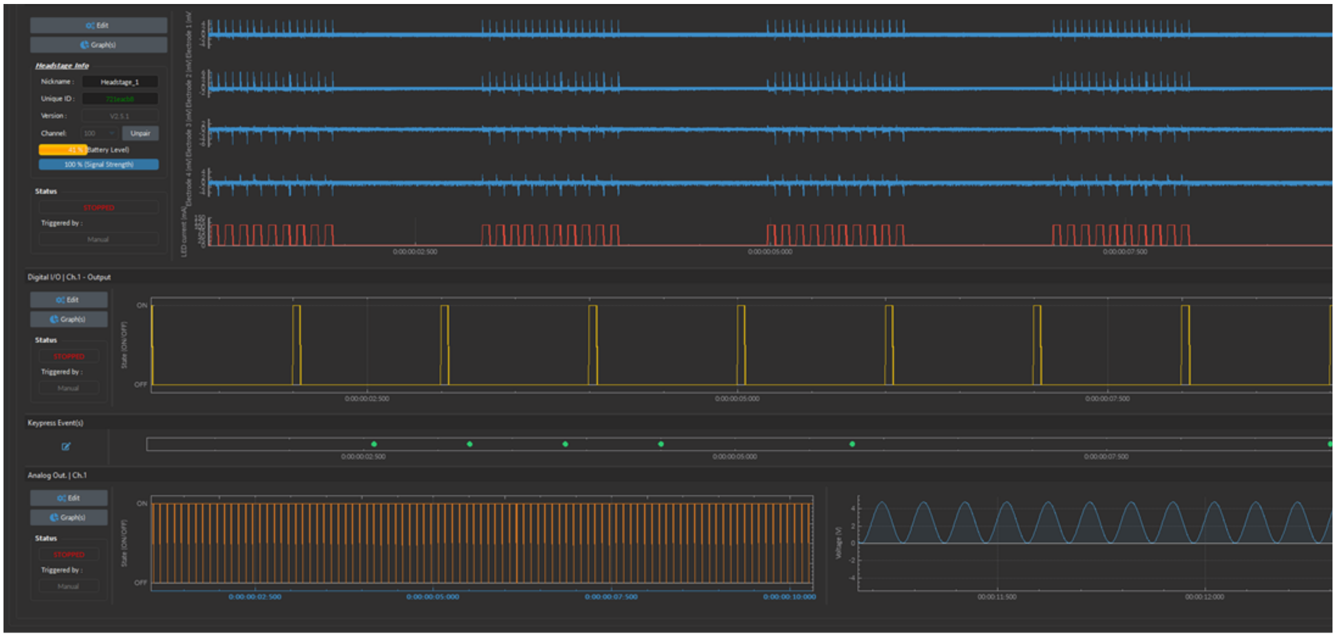


Figure 9.55: *Acquisition View - Graph*

- *Axis Options* - Each **Graph** (Fig. 9.51) 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 9.56: *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. 9.57). 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.

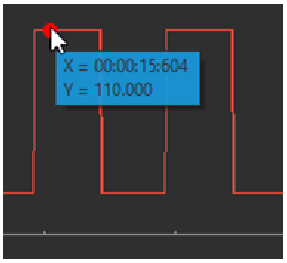


Figure 9.57: *Acquisition View - Instant values*

- 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. 9.58, 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.9.58, right panel), showing a preview of the pulse sequence.

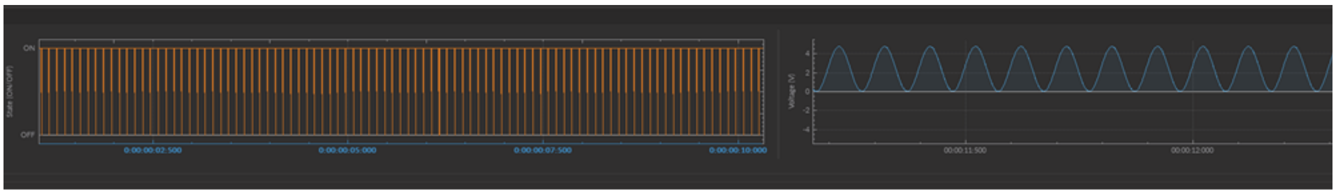


Figure 9.58: *Acquisition View - Output graph*

10

Optogenetics TTL Pulse Generator

The *Optogenetics TTL Pulse Generator* (OTPG; 4 or 8 channels) are controlled by the *Doric Neuroscience Studio* software. Various pulses train sequences can be easily created to combine and synchronize optogenetic and behavior data collection.

The *OTPG* user interface (Fig. 10.1) is split into two main sections: the **Controls & Settings** (Section 10.1) and the **Acquisition view** (Section 10.3).

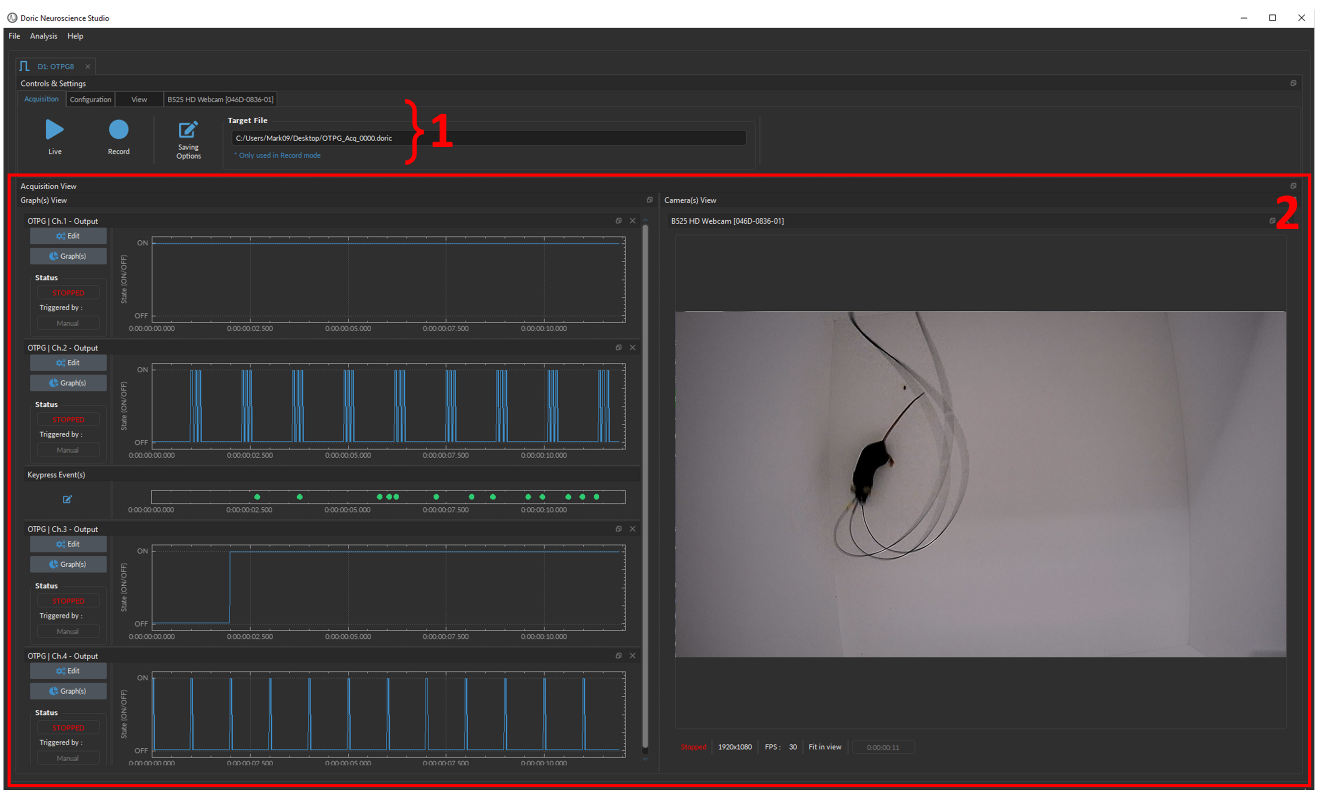


Figure 10.1: *Optogenetics TTL Pulse Generator Interface.*

10.1 Control and Settings

The **Control and Settings** box is used to manage the different parts of the software. It contains three tabs, the **Acquisition** (Section 10.1.1), **Configuration** (Section 10.1.2), and **View** Tabs (Section 10.1.3).

10.1.1 Acquisition Tab

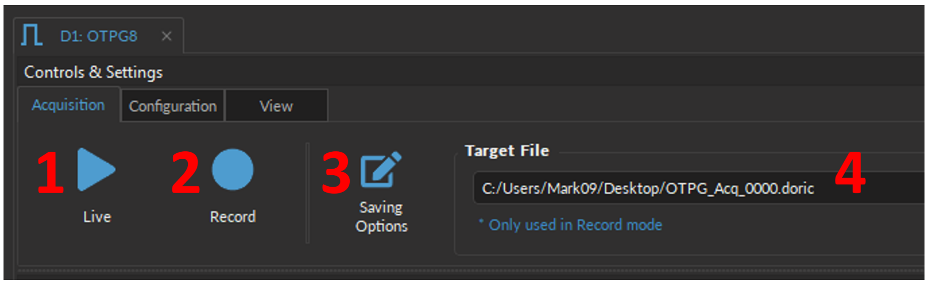


Figure 10.2: *Acquisition Tab*

The different buttons and fields of the **Acquisition Tab** are shown in Figure 10.2 and their functions are explained below.

1. The **Live** button (Fig. 10.2, 1) starts all the configured channels without recording their signal.
2. The **Record** button (Fig. 10.2, 2) starts all the configured channels and records the signal for each of them at the defined **Sampling Frequency**. The recorded data 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 **Saving Options** button (Fig. 10.2, 3) opens the **Saving Options** window (Fig. 10.3 & 10.4) where users can specify **File Settings** or **Decimation settings**. See Section 10.1.1.1 for more details.
4. The **Target File** (Fig. 10.2, 4) field indicates the saving path and filename of the channels signal file.

10.1.1.1 Saving Options

The **Saving Options** window is split into two tabs:

1. The **General** tab (Fig. 10.3) contains the **File Settings** parameters, including:

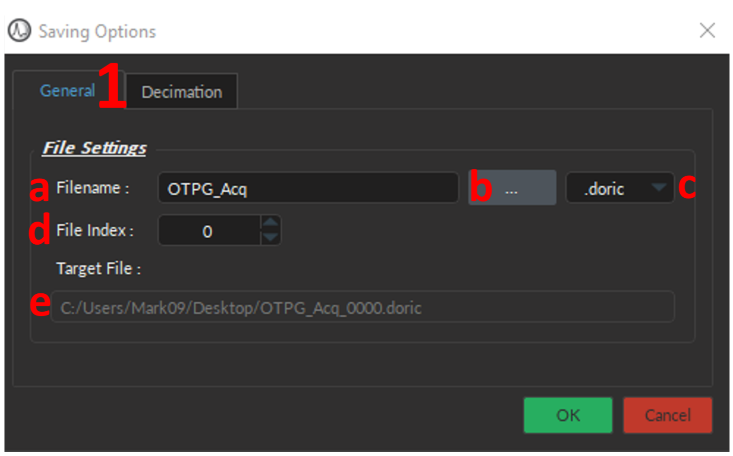


Figure 10.3: *Saving Options, File Settings*

a) The **Filename** text-box (Fig. 10.3, a) lets users specify the name of the data file that will be saved.

b) The **[...]** button (Fig. 10.3, b) opens a File Explorer window where users can select the folder where the data will be saved.

c) The **File format** (Fig. 10.3, c) 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.

d) The **File Index** (Fig. 10.3, d) 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.

e) The **Target File** (Fig. 10.3, e) displays the absolute path and filename where the data will be saved.

2. The **Decimation** tab (Fig. 10.4) contains the **Decimation Settings** parameters, including:

f) The **Enable decimation** checkbox (Fig. 10.4, f) provides a way to reduce the file sizes. This method conserves one point over a number of data points equal to the **Decimation Factor**.

g) The **Decimation factor** text-box (Fig. 10.4, g) is used to define the number of points saved.[1] Note that decimating a file will reduce the sampling rate of the saved file.

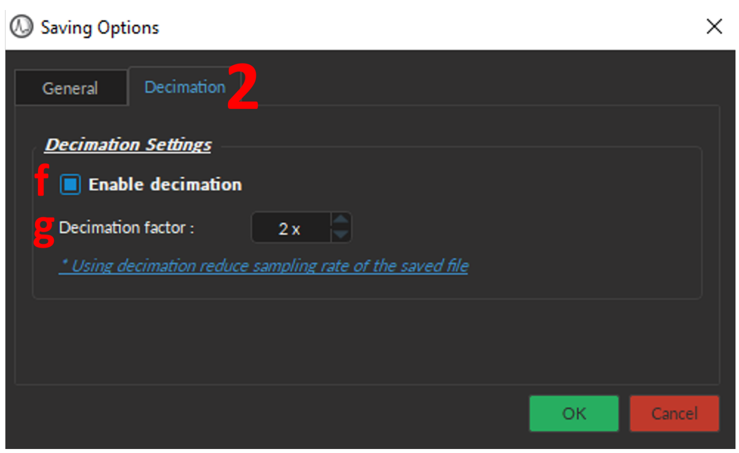


Figure 10.4: *Saving Options, Decimation Settings*

10.1.2 Configuration Tab

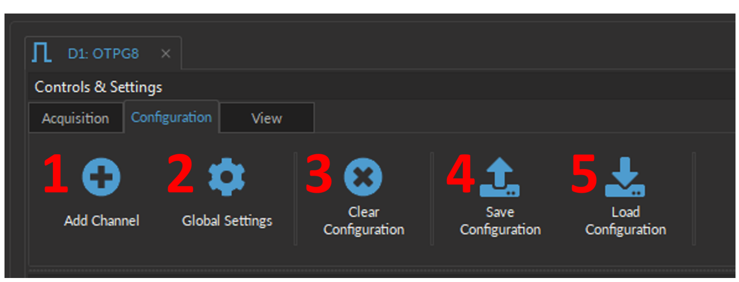


Figure 10.5: *Configuration Tab*

The different buttons of the **Configuration Tab** are shown in Figure 10.5 and their functions are explained below.

1. The **Add Channel** button (Fig. 10.5, 1) opens the **Channels configuration** window to setup the channels. This window is detailed in section 10.2.

[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.

2. The **Global Settings** button (Fig. 10.5, 2) opens a pop-up window (Fig. 10.6) were **DIO sampling rate** and **Master Trigger Options** are specified. See Section 10.1.2.1 for more details.
3. The **Clear Configuration** button (Fig. 10.5, 3) resets the acquisition view and all other parameters set. Any configurations already set will be lost.
4. The **Save Configuration** button (Fig. 10.5, 4) is used to save the OTPG configuration in a **.doric** format.
5. The **Load Configuration** button (Fig. 10.5, 5) allows an OTPG configuration in **.doric** format to be loaded. Recorded data files also contain the configuration used during the experiment and this configuration can be loaded using this button.

10.1.2.1 Global Settings

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

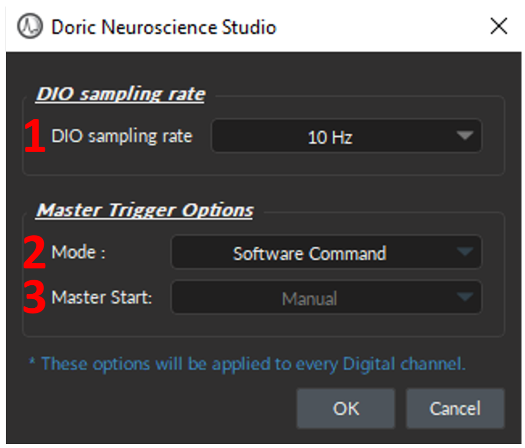


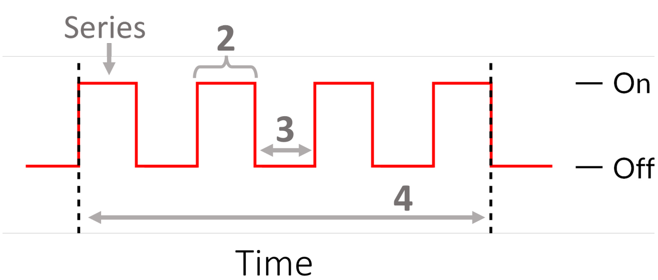
Figure 10.6: *Global Options Window*

1. The **DIO sampling rate** (Fig. 10.6, 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 (100 Hz to 10 kHz). Note that this value ONLY refers to **Digital In/Out** signals. The **effective sampling rate** of each **Camera** channel is displayed at the bottom of the **Camera View** (Fig. 10.34, no. 3).
2. The **Mode** (Fig. 10.6, 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. 10.2, 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 15.10a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 10.7b) pops up. See section 10.1.2.2 for more details.
3. The **Master Start** (Fig. 10.6, 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.***

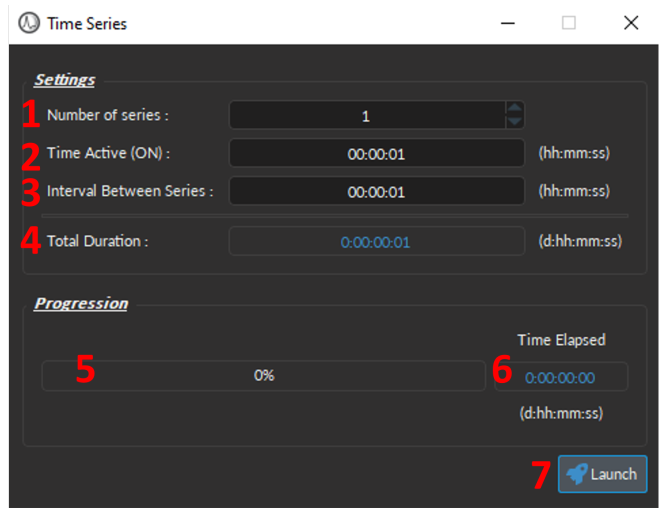
10.1.2.2 Time Series

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 10.7b) can be opened by clicking on the **Record** button (Fig. 10.2, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 10.6, 2). Every **Time series** sequence is automatically saved to the same *.doric* file defined in **Saving Options** (Section 10.1.1.1).



(a) *Time Series Acquisition Timing Diagram*



(b) *Time Series Window*

Figure 10.7: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 10.7b) sets the following parameters:

1. The **Number of series** (Fig. 10.7b, 1) defines the number of times the series is repeated.
2. The **Time Active (ON)** (Fig. 10.7b, 2) defines the duration of the series.
3. The **Interval Between Series** (Fig. 10.7b, 3) defines the number of time between each series, if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 10.7b, 4) displays the total amount of time that the timeseries recording will take place.
5. The **Progression bar** (Fig. 10.7b, 5) indicates the progression of the timeseries (in %).
6. The **Time Elapsed** (Fig. 10.7b, 6) counter indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** (Fig. 10.7b, 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.

10.1.3 View Tab

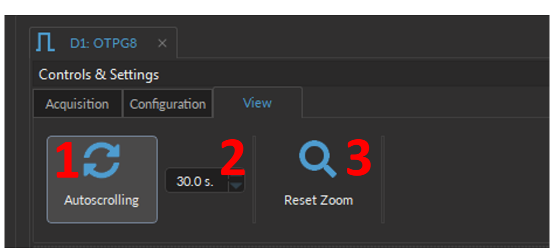


Figure 10.8: *View Tab*

The different buttons and fields of the **View Tab** are shown in Figure 10.8 and sets the graph zoom to the value of choice, specified in the text-box.

1. The **Autoscrolling** button (Fig. 10.8, 1), when enabled, 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 **Zoom range** (Fig. 10.8, 2) sets the graph zoom to the value of choice, specified in the text box.

3. The **Reset Zoom** button (Fig. 10.8, 3) resets the horizontal axis of all graphs displayed in the **Acquisition View** to the duration chosen in the **Zoom range** field.

10.2 Add Channel

10.2.1 New Configuration

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. 10.5, 1). This will open the **OTPG Configuration** window (Fig. 10.9).
To generate a new **Channel** using the **OTPG configuration** window (Fig. 10.9):

1. Select one of the available **Channel Type** icons from the left most column of the **OTPG configuration** window (Fig. 10.9, 1). Table 10.1 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 10.1 for hyperlinks to the relevant sections.

3. Select the **Add/Apply** button (Fig. 10.9, 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.

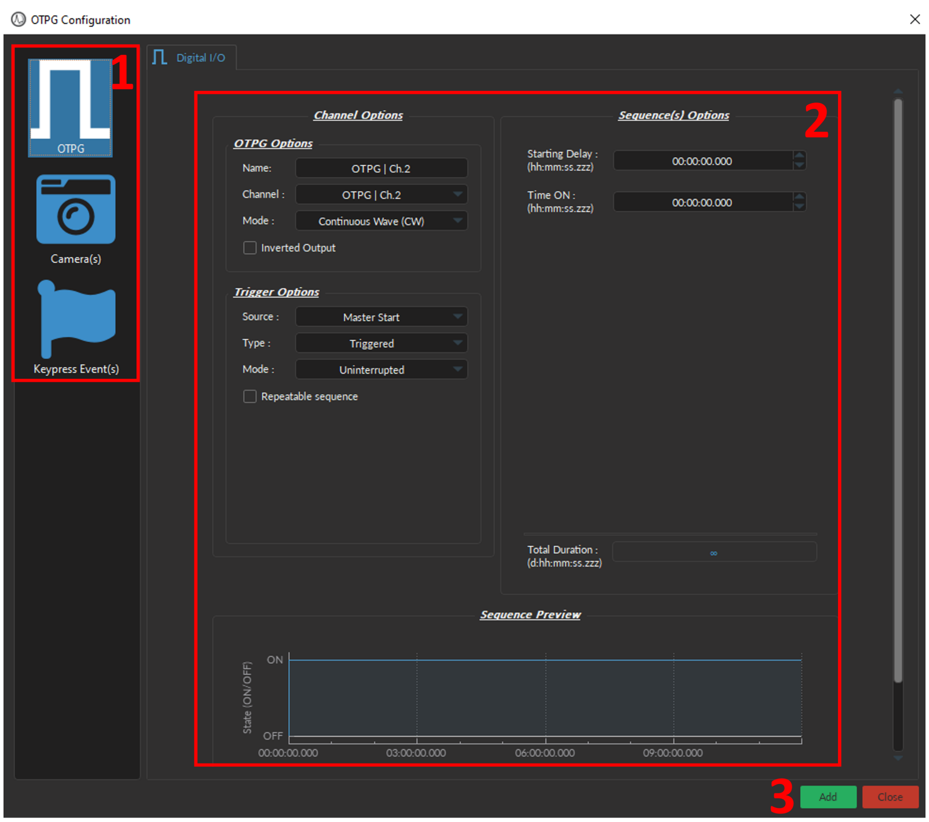


Figure 10.9: *Channel(s) configuration window*

10.2.2 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 10.5). Table 10.1 details the types of inputs and output the OTPG and the software can handle and gives quick access to their sections.

Table 10.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	OTPG	For input and output of TTL signals	10.2.3
	Camera(s)	To collect images for behaviour experiments	10.2.4
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	10.2.5

10.2.3 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 externally driven device used during the experiment (such as the timing of displayed stimuli or a measured behavior).

The *OTPG configuration* window for the **Digital I/O** Channel is divided into three sections (Fig. 10.10): (1) the **Channel Options** (Section 10.2.3.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 10.2.3.2).

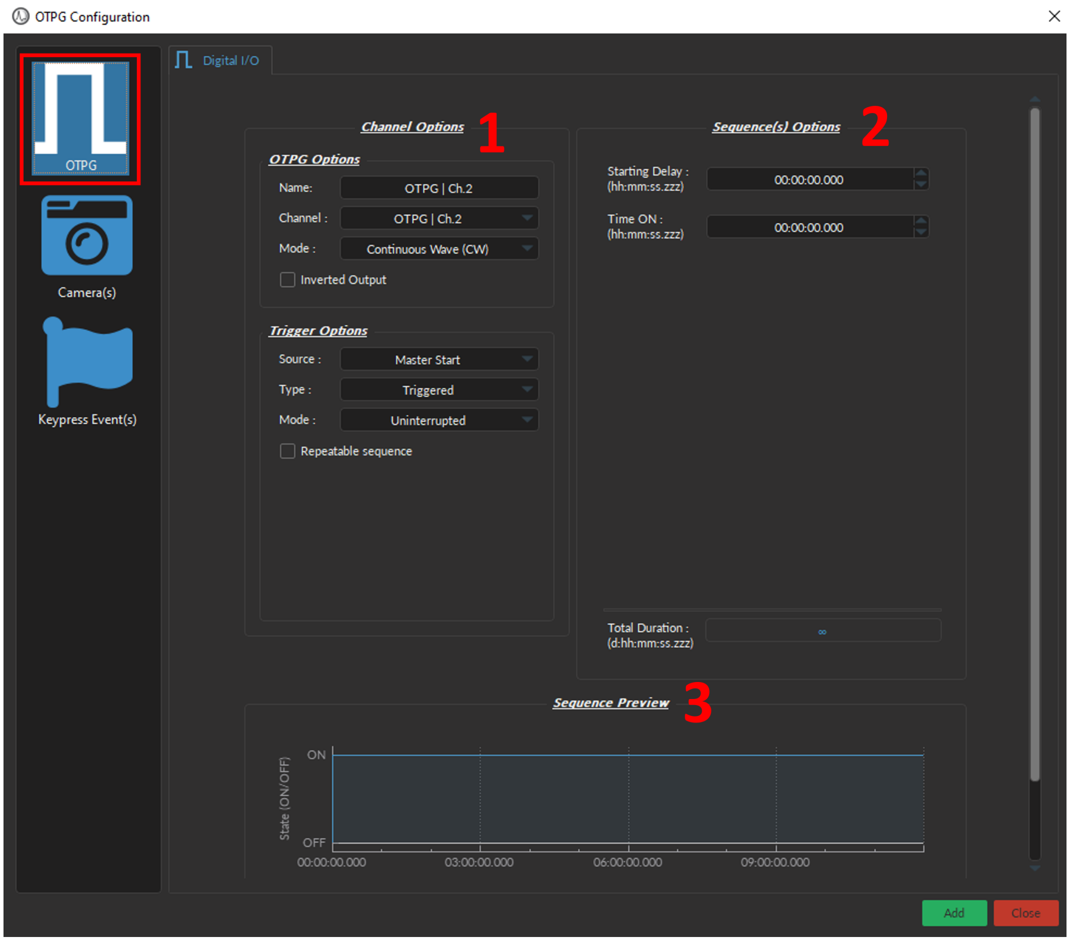


Figure 10.10: *OTPG Configuration Window, Digital I/O*

10.2.3.1 Channel Options

The **Channel Options** defines the channel, source, and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.

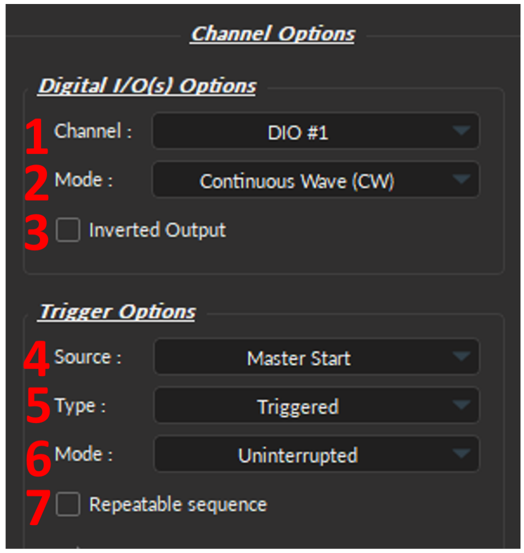


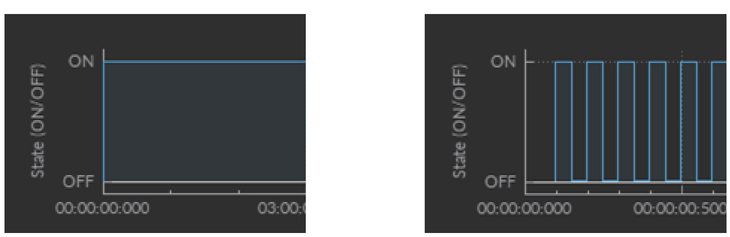
Figure 10.11: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel** (Fig 10.11, 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 OTPG corresponds to the same number of the digital channel within the software.

2. The **Mode** (Fig 10.11, 2) identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). Three modes are available:

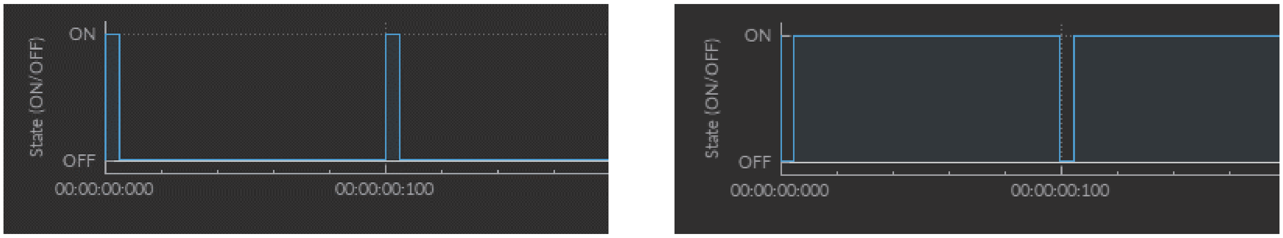
   - The **Continuous wave (CW)** Mode (Fig. 10.12a);
   - The **Square (TTL)** Mode (Fig. 10.12b);
   - The **Input** mode receives a signal that are either 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the OTPG (See Section 10.1.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.



(a) *Continuous* (b) *Square*

Figure 10.12: *Channel Options - Output Modes*

3. The **Inverted Output** checkbox (Fig 10.11, 3), when enabled, will convert every 0 to 1 and 1 to 0, such as in Fig. 10.13.



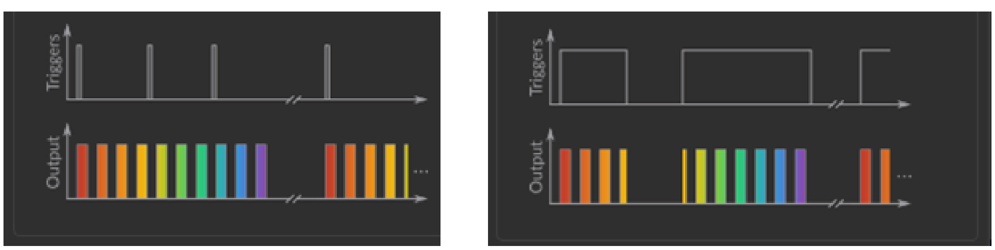
(a) *Disabled* (b) *Enabled*

Figure 10.13: *Inverted Output*

Trigger Options:

4. The **Source** trigger option (Fig 10.11, 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 10.11, 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. 10.14a), 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. 10.14b), the sequence will start once the voltage reaches 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***



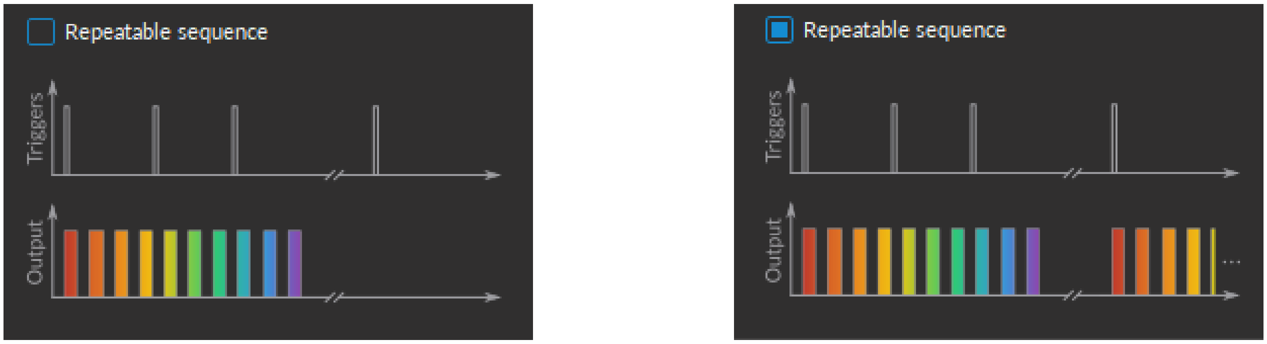
(a) *Trigger* (b) *Gate*

Figure 10.14: *Trigger Options Modes*

6. The **Mode** (Fig 10.11, 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 10.11, 7), when enabled, will run the sequence when additional TTL pulses are received (Fig. 10.15).



(a) *Disabled* (b) *Enabled*

Figure 10.15: *Repeatable sequence*

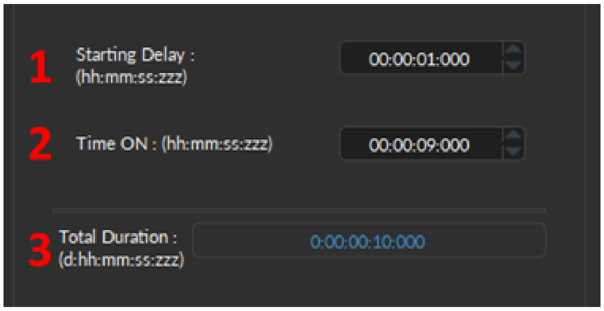
10.2.3.2 Sequence Options & Preview

The **Sequence options** section (Fig. 10.16a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 10.16b) 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**.

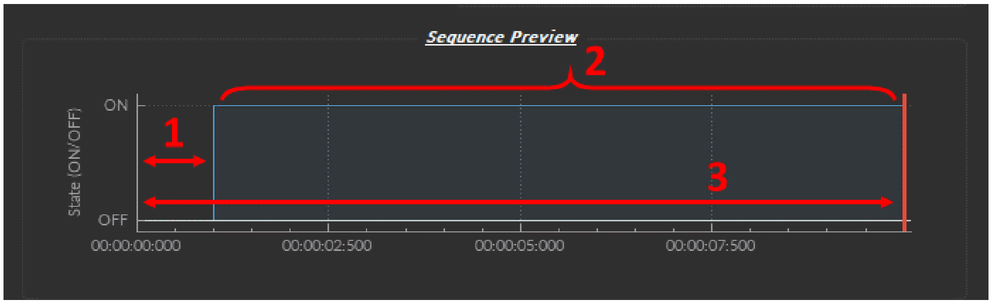
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 10.11), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 10.12a) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

    1. The **Starting Delay** (Fig 10.16, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Time ON** (Fig 10.16, 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 10.16, 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*



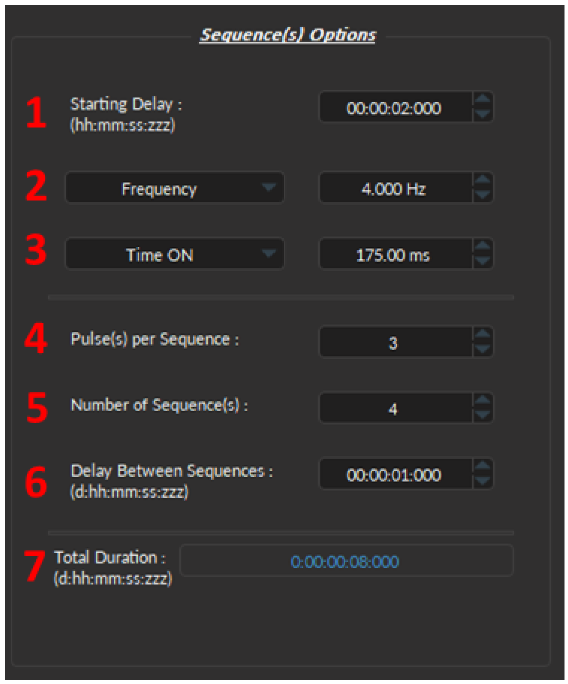
(b) *Sequence Preview*

Figure 10.16: *Channel(s) configuration window, Digital I/O - CW Mode*

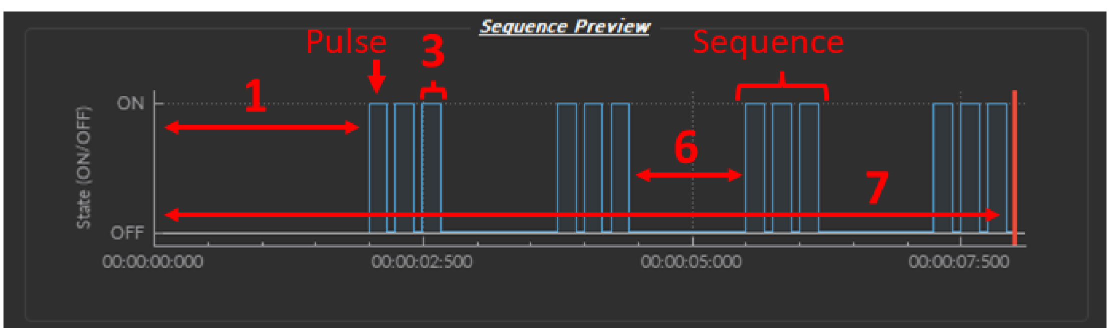
- The **Square** channel mode (Fig. 10.12b) allows the creation of a square TTL pulse sequence. The elements included in the Sequence Option box are as follows (Fig. 10.16, 1-3):

    1. The **Starting Delay** (Fig 10.17, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Frequency** (Fig. 10.17, 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. 10.17, 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. 10.17, 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. 10.17, 5) sets the number of times that the sequence will be repeated.
6. The **Delay between sequences** (Fig. 10.17, 6) sets the amount of time separating any two sequences (excluding the **Starting Delay**).
7. The **Total Duration** (Fig 10.17, 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 10.17: *Channel(s) configuration window, Digital I/O - Square Mode*

10.2.4 Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for their measurement.

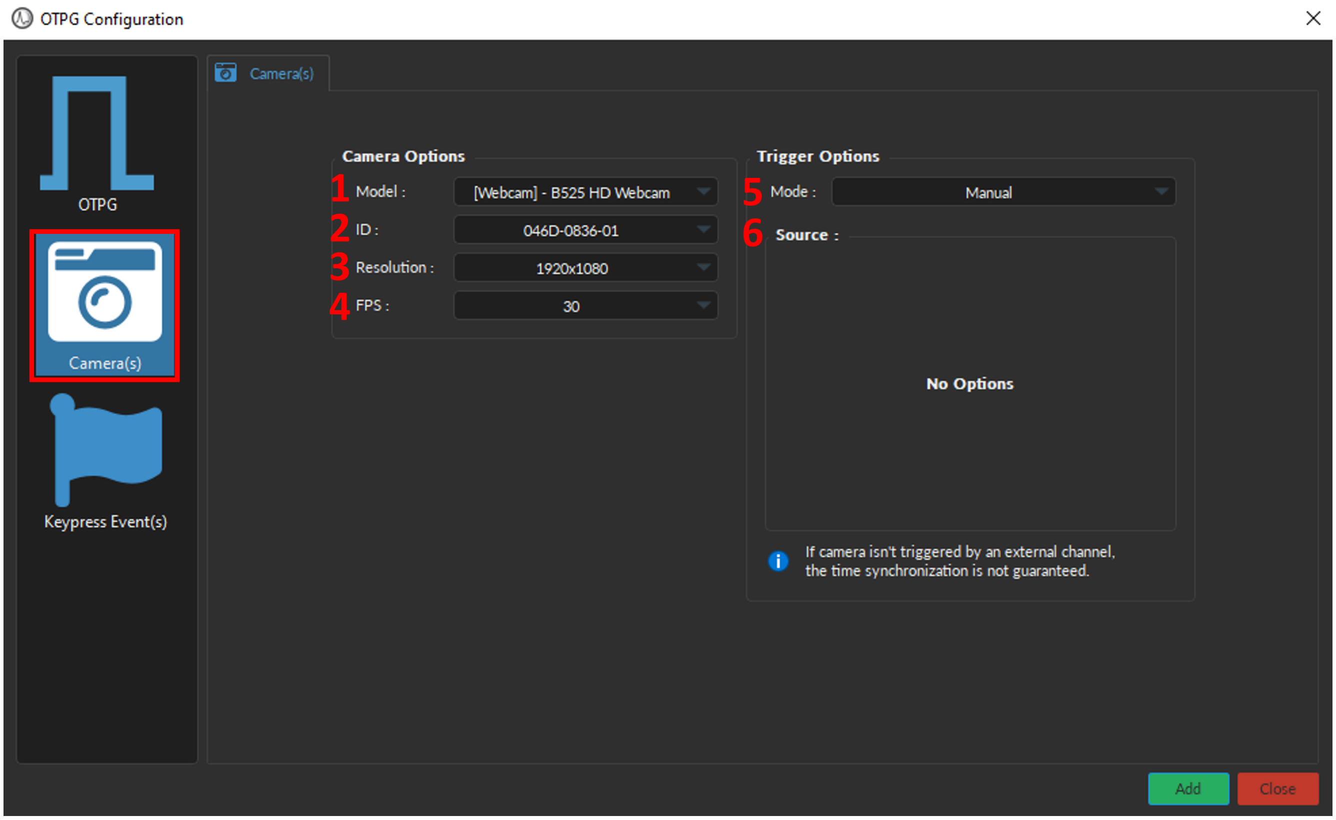


Figure 10.18: *Channel(s) configuration window, Camera*

WARNING:

A camera cannot be used for BOTH **OTPG** and **Camera** modules.
When creating a Camera Channel, if *No available camera detected...*, disconnect the camera in the **Device Selection** window to close the extra module.

Camera Options:

1. The **Model** (Fig. 10.18, 1) allows you to select the camera of choice based on the type of camera.

2. **ID** drop-down list (Fig. 10.18, 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. 10.18, 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. 10.18, 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. 10.18, 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 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. 10.19). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

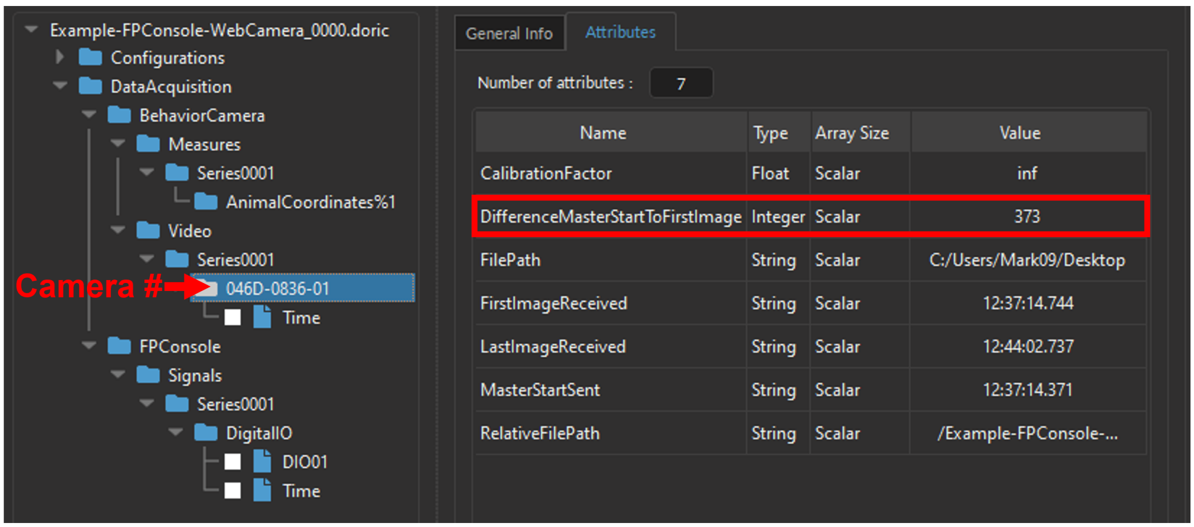


Figure 10.19: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **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. 10.18, 6 & Fig. 10.20) 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. 10.20). For a detailed description of each Digital I/O parameter see Section 10.2.3. Briefly, key parameters include:

   a) The **Channel** (Fig. 10.20, a) corresponds to the physical Digital I/O channel number on the OTPGthat is connected to the trigger cable of the *Doric Behavior Camera*.

   b) The **Mode** (Fig. 10.20, 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. 10.20, 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. 10.20, d) is by default 50%. The frame will be taken at the start of each square pulse.

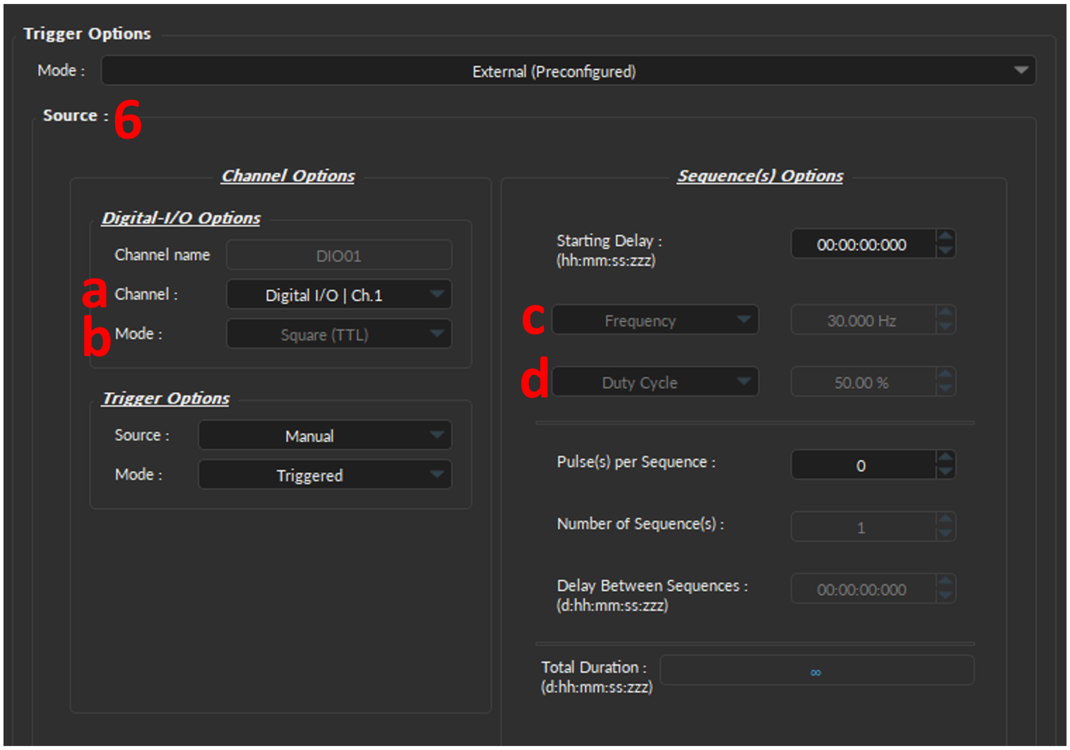


Figure 10.20: *Channel(s) configuration window, Camera - External (Preconfigured)*

10.2.5 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.
- Records 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 bottom of the window (Fig. 10.21, left). To remove a KeyPress, use the - button (Fig. 10.21, 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. 10.21).

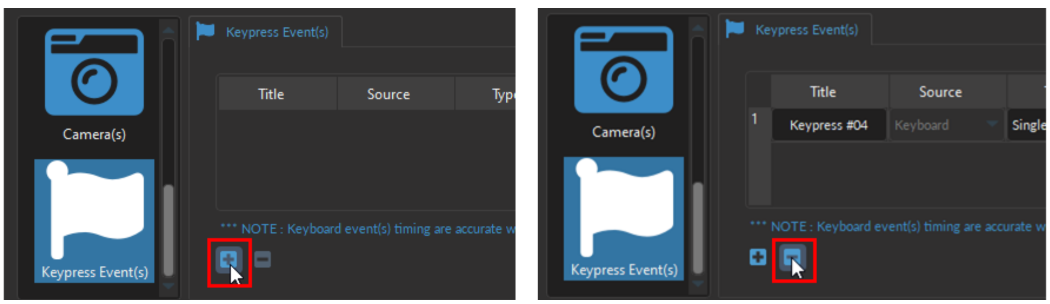


Figure 10.21: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 10.22) in the **Acquisition View**.

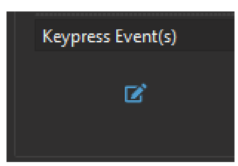
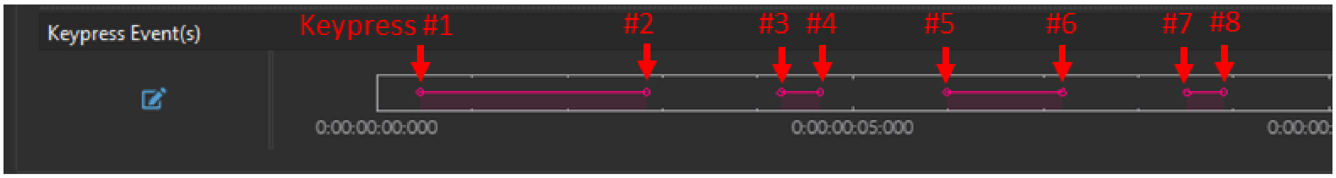


Figure 10.22: *Edit Keypress Event(s) Channel*

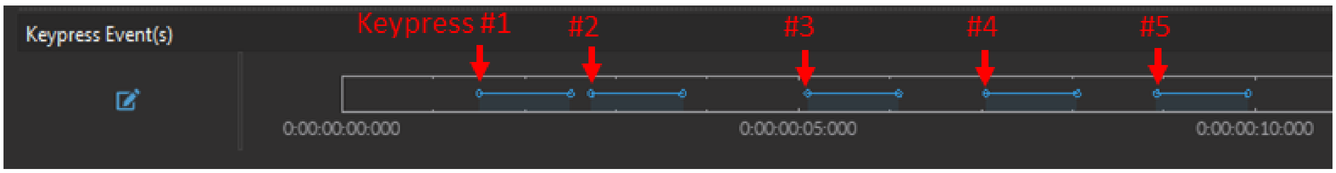
The following are the configurable parameters of a **Keypress Event,** per Fig. 10.24:

1. The **Title** (Fig. 10.24, 1) allows you to give a name for the Keypress event.
2. The **Source** (Fig. 10.24, 2) is by default *Keyboard*.
3. Three **Types** of Keypress Event(s) (Fig. 10.24, 3) can be specified with the drop-down list:
   - **Single** - Records single event at the touch of a key (Fig. 10.23a).
   - **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. 10.23b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 10.23c). 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 10.23: *Three types of Keypress Event(s)*

4. The **Duration** (Fig. 10.24, 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. 10.24, 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. 10.24, 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. 10.24, column 6).
7. The **Information** column (Fig. 10.24, 7) provides space to make notes or write a short description of the Keypress Event.

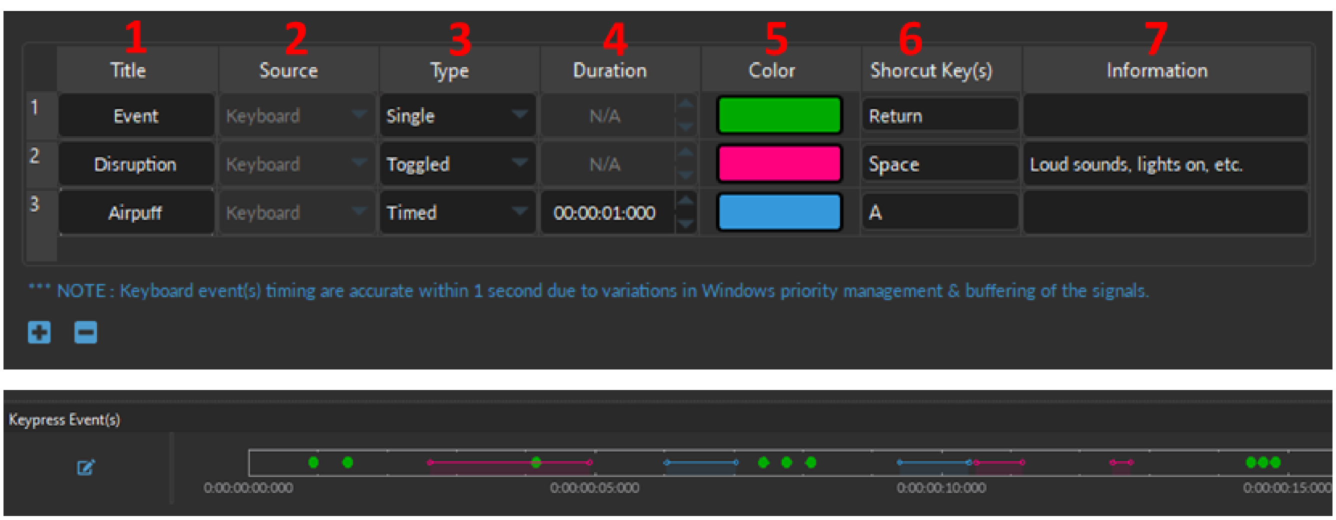


Figure 10.24: *Channel(s) configuration window, KeyPress Event(s)*

10.3 Acquisition View

The **Acquisition View** (Fig. 10.25) is split into two separate divisions (when **Camera Channel** is used), each of which visualizes different types of data in the following sections:

1. The **Graph View** (Fig. 10.25, 1) - Section 10.3.1;
2. The **Camera View** (Fig. 10.25, 2) - Section 10.3.2;

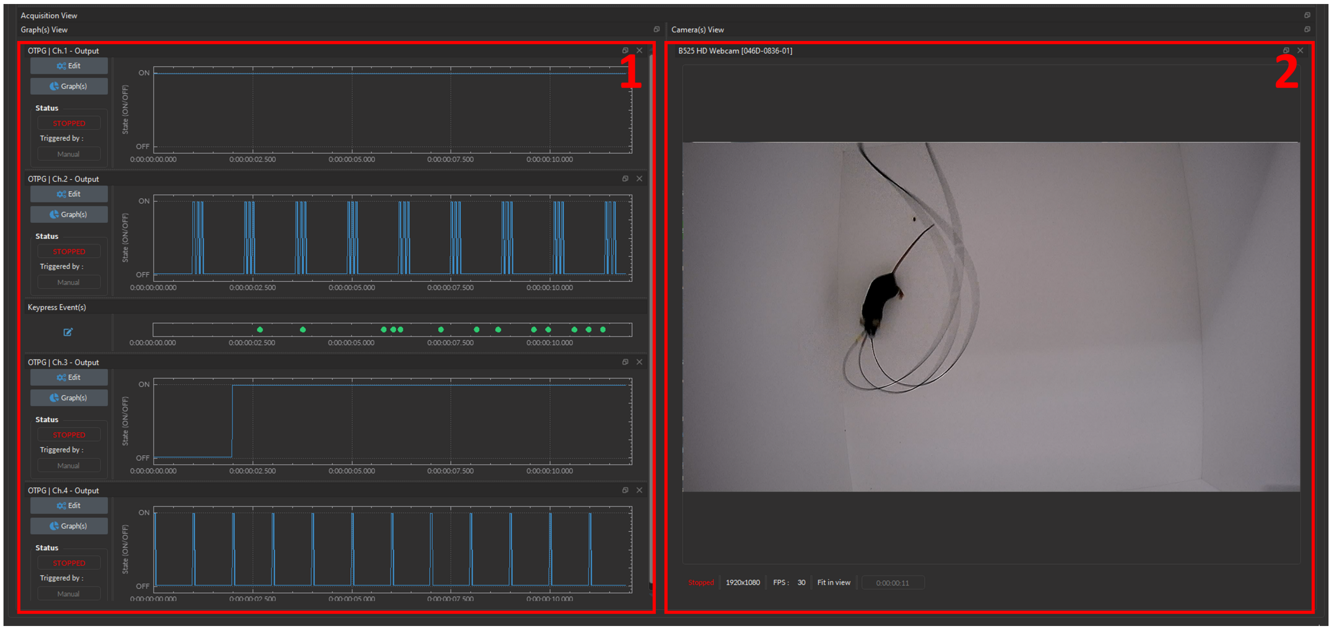


Figure 10.25: *Acquisition View*

10.3.1 Graph(s) View

The **Graph(s) View** displays the active Digital channels. Each Digital I/O channel includes: (1) a **Control Box** (Fig. 10.26, 1), and (2) a **Graph** (Fig. 10.26, 2).

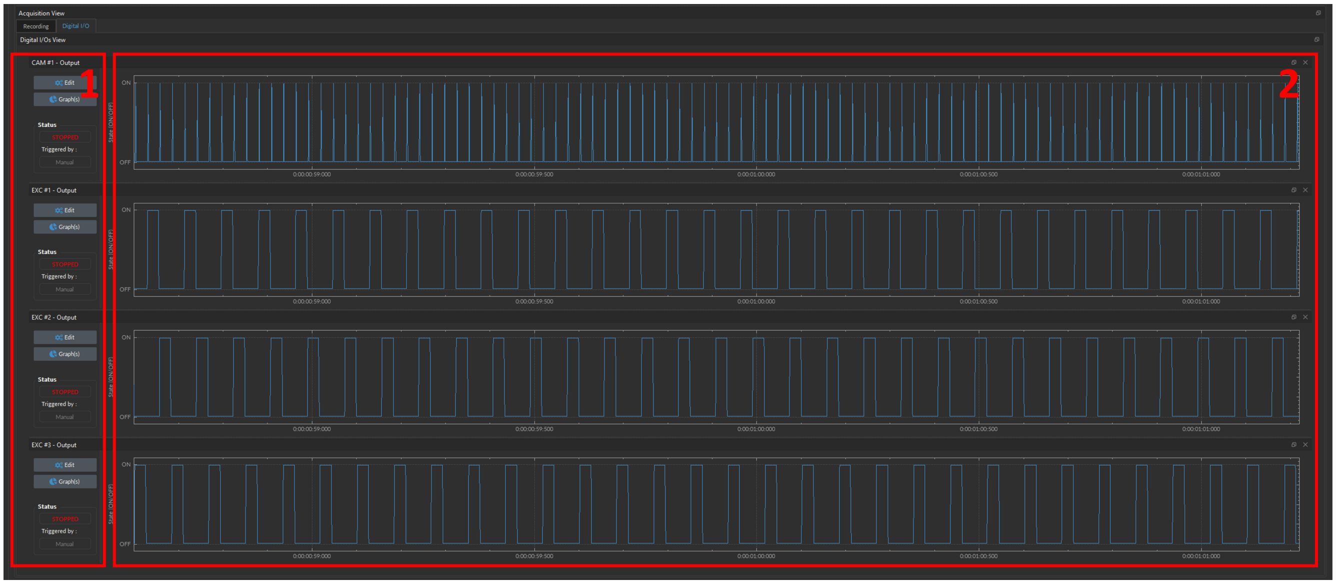


Figure 10.26: *Graph(s) View*

10.3.1.1 Control Box

The **Control box** of each channel allows users to track the status and edit the graph trace or the channel parameters.

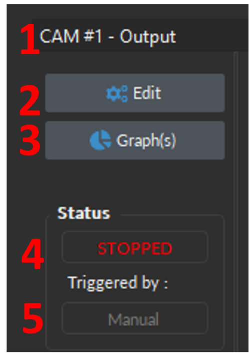


Figure 10.27: *Digital I/O View, Control box*

The following elements are contained within the **Control Box** of every Digitial channel (Fig. 10.27):

1. The **Channel name** (Fig. 10.27, 1) is located on the upper left of the **Control box**, identifying the type of channel and its number, corresponding to that on the *OTPG*.
2. The **Edit** button (Fig. 10.27, 2) opens the **Channel Configuration** window, where parameters can be modified (Fig. 10.28). For details on individual parameters, see Section 10.2.3.

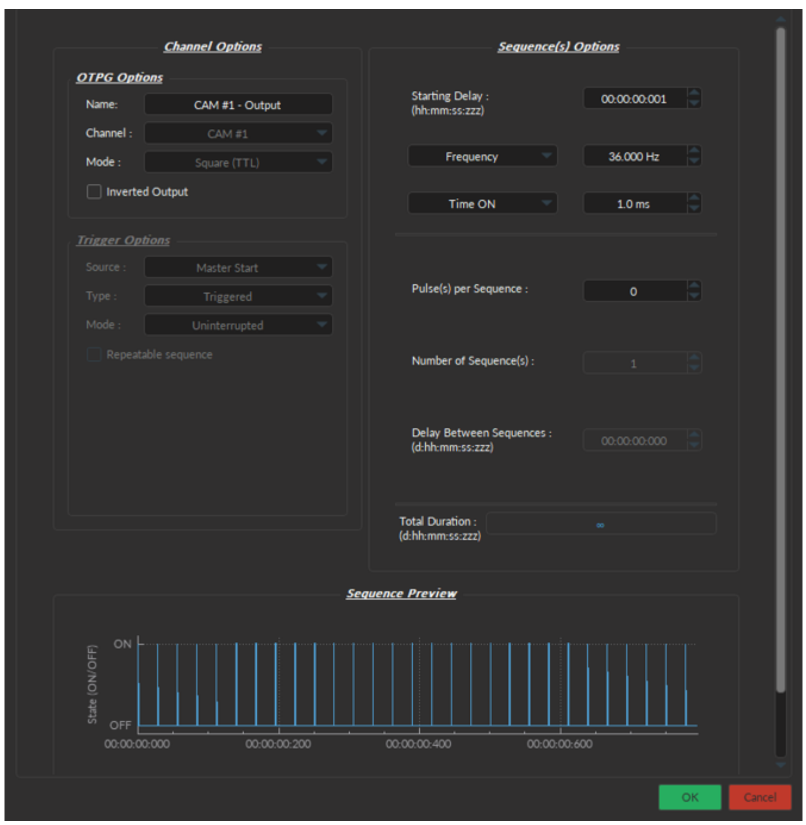


Figure 10.28: *Edit configuration*

3. The **Graph(s)** (Fig. 10.27, 3) button opens the **Graph Options** window (Fig. 14.36) 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:

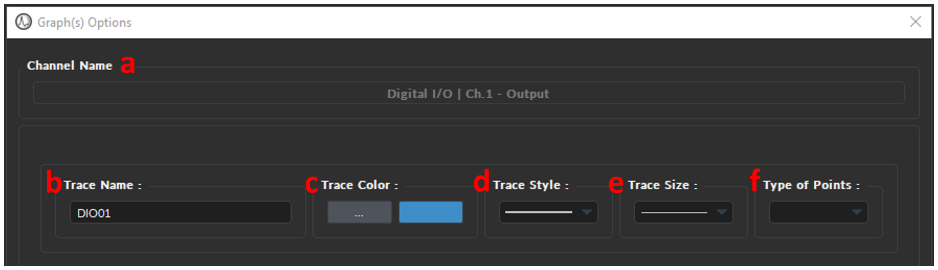


Figure 10.29: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 14.36, a) 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. 14.36, b) allows users to specify a name for the trace, instead of the default name generated by the software.
c) The **Trace Color** button (**...**) (Fig. 14.36, c) opens the **Color Select** window (Fig. 14.37), 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. 14.36, d) 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. 14.36, e) 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. 14.36, f) 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.

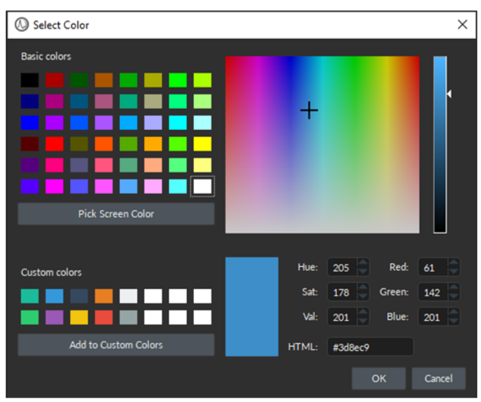


Figure 10.30: *Select Color Window*

4. The **Status** bar (Fig. 10.27, 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 10.1.2.1, no. 3).
5. The **Triggered by:** (Fig. 10.27, 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.

10.3.1.2 Graph

The **Digital I/O** traces are displayed in the **Graph** box (Fig. 10.26, 2). Each channel graph includes the following components:

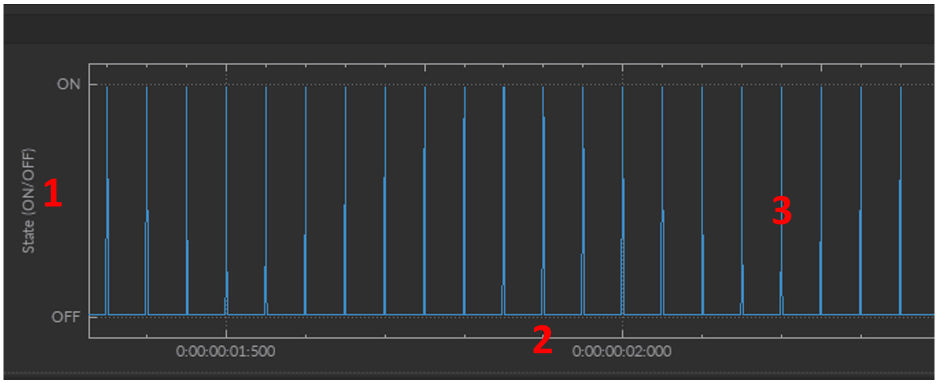


Figure 10.31: *Graph(s) View - Graph*

1. The **Y-axis** (Fig. 10.31, 1) displays the Digital **State** of the channel, which can be either ON (1) or OFF (0).
2. The **X-axis** (Fig. 10.31, 2) displays the time in d:hh:mm:ss:zzz.
3. The **Trace** (Fig. 10.31, 3) can be edited by selecting the **Graph** button in Section 10.3.1.1, no. 3.

While Section 10.3.1.1, no. 3 allow users to control the trace display, there are other features of **Graph** view that can be directly manipulated by selecting elements of the **Graph** itself, such as:

- *Axis Options* - Each **Graph** (Fig. 14.39) 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. 14.39) 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 10.32: *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. 14.40). 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 an instantaneous value, double-click on the dot.

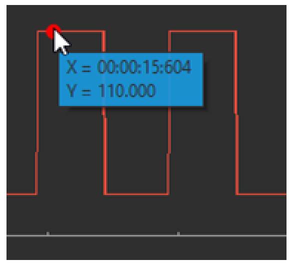


Figure 10.33: *Acquisition View - Instant values*

10.3.2 Camera View

The **Camera View** displays the live video feed from the *Behavior Camera* or *Web Camera*. This view contains the following components:

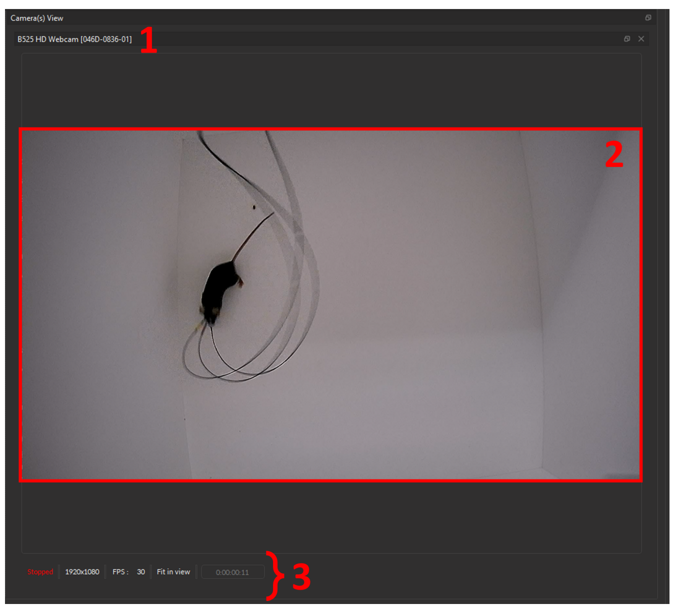


Figure 10.34: *Camera View*

1. The **Camera Name** (Fig. 10.34, 1) displays the serial number of the camera, which is particularly useful if multiple camera channels are used.
2. The **Camera Feed** (Fig. 10.34, 2) displays the live image of the camera.
3. The **Live Feed Monitoring Bar** (Fig. 10.34, 3) allows the user to quickly track the status of the camera feed and includes:

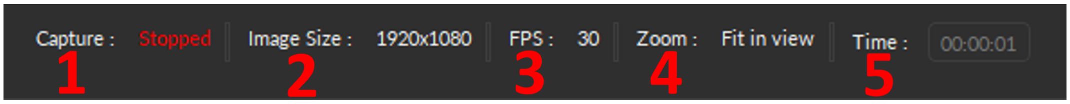


Figure 10.35: *Camera Channel - Live Feed Monitoring bar*

- The **Capture** status displays whether the camera is *Stopped*, *Active* or if using the **External Trigger Mode**, *Waiting for image*.
- The **Resolution** or **Image Size** displays the value selected in Section 10.2.4 - no. 3.
- The **FSP** displays the value selected in Section 10.2.4 - no. 4.
- The **Zoom** displays the magnification percentage of the image. If the **Fit Image in View** checkbox in the **Camera Options** was enabled, the percentage will be replaced by: *Fit in View*.
- The **Time** displays the time since the camera was turned on.

11

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.

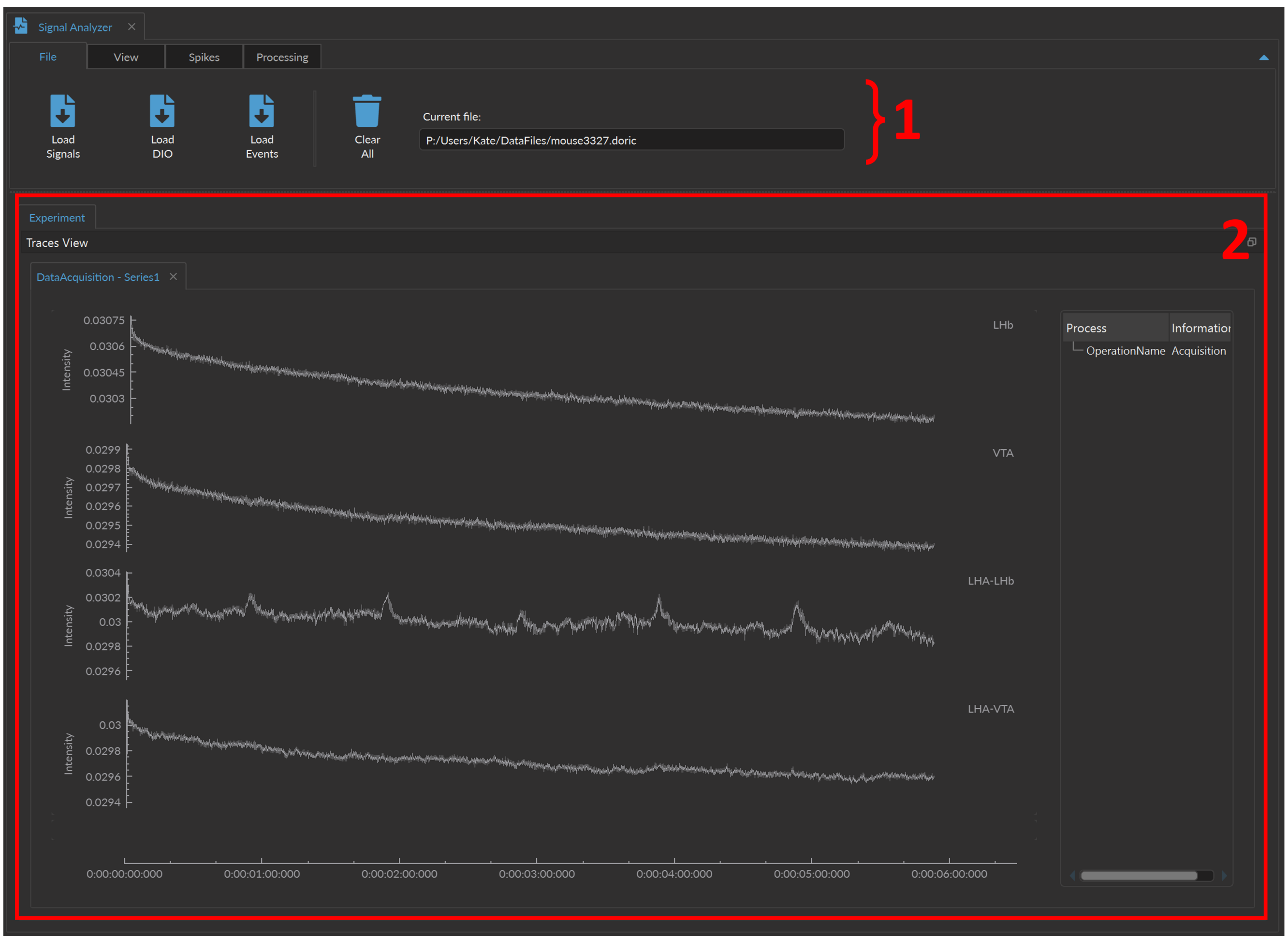


Figure 11.1: *Signal Analyzer Module*

The **Signal Analyzer** module can be accessed through the **Analyse** Tab at the top right of the main window (Fig. 11.2).

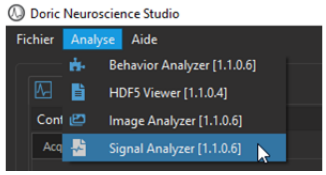


Figure 11.2: *Open the Signal Analyzer module*

The **Signal Analyzer module** (Fig 11.1) is separated into two main sections.

1. The **Control section** (Fig. 11.1, 1) contains all controls, separated into the **File**, **View**, **Spikes** and **Processing** tabs.
2. The **Trace View** (Fig. 11.1, 2) contains all currently displayed graphs as well as timestamped notes.

11.1 File Tab

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:

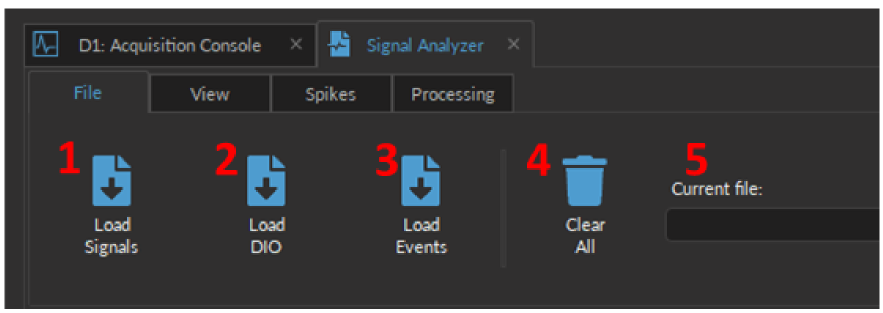


Figure 11.3: *File Tab*

1. The **Load Signals** button (Fig. 11.3, 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. 11.4), 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.

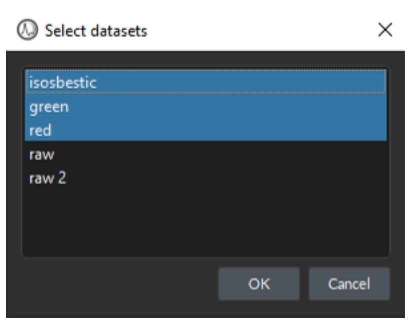


Figure 11.4: *Load selected channels*

2. The **Load DIO** button (Fig. 11.3, 2) allows users to import Digital Input/Output signals from a .doric file into *Trace View*.

3. The **Load Events** (Fig. 11.3, 3) allows users to display **Keypress Events** from a .doric file.
4. The **Clear all** button (Fig. 11.3, 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. 11.3, 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.

11.2 View Tab

The **View** tab (Fig. 11.5) is used to adjust the view in the **Graphs box**. The following details the specific features of each button, as per Fig. 11.5:

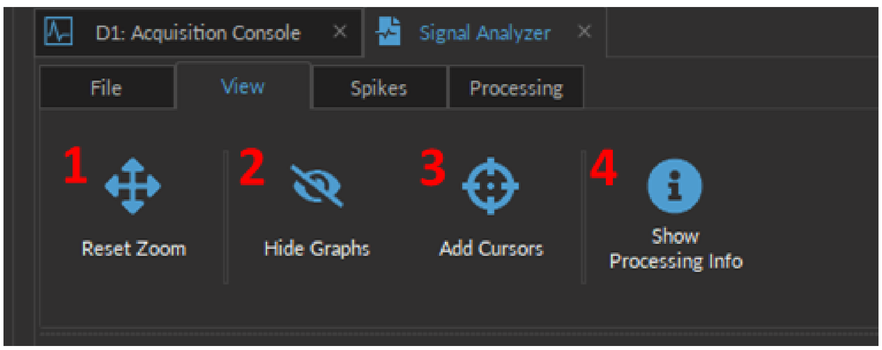


Figure 11.5: *View Tab*

1. The **Reset zoom** button (Fig. 11.5, 1) resets the axis so that the entire recording is visible.
2. The **Hide graph** button (Fig. 11.5, 2) opens the **Show/hide graphs** window (Fig. 11.6). Any checked data sets will be displayed in the **Graph box**.

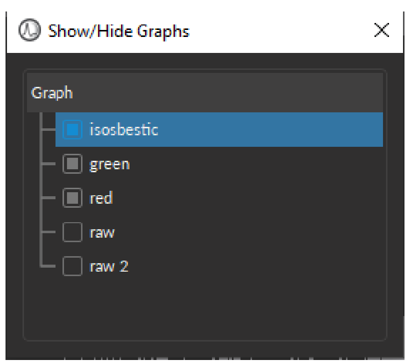


Figure 11.6: *Show/hide Graphs Window*

3. The **Add cursors** button (Fig. 11.5, 3), when selected, allows users to add 1-2 markers on the graph and displays the coordinates of the chosen point (Fig. 11.8). 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. 11.8, red box). To remove the cursors, click the **Remove cursors** button (Fig. 11.7) (previously the **Add cursors** button).

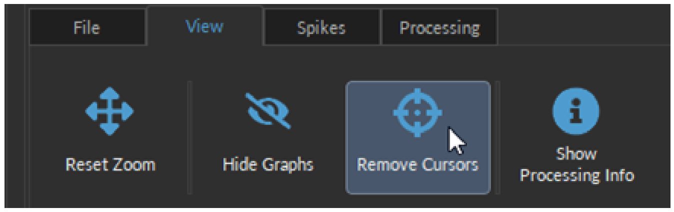


Figure 11.7: *Remove Cursors Button*

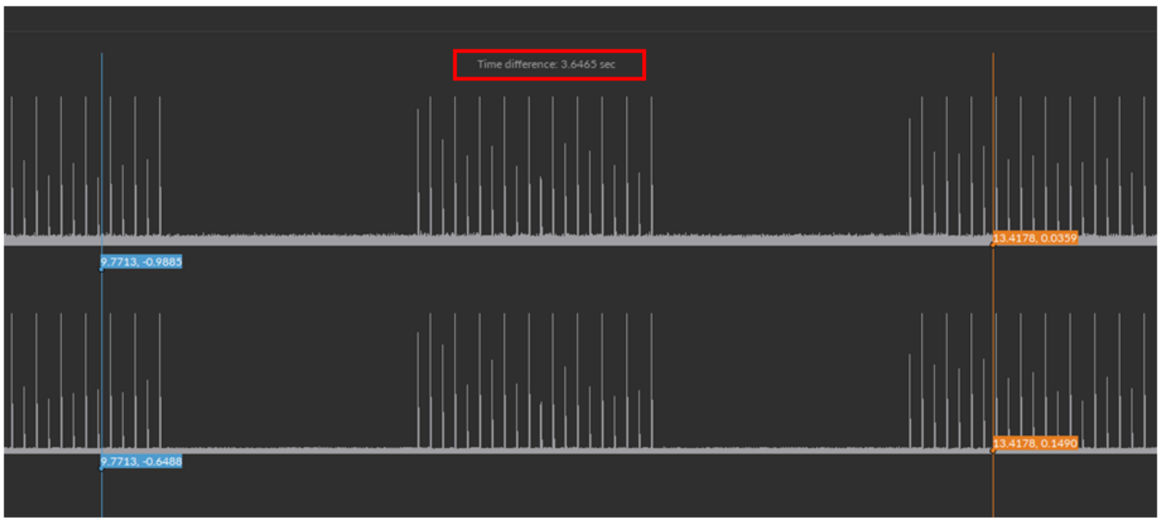


Figure 11.8: *Add Cursors*

4. The **Show Processing Info** button (Fig. 11.5, 4) opens a box on the right side of **Traces View** (Fig. 11.9). 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.

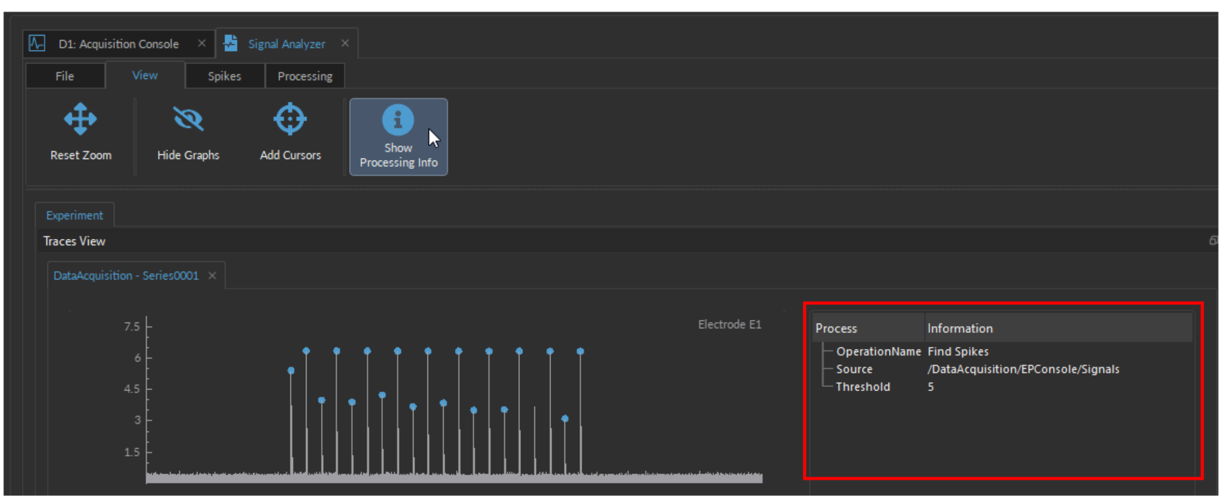


Figure 11.9: *Processing Info Box*

11.3 Spikes Tab

The **Spikes** tab (Fig. 11.10) 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.

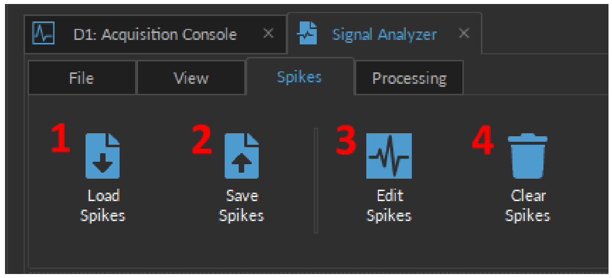


Figure 11.10: *Spikes Tab*

1. The **Load Spikes** button (Fig. 11.10, 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. 11.10, 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. 11.10, 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. 11.10, 4) will erase the Spike file currently loaded in the module.

11.4 Processing Tab

The **Processing** tab (Fig. 11.11) 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. 11.11, 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:

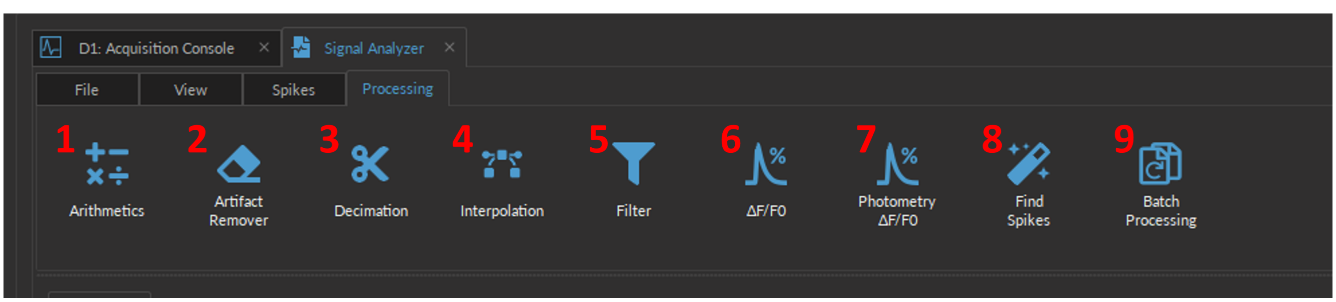


Figure 11.11: *Processing Tab*

1. The **Arithmetics** button (Fig. 11.11, 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 ΔF/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 ΔF/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 (+,-,$\times$ or $\div$) will be performed on the datasets.

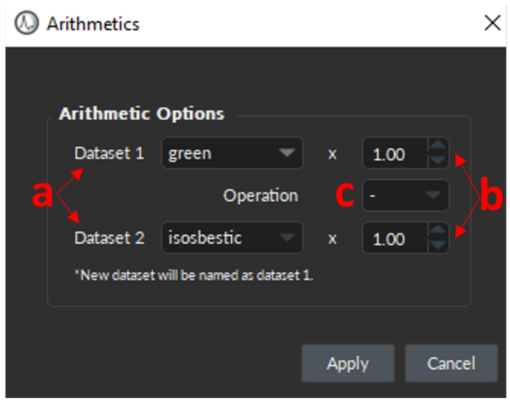


Figure 11.12: *Arithmetic Window*

2. The **Artifact Remover** button (Fig. 11.11, 2) opens the **Artifact Remover** window (Fig. 11.13). 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. 11.3-1 and Fig. 11.4). For more information on this algorithm and the principles behind the artifacts, see section 9.4.3.1, no. 5, LED Artifacts Remover).

   a) Using the electrode selector box (Fig. 11.13, 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. 11.13, 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. 11.13, 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.

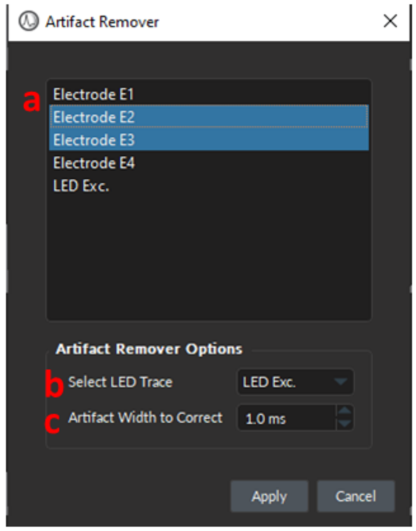


Figure 11.13: *Artifact Remover Window*

3. The **Decimation** button (Fig. 11.11, 3) opens the decimation window (Fig. 11.14). 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. 11.14, a) specifies the channel that will be decimated. Multiple channels can be decimated at once.
   b) The **Decimation Factor** (Fig. 11.14, b) defines the number of points saved. One point is conserved over a number of data points equal to the **Decimation Factor**.

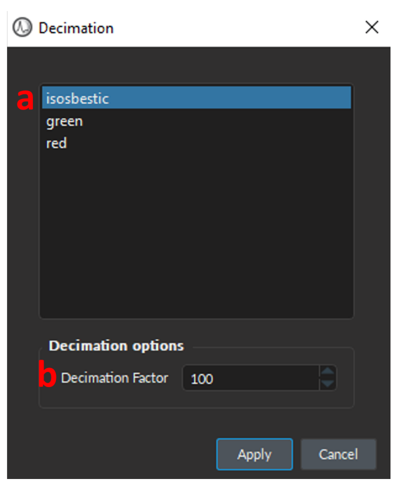


Figure 11.14: *Decimation Window*

4. The **Interpolation** button (Fig. 11.11, 4) opens the interpolation window (Fig. 11.15). 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. 11.15, a) specifies the channel where the function will be applied. Multiple channels can be selected at once.
   b) The **Interpolation Type** (Fig. 11.15, 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.

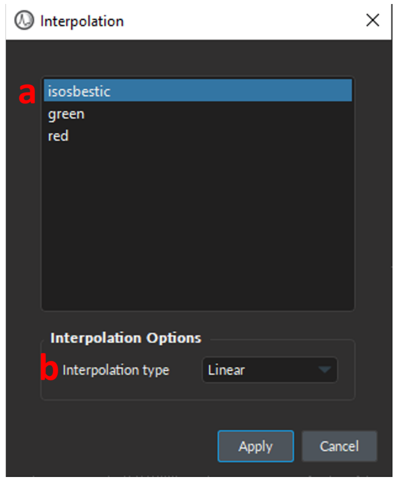


Figure 11.15: *Interpolation Window*

5. The **Filter** button (Fig. 11.11, 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. 11.16) 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. 11.16, a).
   b) The **Filter type** (Fig. 11.16, b) defines whether the filter is low-pass, high-pass or bandwidth.
   c) The **Cutoff frequency** (Fig. 11.16, 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. 11.16, d) displays a visualization of the filter in use, with the specified low and/or high pass filter cutoffs.

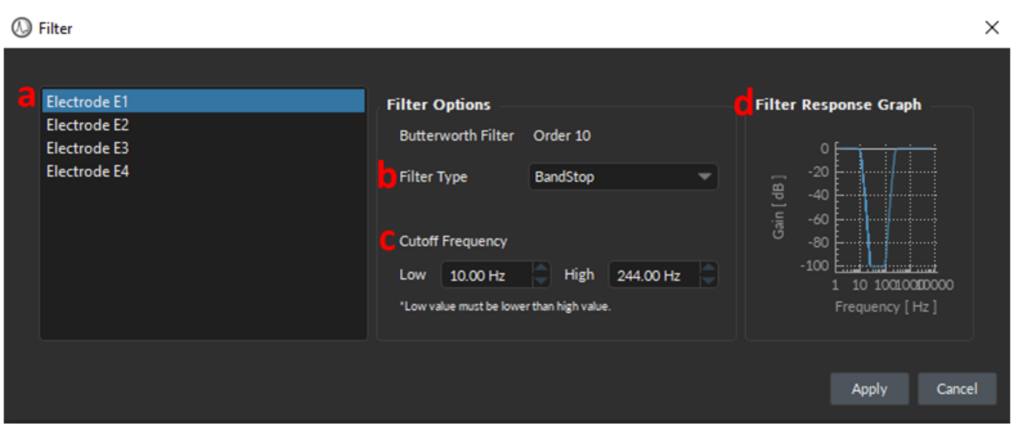


Figure 11.16: *Filter Window*

6. The **ΔF/F$_0$** button (Fig. 11.11, 6) opens the simple ΔF/F$_0$ window (Fig. 11.17). 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. 11.17, 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. 11.17, 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. 11.17, 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.***

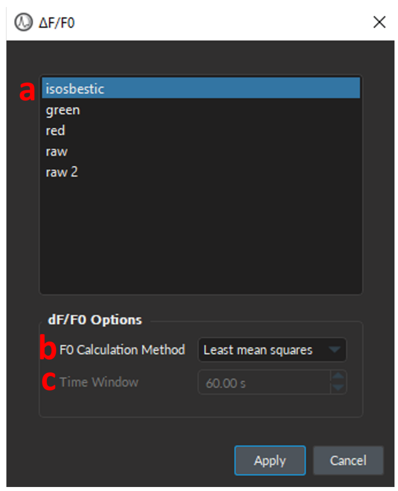


Figure 11.17: $\Delta F/F_0$ *window*

7. The **Photometry ΔF/F$_0$** button (Fig. 11.11, 7) opens the Photometry ΔF/F$_0$ window (Fig. 11.19), 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. 11.19, 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. 11.19, 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. 11.19, 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. 11.19, h), but does not pick up the peaks in the calcium-dependent trace (Fig. 11.19, 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. 11.19, 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. 11.18), 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.

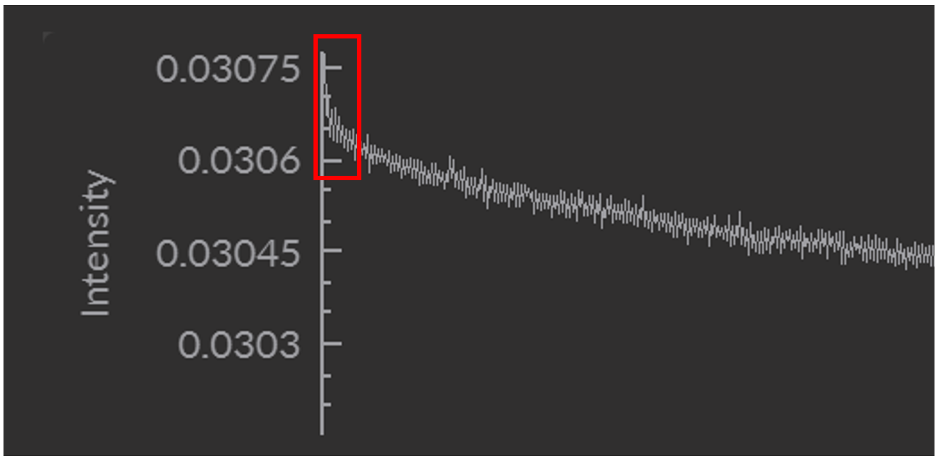


Figure 11.18: *Discard steep drop in the signal*

e) The **Fit Signals** (Fig. 11.19, e) specified the residual threshold used when fitting the line between calcium-dependent and calcium-independent signal (Fig. 11.19, 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. 11.19, 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. 11.19, h and the new line of best fit in Fig. 11.19, i.

h) The **Processing Example View Graphs** (Fig. 11.19, g) displays the original raw trace and smoothed curve for both the *Calcium independent* (Fig. 11.19, h) and *Calcium dependent* signal (Fig. 11.19, i). The bottom graph displays the output $\Delta F/F_0$ trace (Fig. 11.19, j).

i) The **Signal Fit Line Graph** (Fig. 11.19, 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.

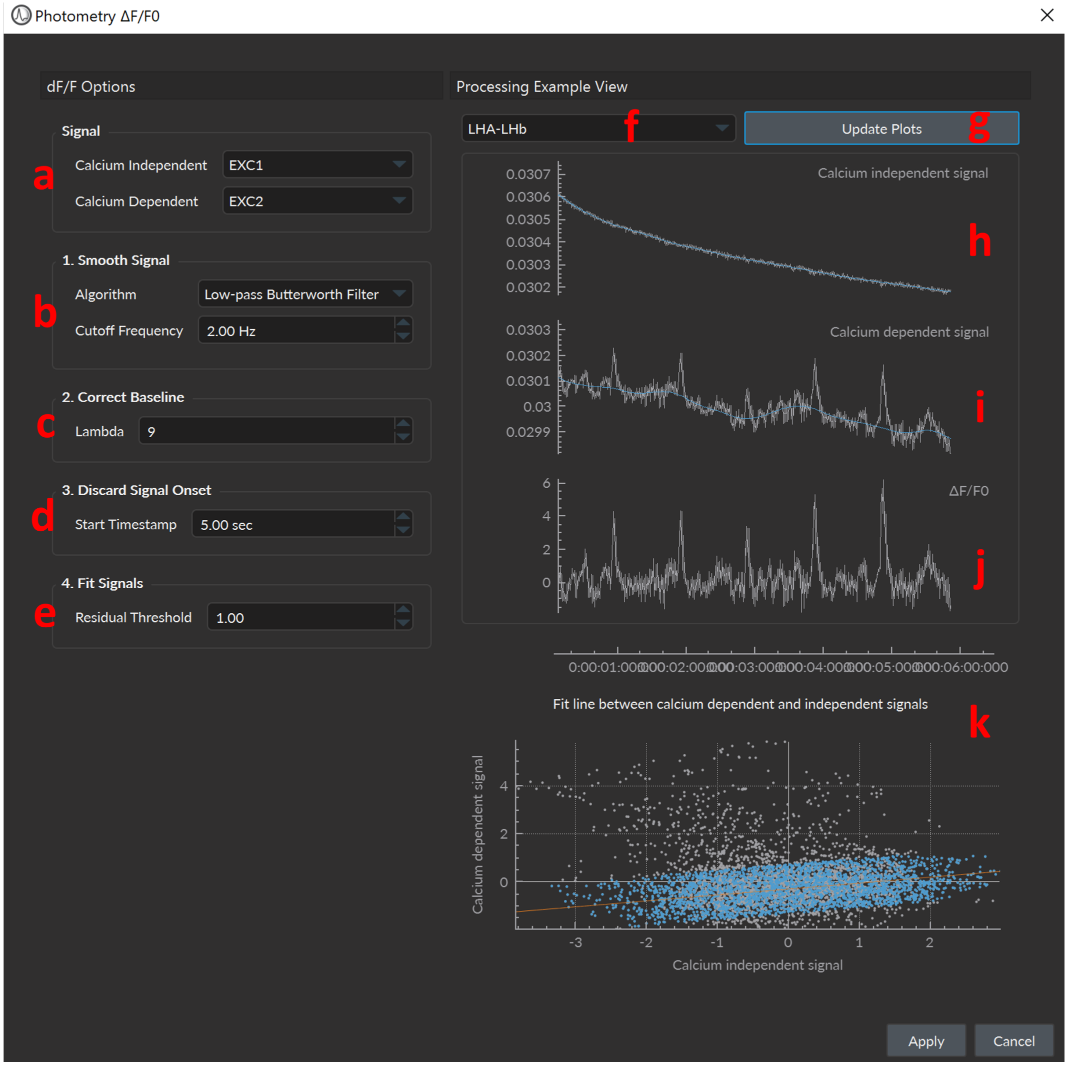


Figure 11.19: *Photometry $\Delta F/F_0$ Window*

8. The **Find spikes** button (Fig. 11.11, 8) identifies peaks in the data over one or multiple electrode/channels (Fig. 11.20). 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. 11.20). 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. 11.21).

   Note: For electrophysiological data, all detected spikes correspond to multi-unit activity since the algorithm used does not differentiate between different spike shapes.

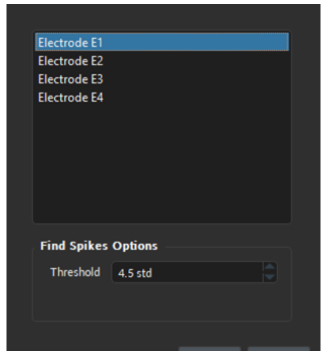


Figure 11.20: *Find Spikes Window*

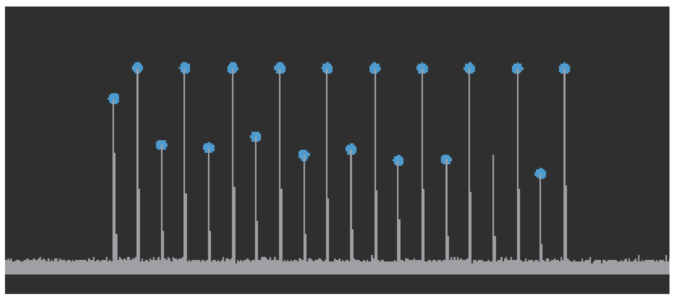


Figure 11.21: *Spikes are identified on the signal trace with blue dots*

9. The **Batch Processing** button (Fig. 11.11, 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. 11.23, 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. 11.23, 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. 11.22. 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.

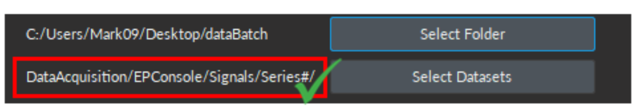


Figure 11.22: *Automatic FiWi Dataset selection*

   c) If the **Save Intermediate Files** options (Fig. 11.23, c) is selected (**Yes**) batch processing will generate a file after every operation, and for each recording (Fig. 11.24). Select **No** if intermediate files are not required.

   d) The **Available Operations** box (Fig. 11.23, 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. 11.11).

   e) The **Workflow** box (Fig. 11.23, 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. 11.23, 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 11.4: 1-8).

NOTE: Some operations use data-set names. Make sure that the names are the same across all the files.

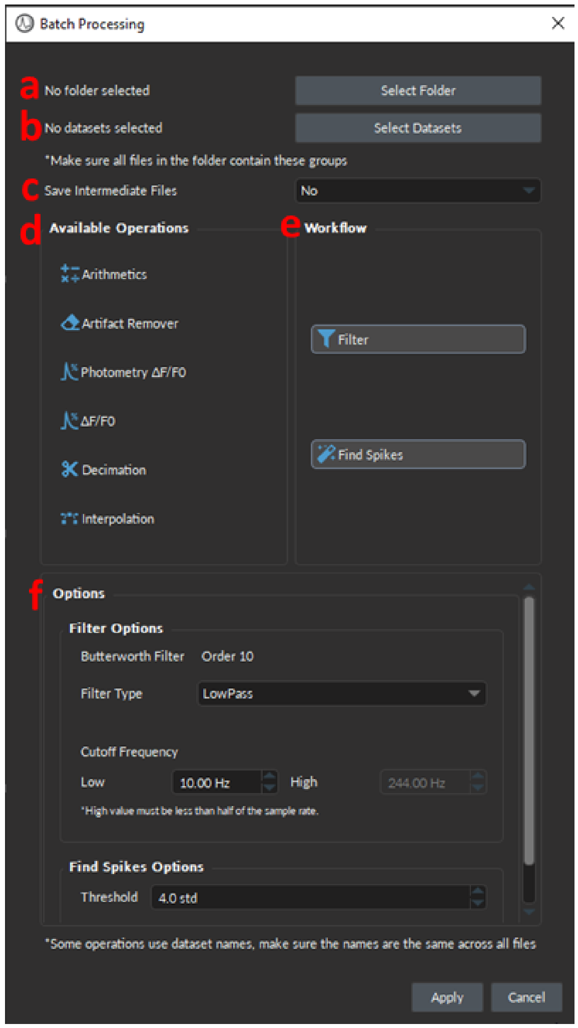


Figure 11.23: *Batch Processing Window*

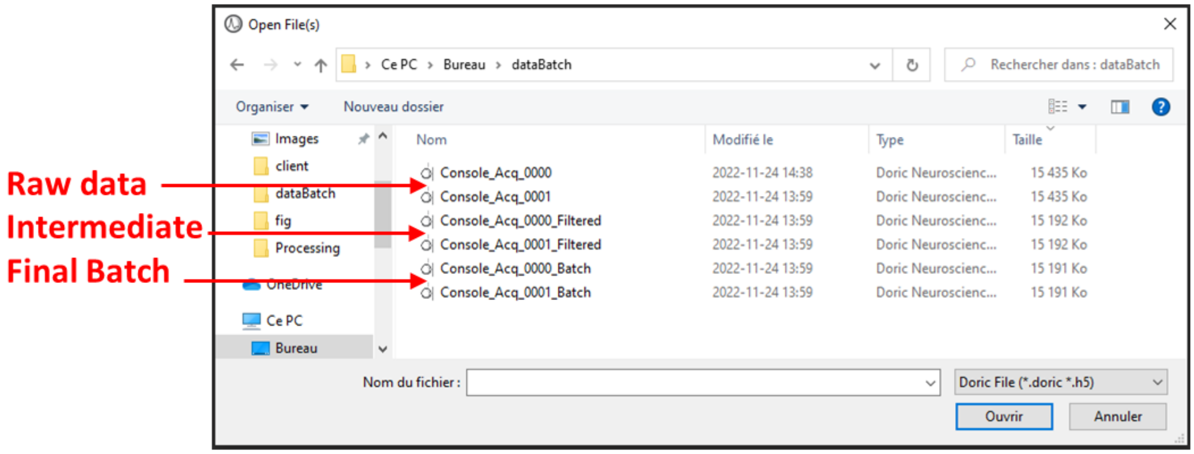


Figure 11.24: *Batch Processing Output*

12

Behavior Analyzer

The **Behavior Analyzer** module allows simultaneous observation of behavior video with traces from experimental measurements. Note that video data must be either in **.avi, .mp4, .mkv, .mpeg, .doric** 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.

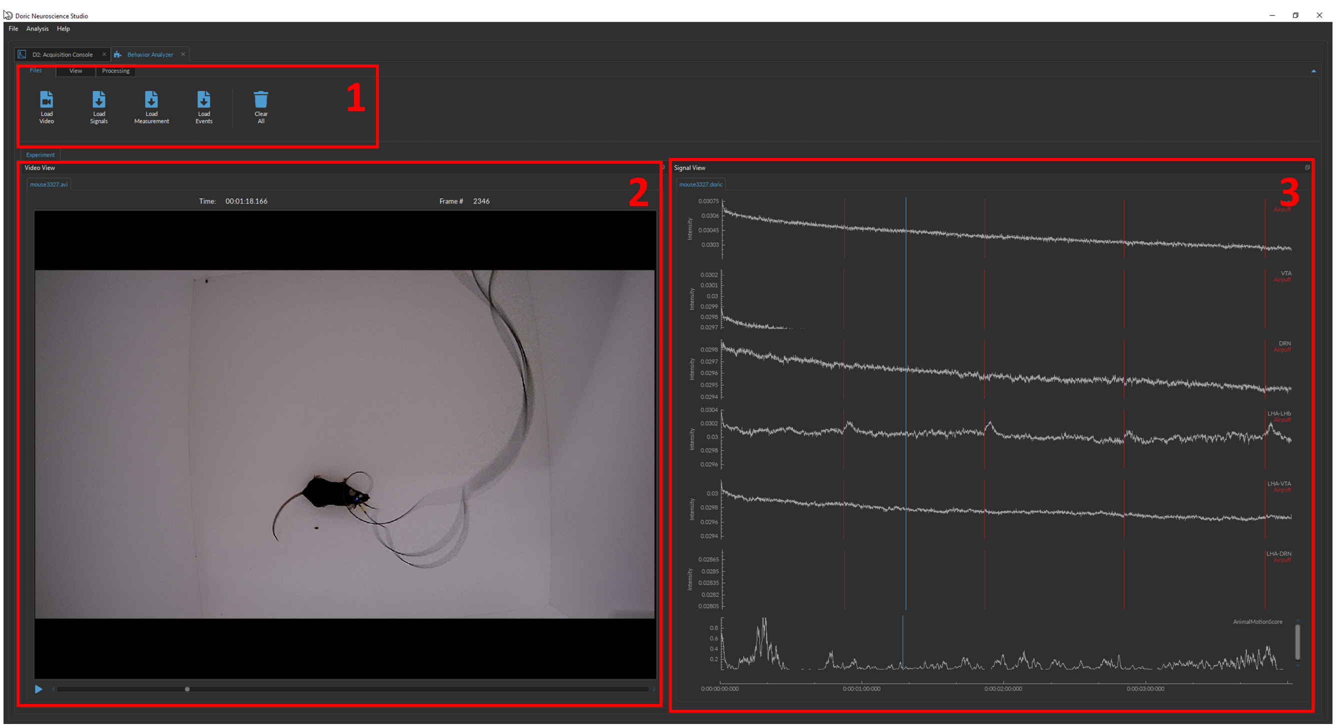


Figure 12.1: *Behavior Analyzer Module Interface*

To open the **Behavior Analyzer** module, select **Analysis** and the module name from the drop-down menu, as per Fig. 12.2.

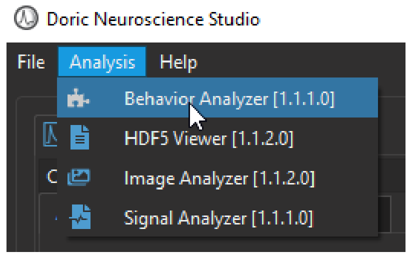


Figure 12.2: *Open Behavior Analyzer module*

The interface can be separated into three major sections (Fig 12.1):

1. The **Tabs** (Fig 12.1, 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 12.1
    - *View Tab* - Section 12.2
    - *Processing Tab* - Section 12.3
2. The **Video View** (Fig 12.1, 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 12.4 for more details.
3. The **Signal View** (Fig 12.1) 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 12.5 for more details.

12.1 Files Tab

The **File** tab (Fig. 12.3) is primarily used to load and save the data. The following details the specific features of each button, as per Fig. 12.3:

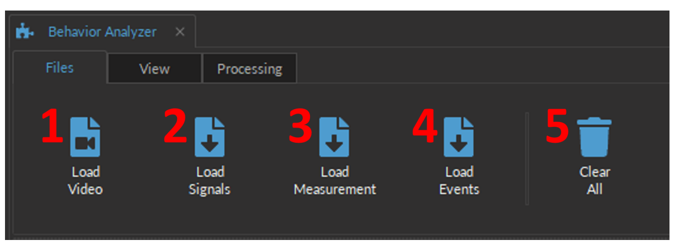
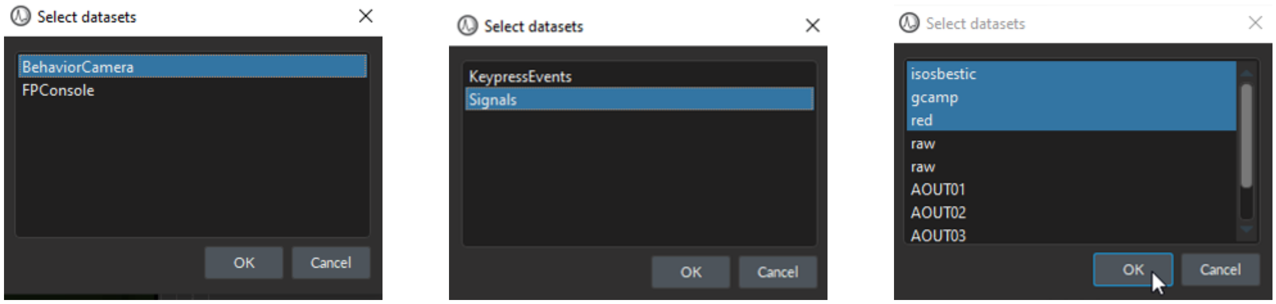


Figure 12.3: *Behavior Analyzer, Files tab*

1. The **Load Video** button (Fig. 12.3, 1) will open the 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. 12.3, 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. 12.4), allowing the user to specify which device (Fig. 12.4a), signal type (Fig. 12.4b), and the channels (Fig. 12.4c) 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. 12.3, 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 the .doric file.



(a) *Device* (b) *Type* (c) *Channels*

Figure 12.4: *Select Datasets*

4. The **Load Events** (Fig. 12.3, 4) allows users to display **Keypress Events** from 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. 12.4b) to import them into the module.

5. The **Clear all** button (Fig. 12.3, 5) deletes all data currently loaded in the module. This data cannot be recovered, so ensure the data is properly saved before clearing it.

12.2 View Tab

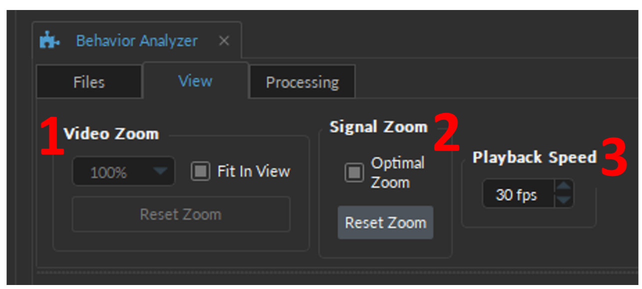


Figure 12.5: *Behavior Analyzer, View tab*

The **View** tab (Fig. 12.5) 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. 12.5, 1) sets the image magnification factor.
   a) The **Zoom %** drop-down list specifies the zoom factor for the image display, which ranges between 10% and 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. 12.5, 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. 12.5, 3) sets the frame rate (in FPS) that the video and signal data will be run once the user selects **Play** from the **Video View**.

12.3 Processing Tab

The **Processing** tab (Fig. 12.6) includes two behavior measurements, which will be treated in the following sections:

- *Animal Tracking* (Fig. 12.6, 1) - Section 12.3.1
- *Motion Score* (Fig. 12.6, 2) - Section 12.3.2

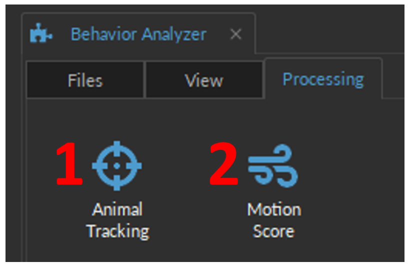


Figure 12.6: *Behavior Analyzer, Processing tab*

12.3.1 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.

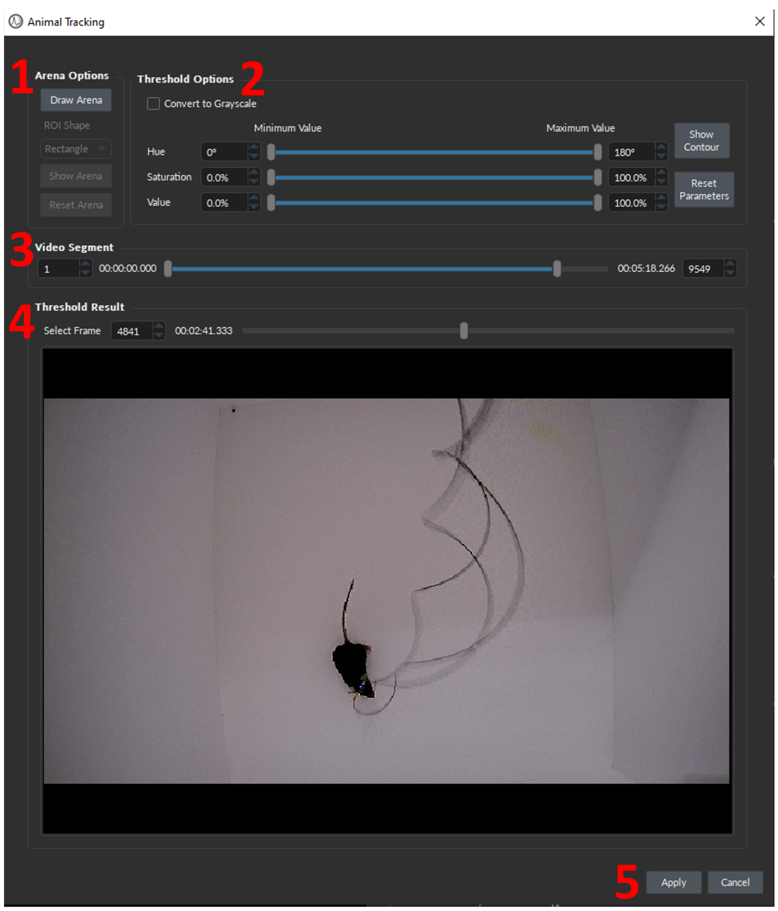


Figure 12.7: *Animal Tracking window*

1. The **Arena Options** (Fig. 12.7, 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. 12.8, a), when selected, allows the 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 becomes a **Done** button, which must be selected to save the current arena area for the algorithm.

   b) The **ROI shape** drop-down menu (Fig. 12.8, b) sets the geometrical shape that will be used when drawing the arena area. The user can select between: *Freehand, Rectangle, Circle or Square*.

   c) The **Show Arena** button (Fig. 12.8, c) will blackout the part of the video that isn't contained within the user-defined arena.

   d) The **Reset Arena** button (Fig. 12.8, d) will remove the video blackout that designates the arena in order to redraw the shape.

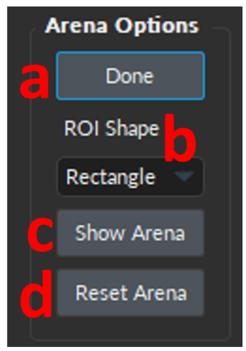


Figure 12.8: *Animal Tracking window, Arena Options*

2. The **Threshold Options** (Fig. 12.7, 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. 12.9). *** There should be high contrast between the animal and the background arena for best results.***

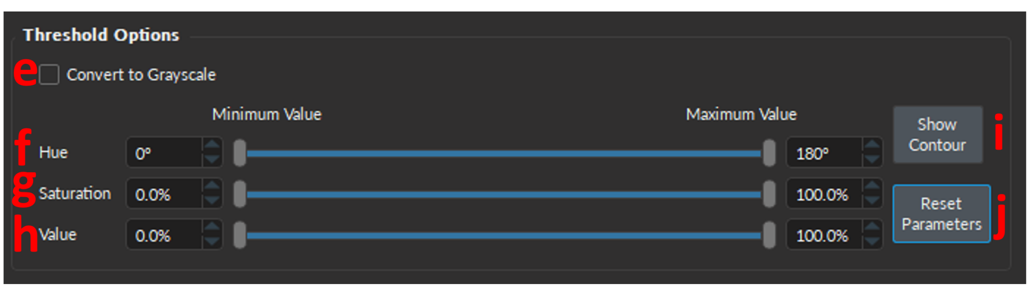


Figure 12.9: *Animal Tracking window, Threshold Options*

   e) The **Convert to Grayscale** checkbox (Fig. 12.9, 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. 12.9, 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. 12.9, g) describes the intensity of the pixel. Saturation is a percentage ranging from 0% (grayscale) to 100% (pure color).

   h) The **Value** (Fig. 12.9, h) sets the pixel value as threshold. The value is a percentage that ranges from 0% (black) to 100% (white).

i) The **Show Contour** button (Fig. 12.9, 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 (Fig. 12.10). A blue outline will surround the detected animal and the small blue circle will be computed as the center of the shape.

j) The **Reset Parameters** button (Fig. 12.9, 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.

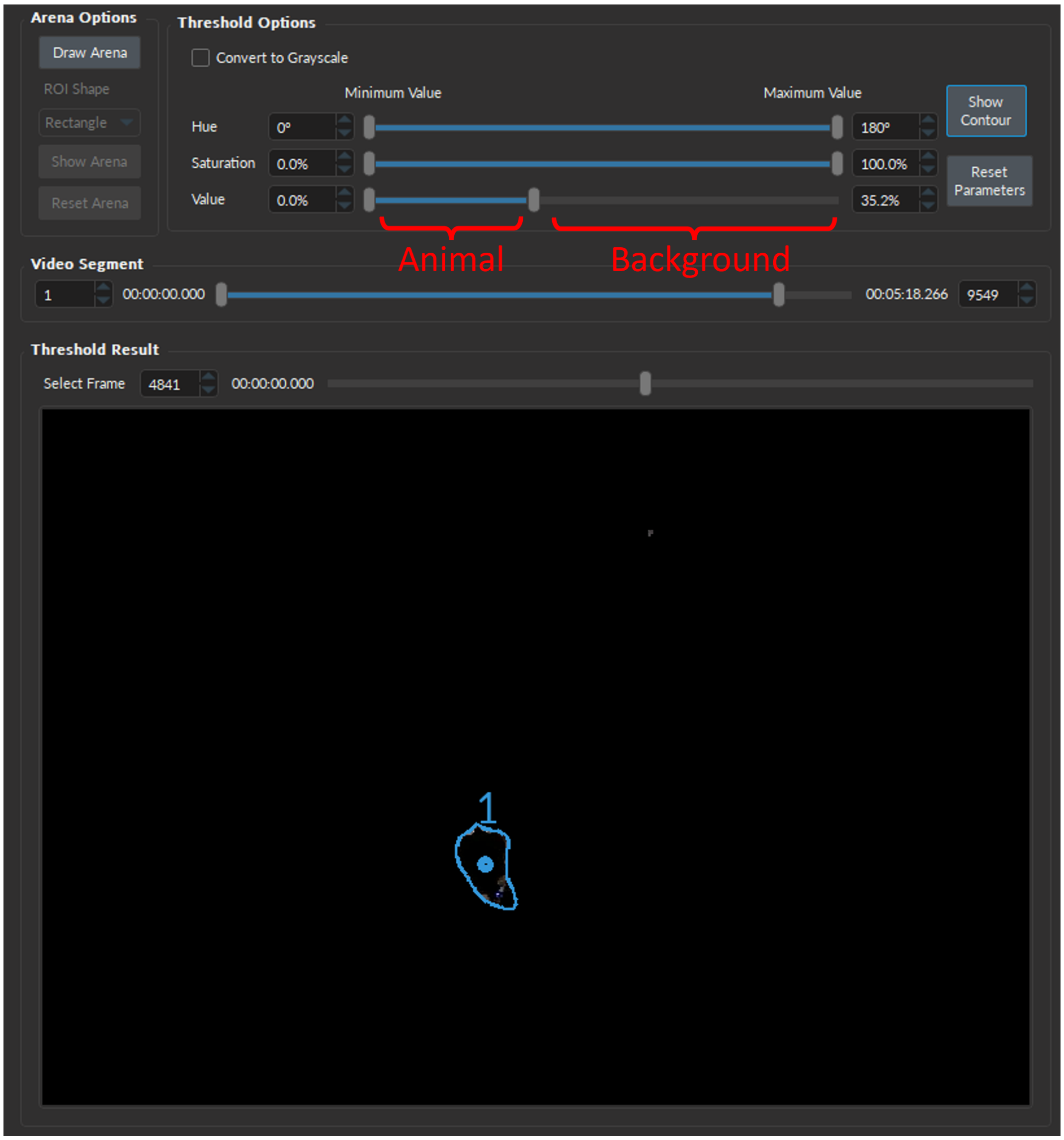


Figure 12.10: *Animal Tracking window, Threshold Options - Show Contour*

3. The **Video Segment** sliding scale (Fig. 12.7, 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 either by specifying a starting and ending frame (Fig. 12.11, k & n respectively) or by using the two sliding scale pointers (Fig. 12.11, l & m respectively).

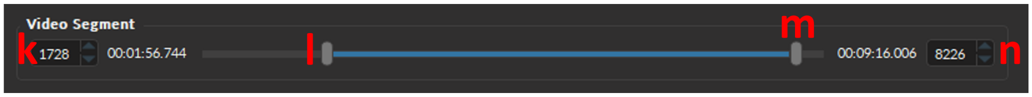


Figure 12.11: *Animal Tracking window, Video Segment*

4. The **Threshold Results** (Fig. 12.7, 4) view displays a still frame of the video where the user will define the arena and view the animal tracking contour. The user 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.

5. The **Apply** button (Fig. 12.7, 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 (instead of the **AnimalMotionScore** in Fig. 12.14).

12.3.2 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 (and thus lead to a small Motion Score).

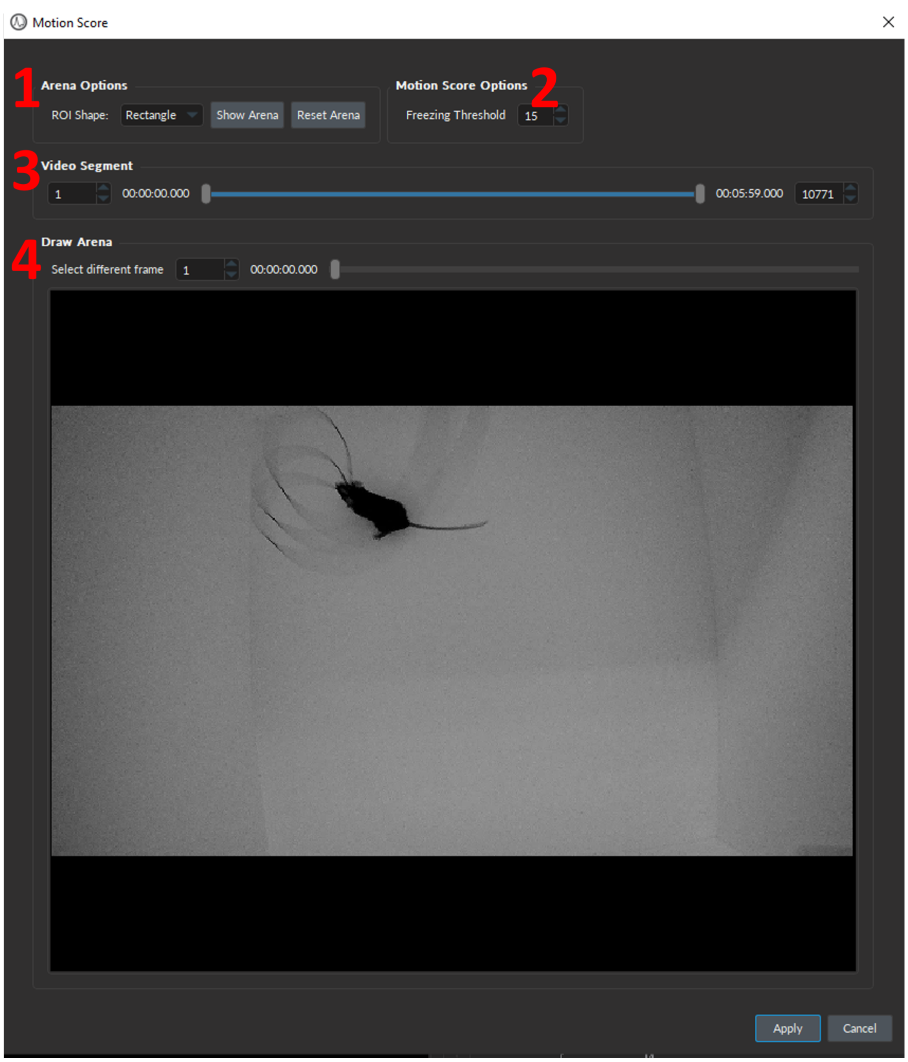


Figure 12.12: *Motion Score window*

1. The **Arena Options** (Fig. 12.12, 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 Arena** button will blackout the part of the video that isn't contained within the user-defined arena.

   c) The **Reset Arena** button will remove the video blackout that designates the arena in order to redraw the shape.

2. The **Motion Score Options** (Fig. 12.12, 2) defines the **Freezing Threshold** pixel value. This value must be between 0 and 255. This is a relative value indicating the value count change that is interpreted as movement.

3. The **Video Segment** sliding scale (Fig. 12.12, 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** (Fig. 12.12, 4) displays a still frame of the video where the user will define the arena which will be the input to the **Motion Score** algorithm.

12.4 Video View

The **Video View** (Fig. 12.13) 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.

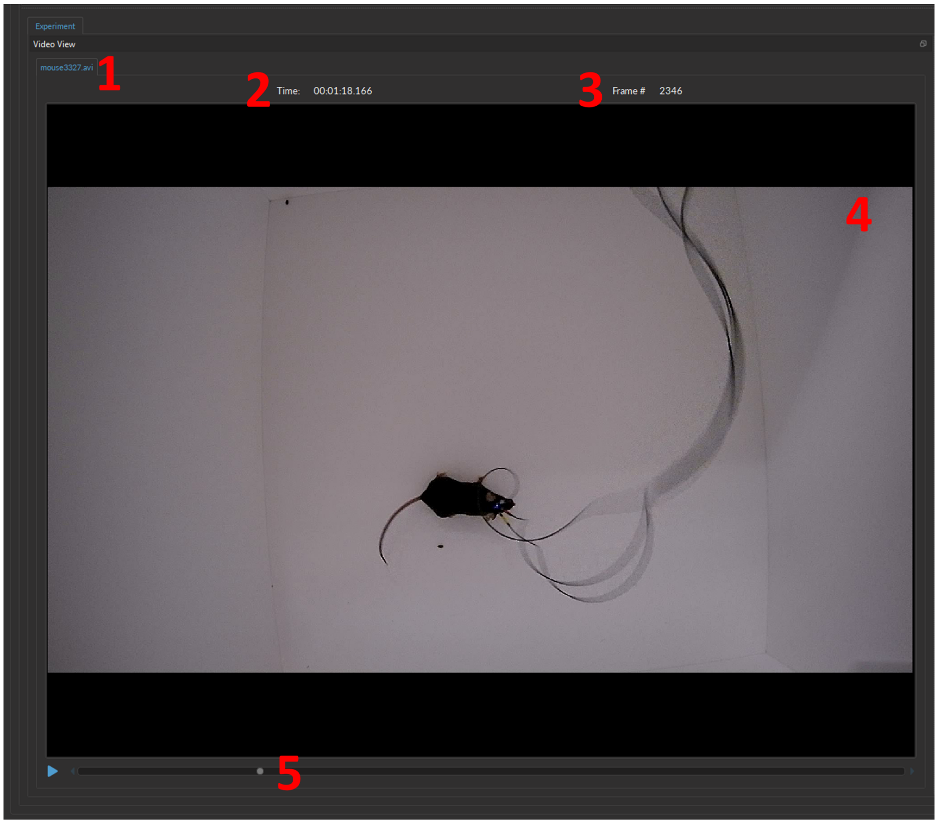


Figure 12.13: *Video View*

1. The **Video tab** (Fig. 12.13, 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. 12.13, 2) displays the timestamp associated with the current frame (in hh:mm:ss:zzz).

3. The **Fame #** (Fig. 12.13, 3) displays the index of the current frame within the entire video.

4. The **Current frame** (Fig. 12.13, 4) is where the image associated with the **Frame #** of the video is displayed.

5. The **Current Time Bar** (Fig. 12.13, 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 the cursors in the **Video View** will automatically move the cursor in the **Signal View**, facilitating checking the correlation between signal and behavior.

12.5 Signal View

The **Signal View** (Fig. 12.14) 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.

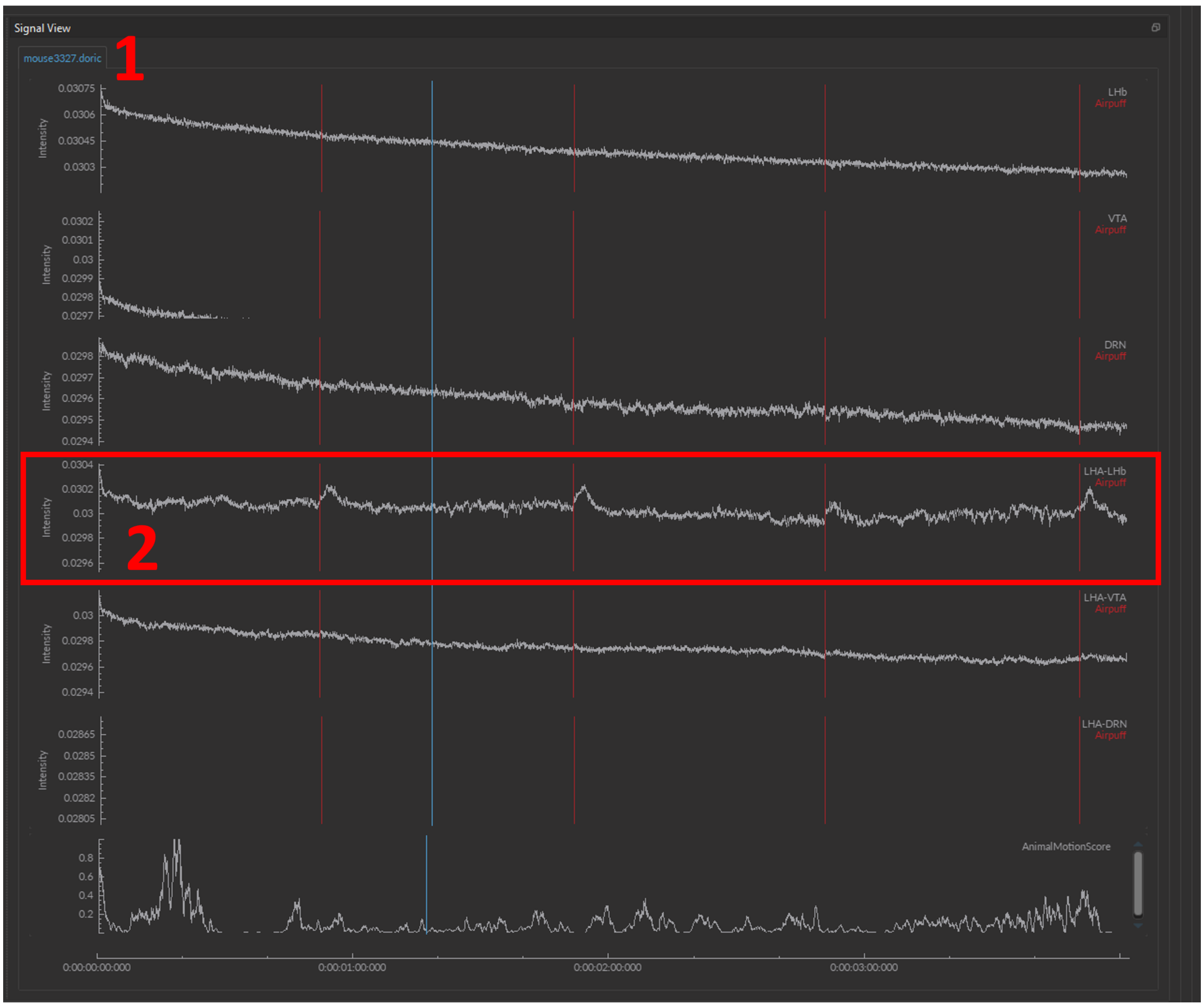


Figure 12.14: *Signal View*

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:

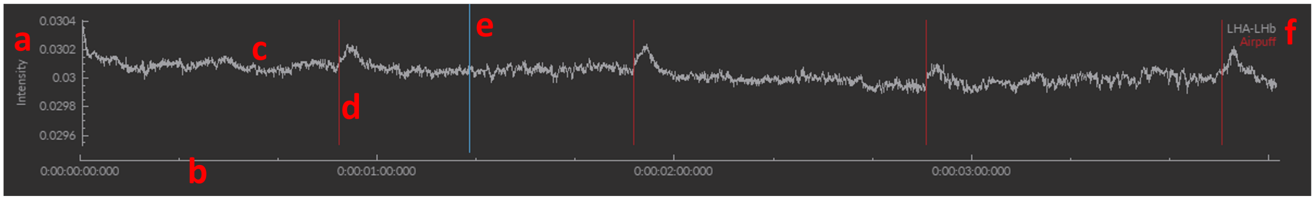


Figure 12.15: *Signal View, graph*

a) The **Y-axis** (Fig. 12.15, a) for loaded signal, represents the intensity of the pixel, which is unitless. For the **Animal Speed**, the unit is in pixel/second, while the **Animal Motion Score** is plotted with Arbitrary Units (AU).

b) The **X-axis** (Fig. 12.15, b) represents the time in d:hh:mm:ss:zzz.

c) The **Trace** (Fig. 12.15, 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. 12.15, d) are the overlayed **Keypress Events**, which can be imported using the **Load Events** button (Fig. 12.3, 4).

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. 12.15, f) displays the color code of the graph traces. The trace name of the signal is always included and, when present, keypress event(s) are also displayed.

13

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: 13.1).

Figure 13.1: *Image Analyzer*

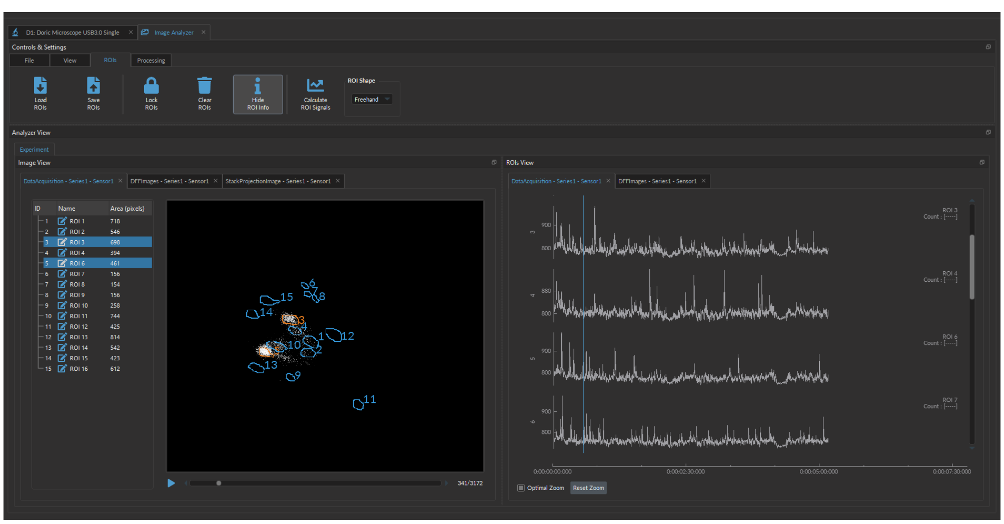


Figure 13.2: *Image Analysis Module Interface*

The Image Analyzer is composed of 2 main parts:

1. The **Controls & Settings** which regroups four tabs developed further in the document: **File** (section 13.1.1), **View** (section 13.1.2), **ROIs** (section 13.1.3), and **Processing** (section 13.1.4).

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 ROI.

13.1 Controls & Settings

13.1.1 File

The **File tab** (Fig. 13.3) is used to load data, obtain information about data, and clear the **Analyser View**.

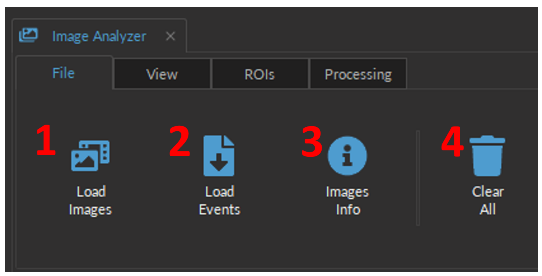


Figure 13.3: *File Tab*

1. The **Load Images** button (Fig. 13.3, 1) loads the images. They must be saved in a .doric file format containing images in a square, 16 bits format.

2. The **Load Events** button (Fig. 13.3, 2) displays **Keypress Events** from a .doric file over the signals in **ROIs View**.

3. The **Images Info** (Fig. 13.3, 3) displays a window with information about the images (Width x Height, Bits Count, Timestamp, Sensor ID, LED power, Exposure, and the Gain).

4. The **Clear All** button (Fig. 13.3, 4) closes the **Analyser View** and the analysis in progress.

13.1.2 View

The **View tab** (Fig. 13.4) is used to manipulate the appearance of an image without changing the base data.

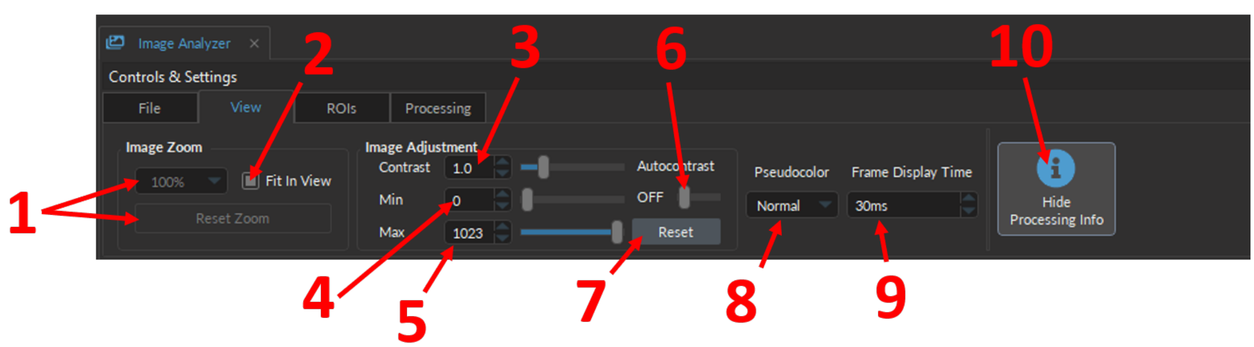


Figure 13.4: *View Tab*

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 13.3.1 for details.
4. The **Min** function applies a lower threshold with the cut-off value defined by the slider. See section 13.3.2 for details.
5. The **Max** function applies an upper threshold with the cut-off value defined by the slider. See section 13.3.2 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 processes operated on the images in the order in which they are applied.

13.1.3 ROI

The **ROI** tab (Fig. 13.5) is used to save/load data relating to regions of interest drawn on an image.

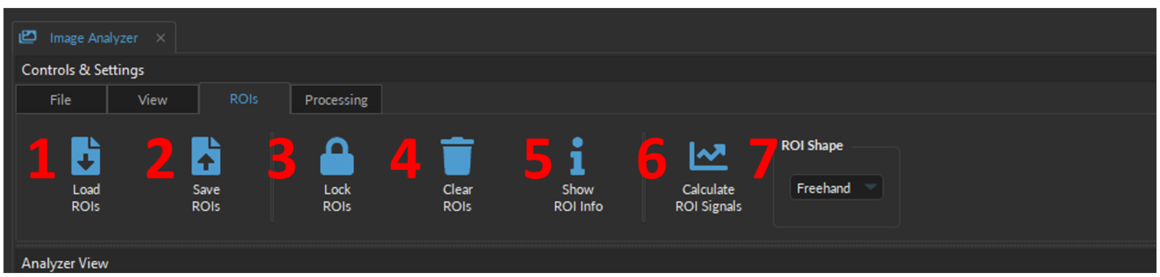


Figure 13.5: *ROI Tab*

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**.

13.1.4 Processing

The **Processing** tab (Fig. 13.6) is used to process the image data.

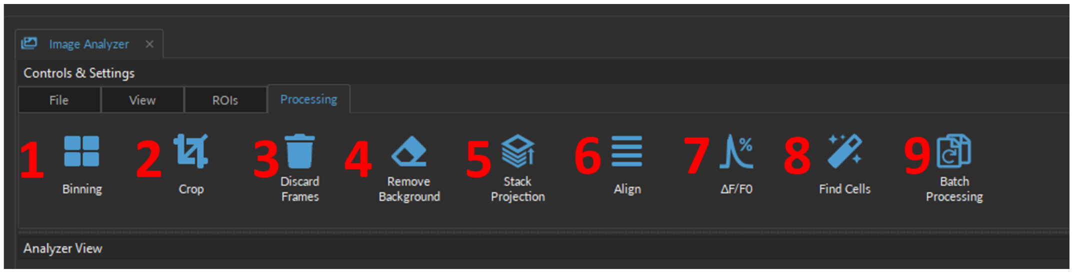


Figure 13.6: *Processing Tab*

1. The **Binning** function combines a cluster of pixels in a single pixel to reduce the amount of data and facilitate 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 **ΔF/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 13.3.6 for details.
6. The **Align Images** function aligns the image stack to the user-defined key frame. See section 13.3.3 for computational details. Selecting this button will open the **Align Images** window (Fig. 13.7). There are 4 different methods available.

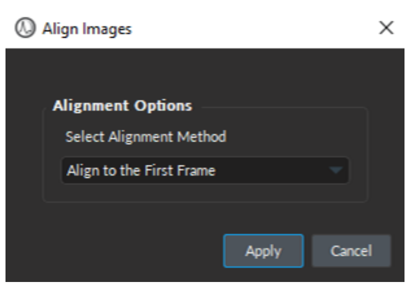
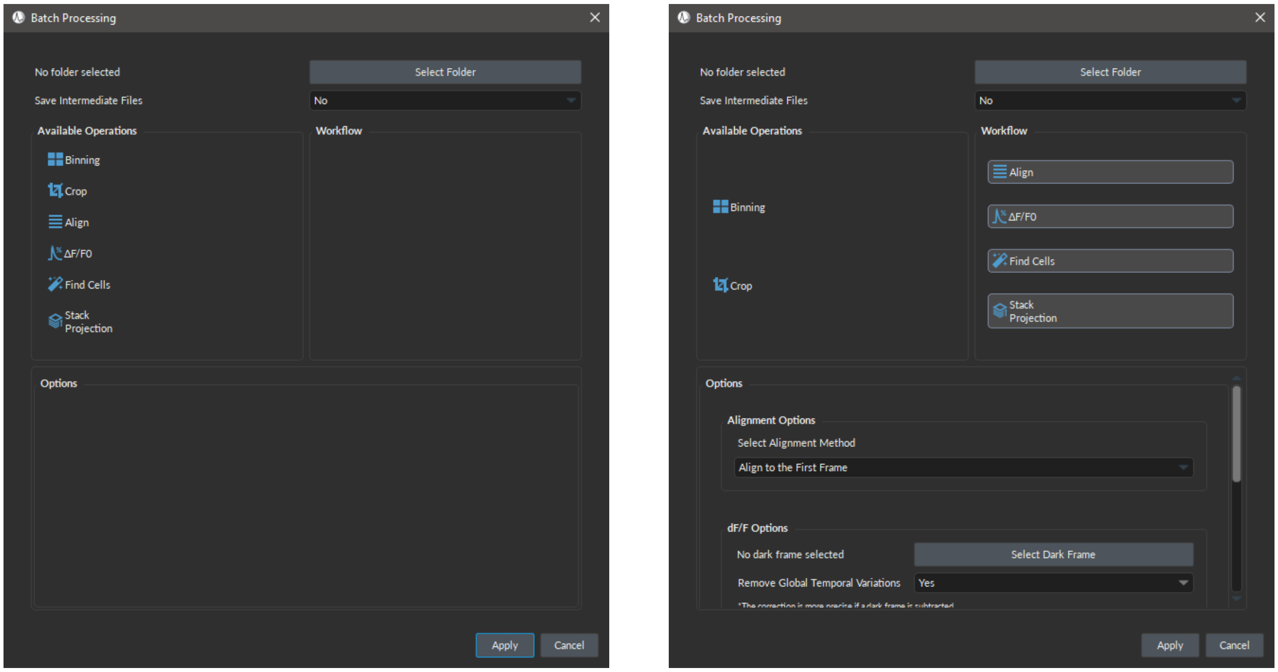


Figure 13.7: *Align Images Window*

- 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 the 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 **Microscope ΔF/F$_0$** function calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 13.3.4 for details.
8. The **Find Cells** function detects the cells and creates the ROI automatically. See section 13.3.5 for details.

9. The **Batch Processing** function opens the **Batch Processing Window** (Fig. 13.8). 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 13.8: *Batch Processing Window*

- The **Available Operations** box lists all processes available. Processes on the list will be greyed out if the workflow 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 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 Images** process aligns the image stack to the user-defined key frame. See section 13.3.3 for computational details.
  - The **Microscope $\Delta F/F_0$** process calculates the normalized fluorescence variation of the images and displays the results in a new tab. See section 13.3.4 for details.
  - The **Find Cells** process detects the cells and creates the ROI automatically. See section 13.3.5 for details.
  - The **Stack Projection** process projects all image frames to a single frame using the method selected in the Settings dialog. See section 13.3.6 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.

13.2 Analyzer View

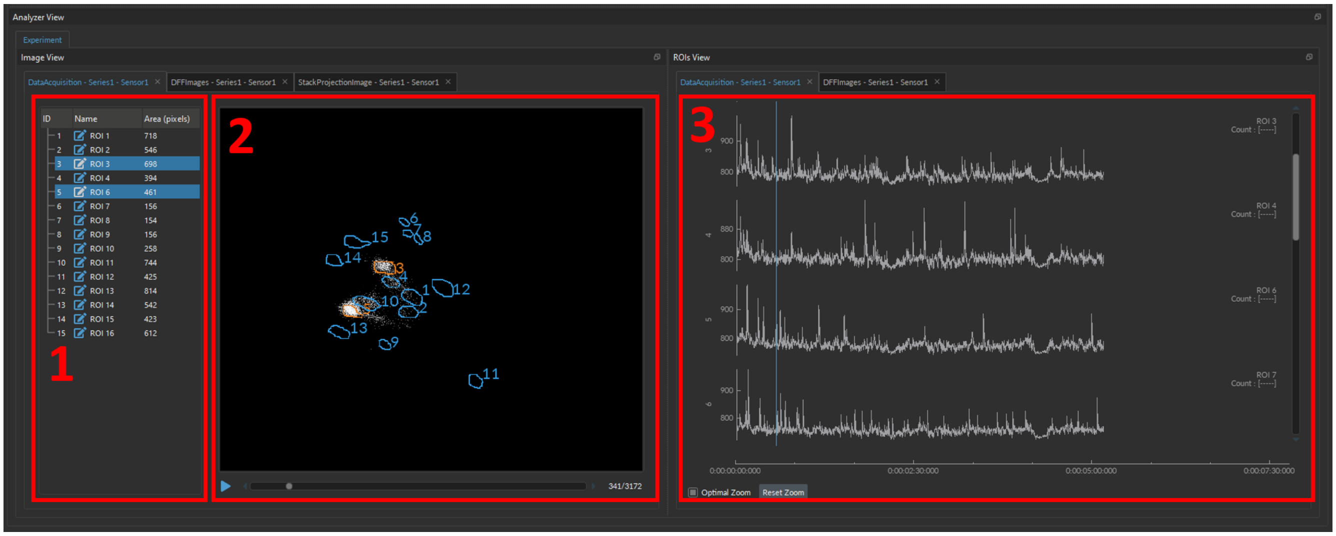


Figure 13.9: *ROI View*

The ROI manager extracts the 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 changed 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 stacks and the ROIs, numbered according to the order they were set. The ROIs can be saved independently from the image stack on the ROI toolbar. The ROIs are drawn directly on the *Image Viewer* in a *freehand* manner. All selected ROIs 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 normalized images. Each trace on a separate graph represents an ROI, allowing for precise intensity measurements (see Fig. 13.10)

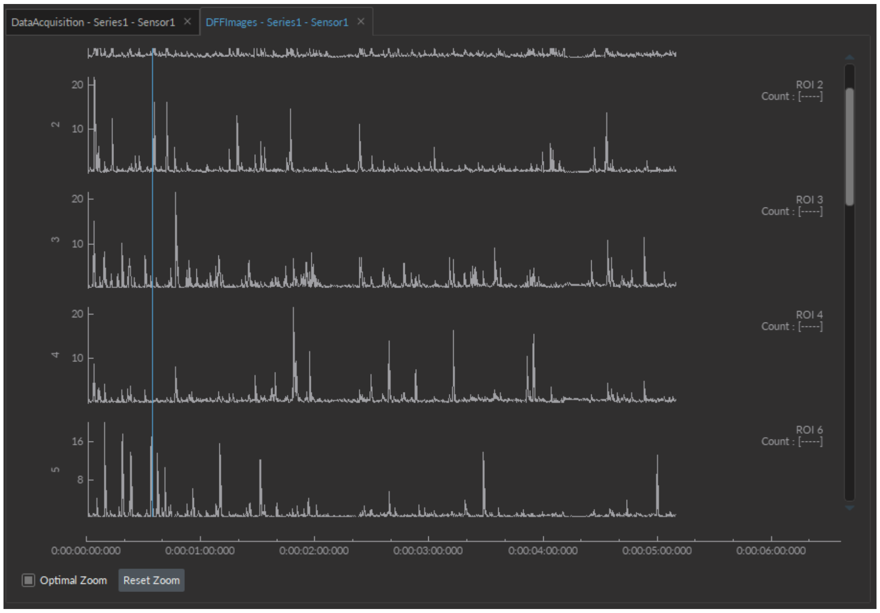


Figure 13.10: *ROI View Graph*

13.3 Algorithms

13.3.1 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.

13.3.2 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.

13.3.3 Image Alignment

The algorithm is inspired by 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 a 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.

13.3.4 Microscope $\triangle$F/F$_0$

The algorithm calculates a standard ΔF/F$_0$ with F_0 corresponding to the temporal average intensity, with an optional preprocessing step to remove the illumination variation artefacts. In order to properly calculate the $\Delta F/F_0$, the algorithm uses a dark frame to account for the sensor electronic offset. Calculating the $\Delta F/F_0$ without subtracting the offset will lead to artificially lower values. To record a dark frame, set the microscope driver to the desired exposure and gain, the LED power to zero, and take a snapshot. Before calculating the F_0, the average temporal variations can be compensated to get a flat temporal average profile (Fig. 13.11). Keep in mind that removing the average temporal profile can also remove global activity patterns.

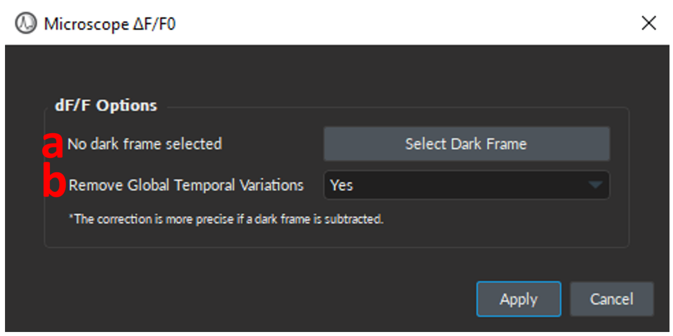


Figure 13.11: ΔF/F$_0$ *Settings*

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 $R(t) = (F(t) - F_0)/F_0$.

13.3.5 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 false positives. The *Find Cells* algorithm uses user-defined boundaries shown in Fig. 13.12. The first parameter is an estimate of the number of cells 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.

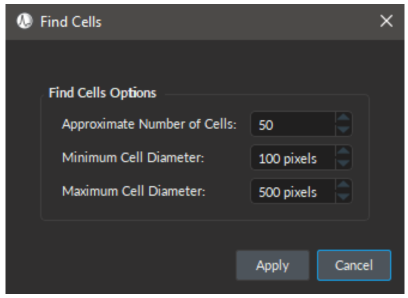


Figure 13.12: *Find Cells Settings*

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.

13.3.6 Stack Projection

This function can be used to help with ROI drawing. It calculates a temporal projection using the user-defined method (see Fig. 13.13).

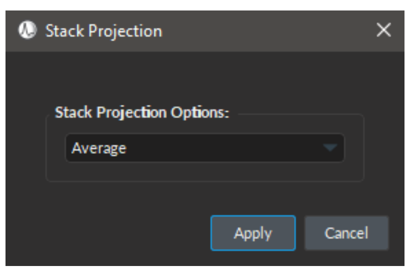


Figure 13.13: *Stack Projection Settings*

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.

14

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.

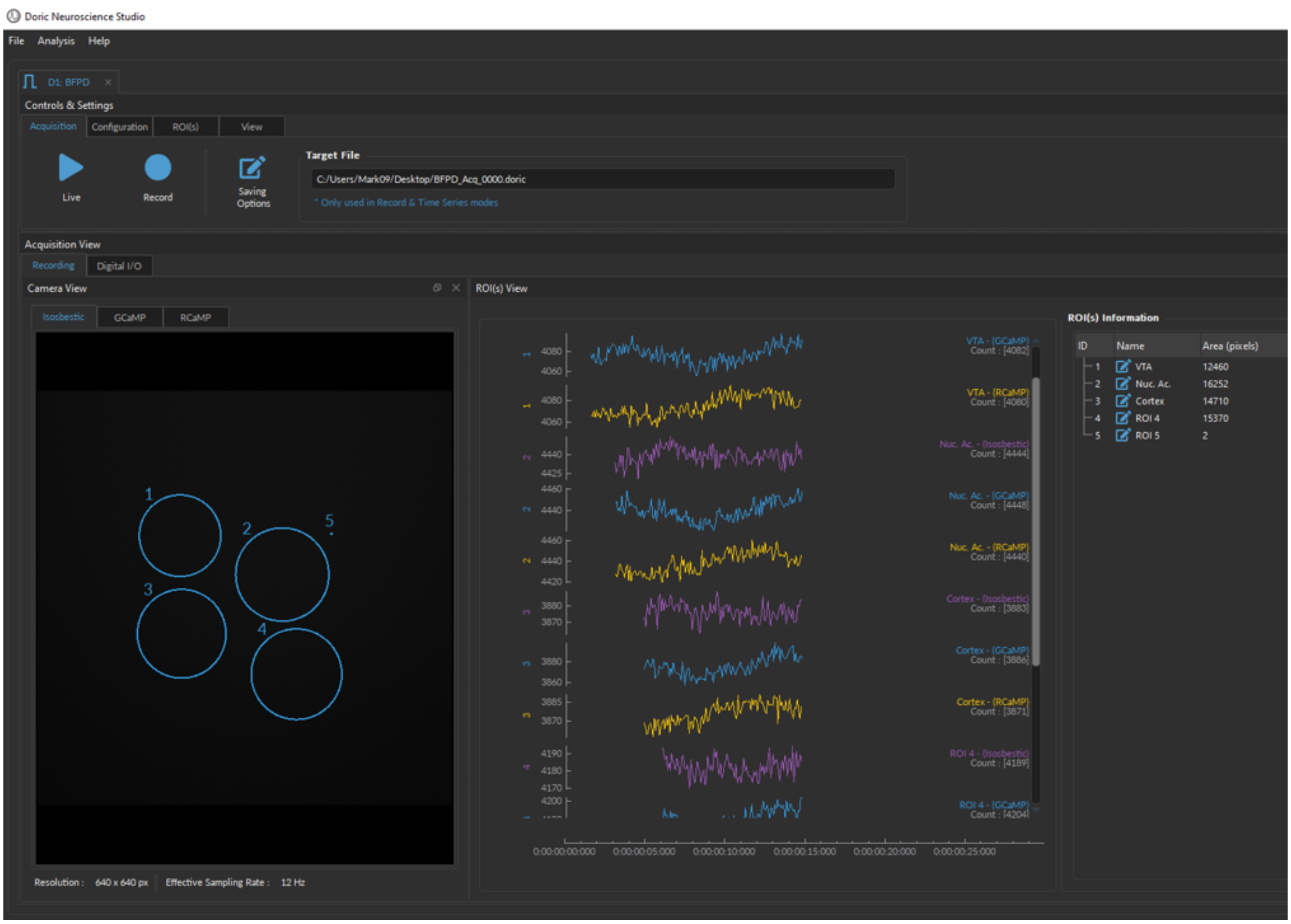


Figure 14.1: *BFPD interface*

14.1 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. 14.2).

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. 14.2).

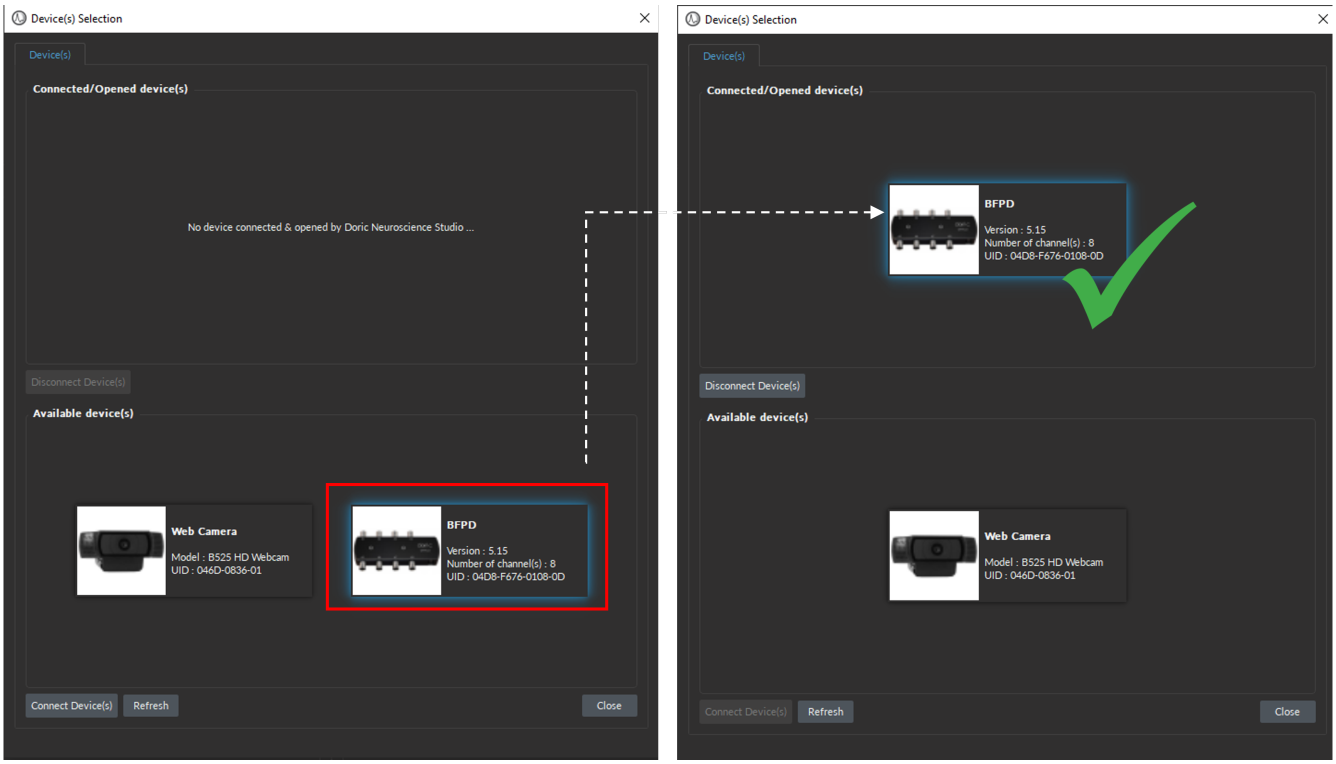


Figure 14.2: *Double click on the device of choice to connect it to DNS*

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. 14.3) or use the hot key: *Ctrl+N*.

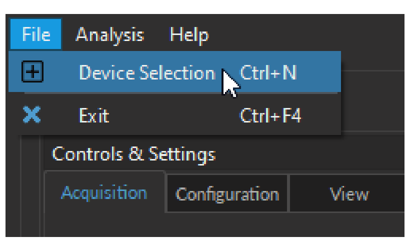


Figure 14.3: *Open Device Selection Window*

14.2 Overview

The **BFPD** interface is split into two sections (Fig. 14.4):

1. **Control and settings tabs** (Section 14.3) are used to manage different parameters and settings of the software (Acquisition, Configuration, ROI, and View).

2. **Acquisition view** (Section 14.5) displays the input and output traces for visualization.

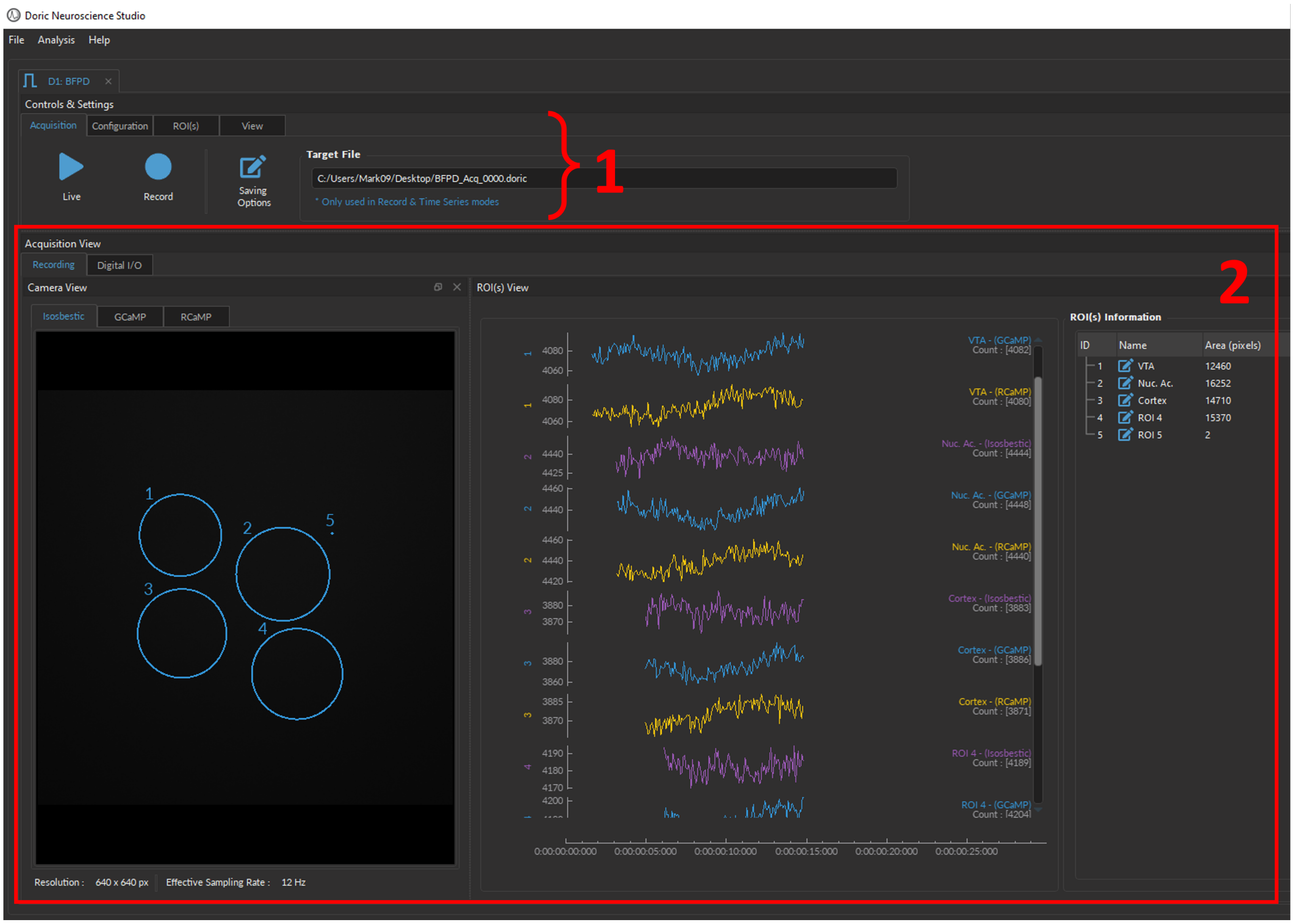


Figure 14.4: *BFPD interface*

14.3 Control and Settings tabs

The **Control and settings tabs** (Fig. 14.4, 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 14.3.1;
- *Configuration* tab - Section 14.3.2;
- *ROI(s)* tab - Section 14.3.3;
- *View* tab - Section 14.3.4.

14.3.1 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 14.4.1 to configure channels for recording.

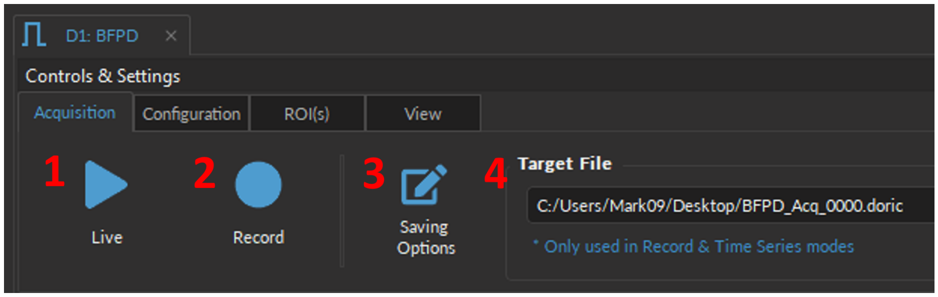


Figure 14.5: *Acquisition Tab*

1. The **Live** button (Fig. 14.5, 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. 14.5, 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. 14.5, 3) button opens the **Saving Parameters** window (Fig. 14.6). See section 14.3.1.1 for more details.
4. The **Target File** (Fig. 14.5, 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.

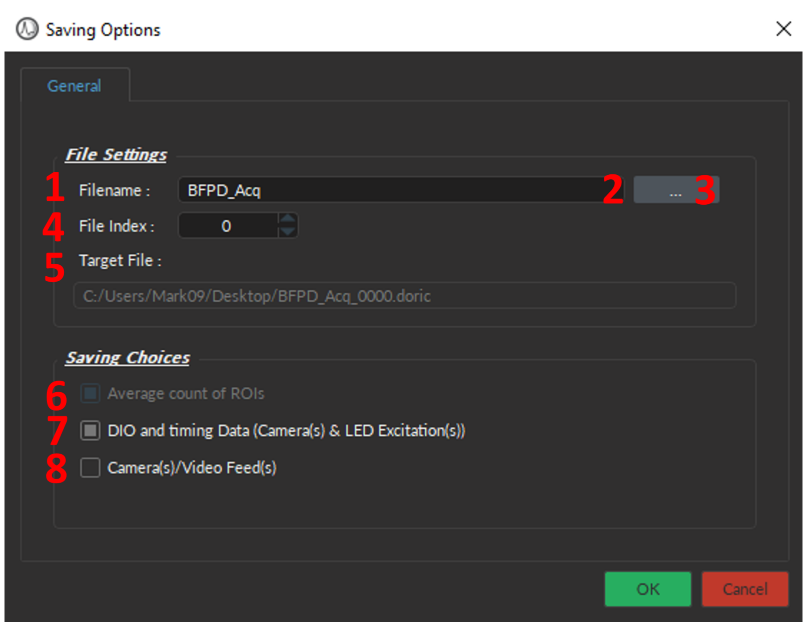


Figure 14.6: *Saving Menu Window*

14.3.1.1 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. 14.5, 3).

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 14.6, 1).
2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 14.6, 2).

3. The **File format** (Fig. 14.6, 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. 14.6, 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. 14.6, 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. 14.6, 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. 14.6, 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. 14.6, 8), if enabled, will save the raw image stacks collected by the CMOS sensor(s). Note that selecting this option will generate large files.

14.3.2 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.

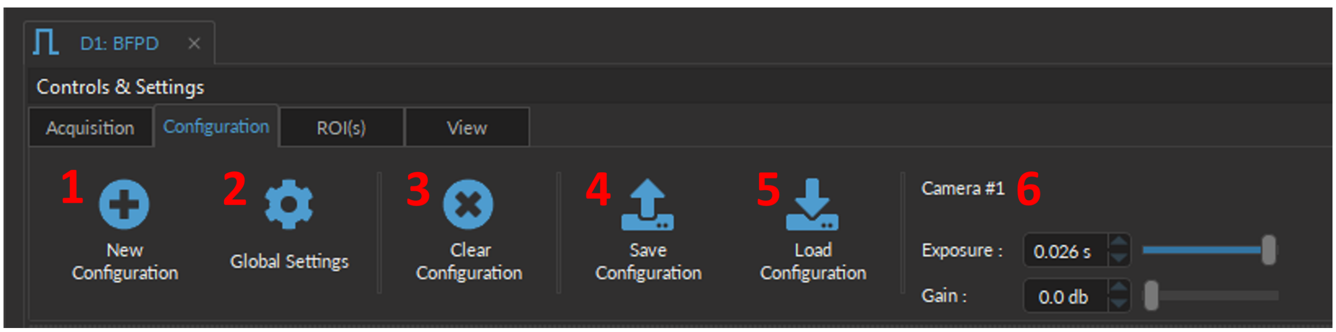


Figure 14.7: *Configuration Tab*

1. The **New Configuration** button (Fig. 14.7, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 14.4. Table 14.1 describes different types of channels available, their use cases and their individual sections.

2. The **Global Settings** (Fig. 14.7, 2) opens the **Global Options** window in Fig. 14.8, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 14.3.2.1 for more details.

3. The **Clear configuration** button (Fig. 14.7, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.

4. The **Save configuration** button (Fig. 14.7, 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. 14.7, 5) imports a pre-configured **.doric** file into the module.

6. The **Camera #** options (Fig. 14.7, 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.

14.3.2.1 Global Settings

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

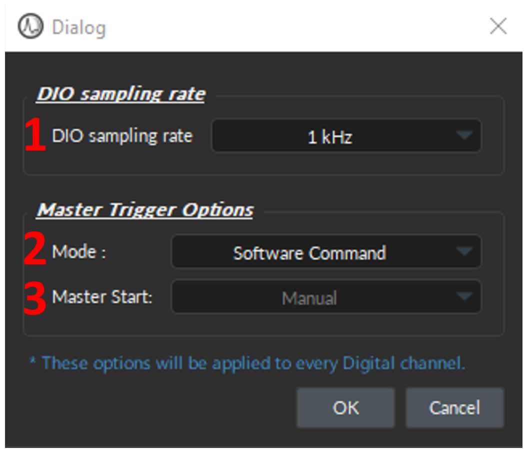


Figure 14.8: *Global Options Window*

1. The **DIO sampling rate** (Fig. 14.8, 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. 14.41, no. 4).

2. The **Mode** (Fig. 14.8, 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. 14.5, 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 15.10a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig 14.9b) pops up. See section 14.3.2.2 for more details.

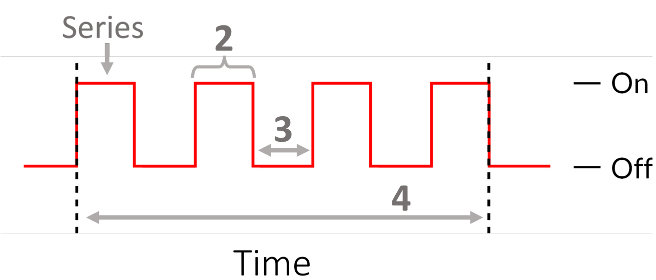
3. The **Master Start** (Fig. 14.8, 3) defines the source that will automatically start the recording. This source can either be:

   - *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.***

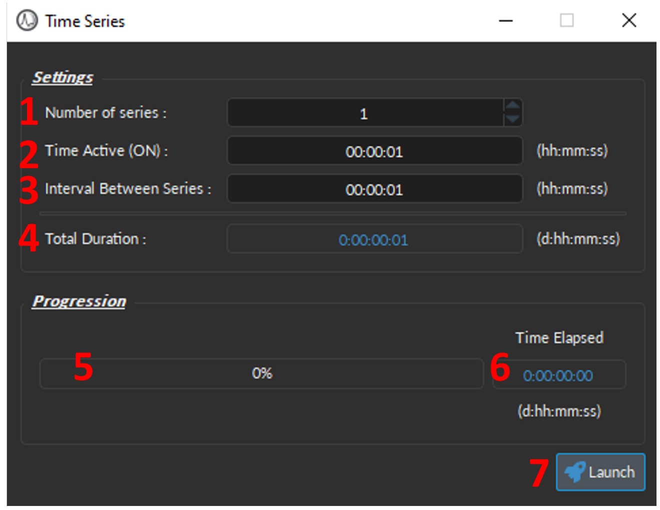
14.3.2.2 Time Series

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 14.9b) can be opened by clicking on the **Record** button (Fig. 14.5, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 14.8, 2). Every **Time series** sequence is automatically saved to the same *.doric* file defined in **Saving Options** (Section 14.3.1.1).



(a) *Time Series Acquisition Timing Diagram*



(b) *Time Series Window*

Figure 14.9: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 14.9b) sets the following parameters:

1. The **Number of series** (Fig. 14.9b, 1) defines the amount of times the series is repeated.
2. The **Time Active (ON)** (Fig. 14.9b, 2) defines the duration of the series.
3. The **Interval Between Series** (Fig. 14.9b, 3) defines the amount of time between each series, if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 14.9b, 4) displays the total amount of time that the timeseries recording will take place.
5. The **Progression bar** (Fig. 14.9b, 5) indicates the progression of the timeseries (in %).
6. The **Time Elapsed** (Fig. 14.9b, 6) counter indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** (Fig. 14.9b, 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.

14.3.3 ROI(s) Tab

The **ROI(s) Tab** (Fig. 14.10) contains parameters to save, load, clear, or edit bundle fiber photometry ROI(s).

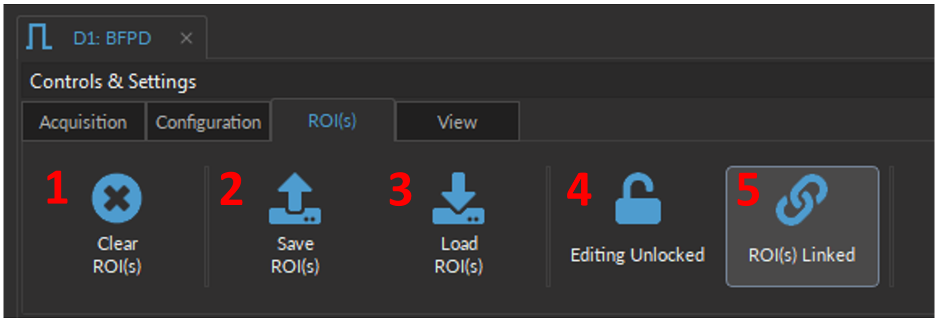


Figure 14.10: *ROI(s) Tab*

The **ROI(s)** parameters are as follows:

1. The **Clear ROI(s)** button (Fig. 14.10, 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. 14.10, 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. 14.10, 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. 14.10, 4) when enabled prevents new ROI(s) from being drawn, but does not prevent moving or reshaping a selected ROI (see section 14.5.2).
5. The **ROI(s) Linked** button (Fig. 14.10, 5) automatically redraws identical ROI(s) in the other CAM# excitation tabs (Fig. 14.41, 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.

14.3.4 View Tab

The **View Tab** (Fig. 14.11) is used to modify the presentation of graphs in the **Acquisition view**.

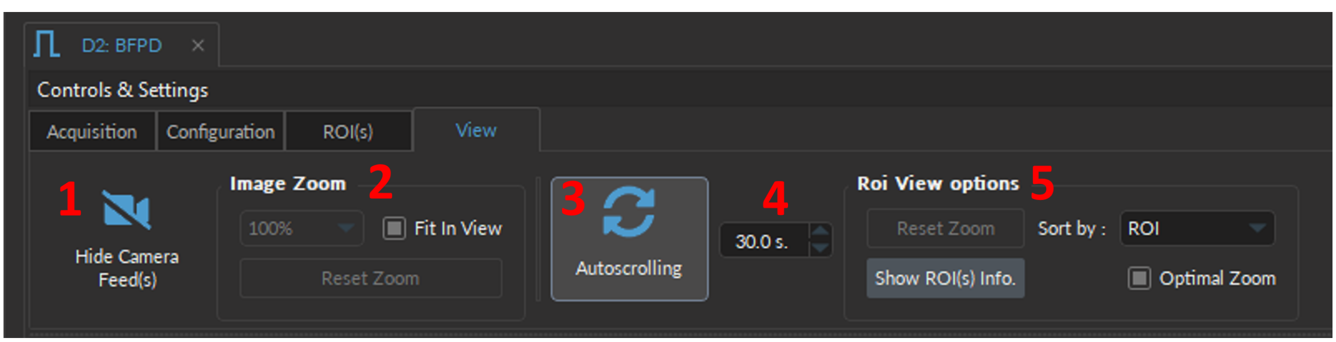


Figure 14.11: *View Tab*

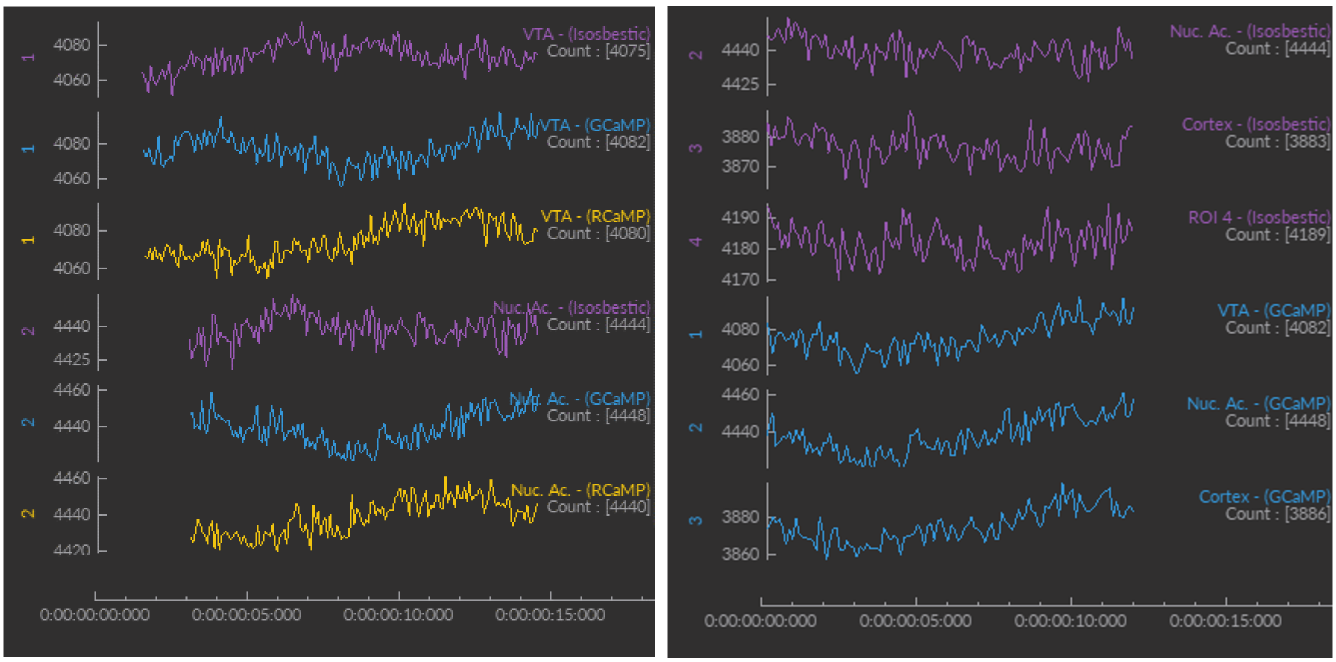
The **View** parameters are as follows:

1. The **Hide Camera Feed(s)** button (Fig. 14.11, 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. 14.11, 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. 14.11, 3), when selected, automatically sets the graphs to scroll as new data appears.
4. the **Autoscrolling range** (Fig. 14.11, 4) sets the graph zoom to the value of choice, specified in the text-box.
5. The **ROI View Options** (Fig. 14.11, 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. 14.12a) or by their *Excitation* type (Fig. 14.12b).



(a) *Sort by ROI*　　(b) *Sort by EXC*

Figure 14.12: *Sort ROI(s) View options*

- The **Show ROI(s) Info.** button opens or closes the **ROI(s) Information** Tab in Fig. 14.43, 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.

14.4 BFPD Configurations

14.4.1 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. 14.7, 1). This will open the **Channel(s) Configuration** window (Fig. 14.13). To generate a new **Channel** using the **Channel(s) configuration** window (Fig. 14.13):

1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 14.13, 1). Table 14.1 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 14.1 for hyperlinks to the relevant sections.

3. Select the **Add/Apply** button (Fig. 14.13, 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.

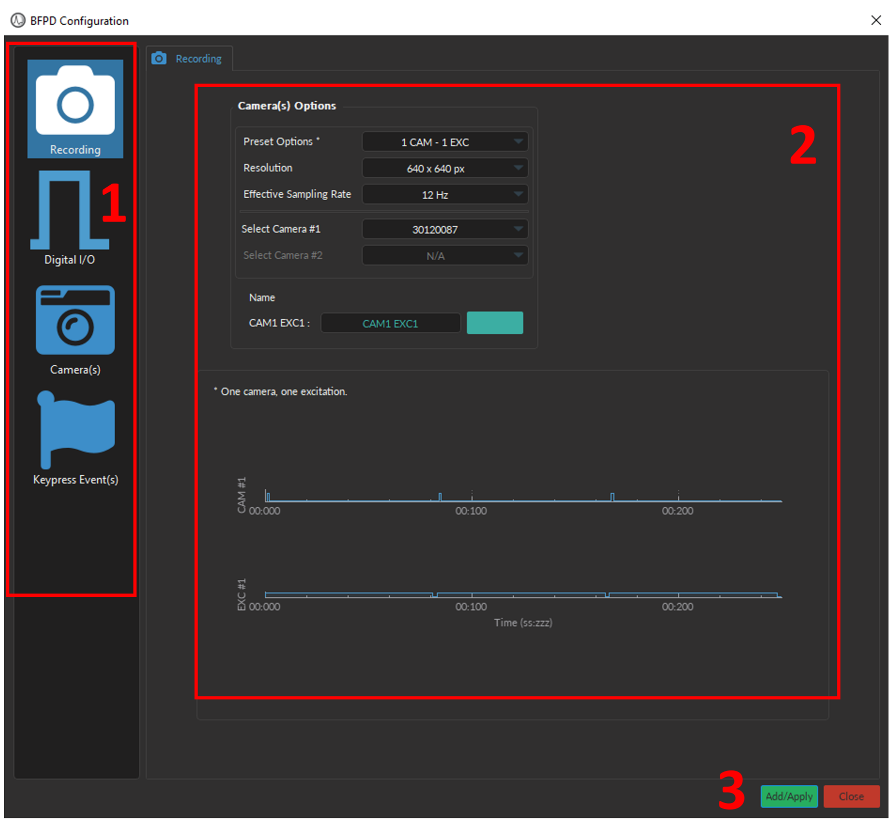


Figure 14.13: *Channel(s) configuration window*

14.4.2 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 14.7). Table 14.1 details the types of inputs and output the BFPD and the software can handle and gives quick access to their sections.

Table 14.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	Recording	To collect the fluorescence signal of *BFMC* ROI(s)	14.4.3
	Digital I/O	For input and output of TTL signals	14.4.4
	Camera(s)	To collect images for behaviour experiments	14.4.5
	Keypress Event(s)	To manually flag events time-locked to the current recording using customized keys	14.4.6

14.4.3 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.

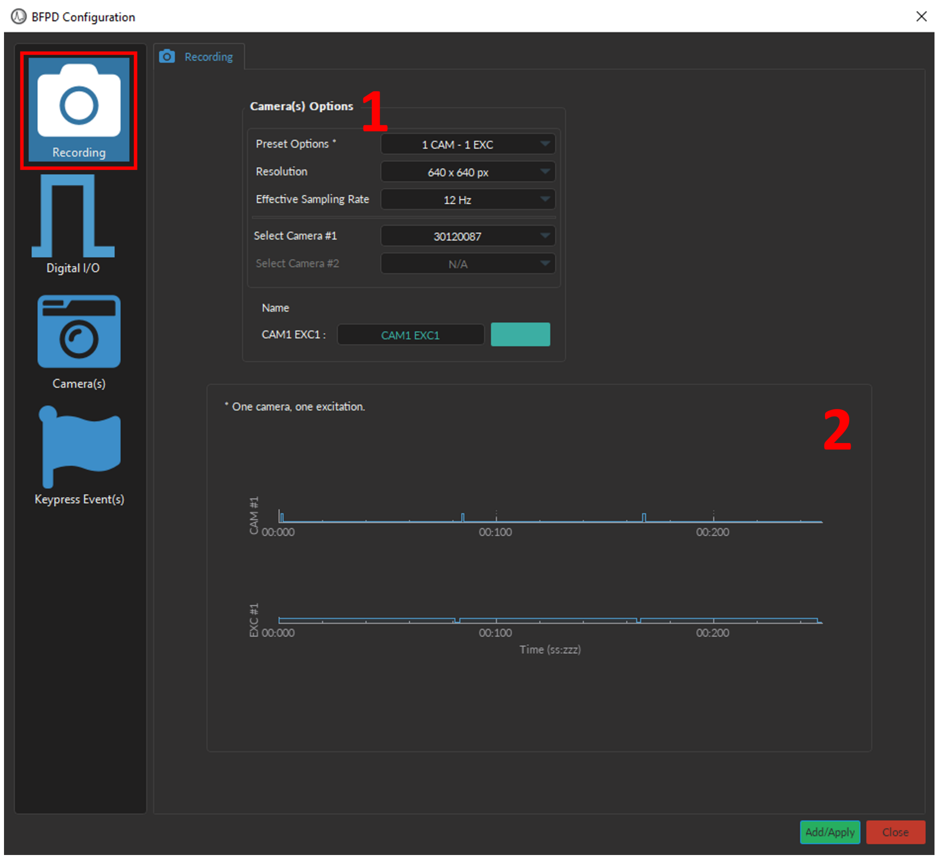


Figure 14.14: *Channel(s) configuration window, Recording*

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. 14.14):

1. The **Camera Options** (Fig. 14.15) defines which preset option to use and allows users to select the proper camera and camera parameters for the recording, such as:

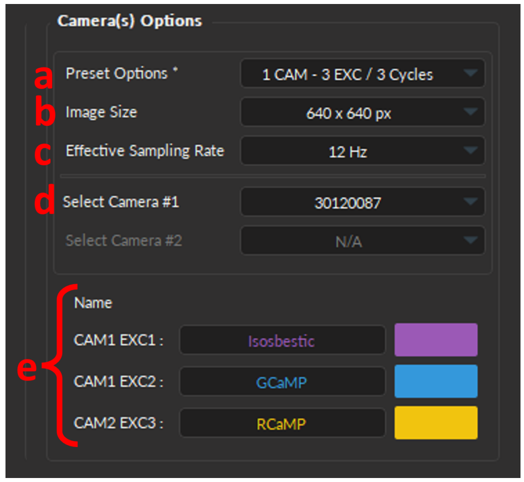


Figure 14.15: *Recording Channel - Camera Options*

a) The **Preset Options** drop-down (Fig. 14.15, a) contains six pre-configured options which are listed in Table 14.2, along with their use cases.

Table 14.2: *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. 14.15, 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. 14.15, 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. 14.15, 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. 14.15, 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. 14.16) displays the TTL output pulses for the **CAM** and **EXC** that will be used during the recording.

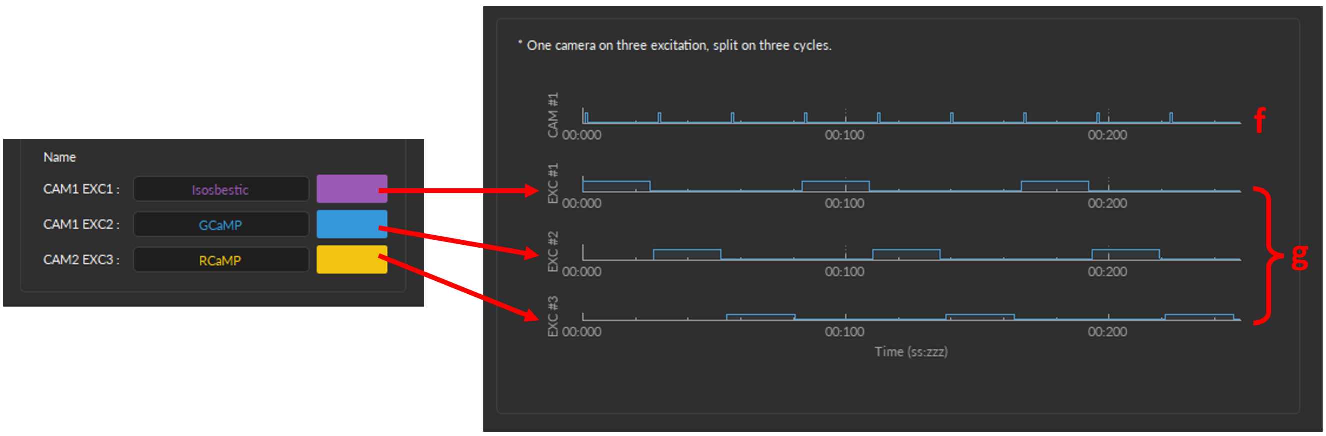


Figure 14.16: *Recording Channel - Preview digital Camera and Excitations outputs*

   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.

14.4.4 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. 14.17): (1) the **Channel Options** (Section 14.4.4.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 14.4.4.2).

Figure 14.17: *Channel(s) configuration window, Digital I/O - CW mode*

14.4.4.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.

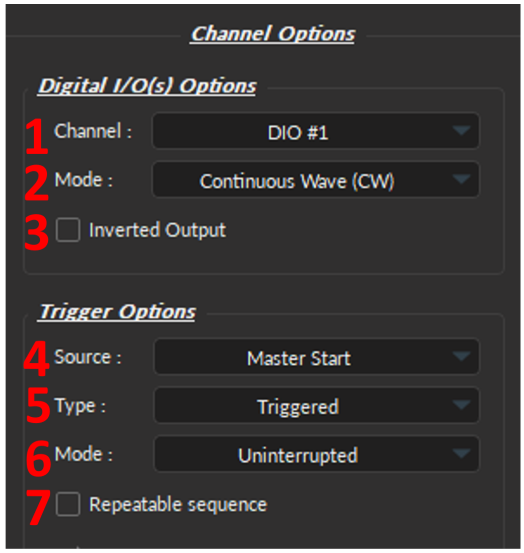


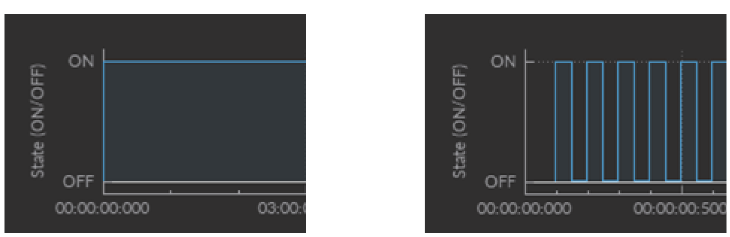
Figure 14.18: *Channel(s) configuration window, Digital I/O Channel Options*

Digital I/O Options:

1. The **Channel** (Fig 14.18, 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 14.18, 2) identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). Three modes are available:

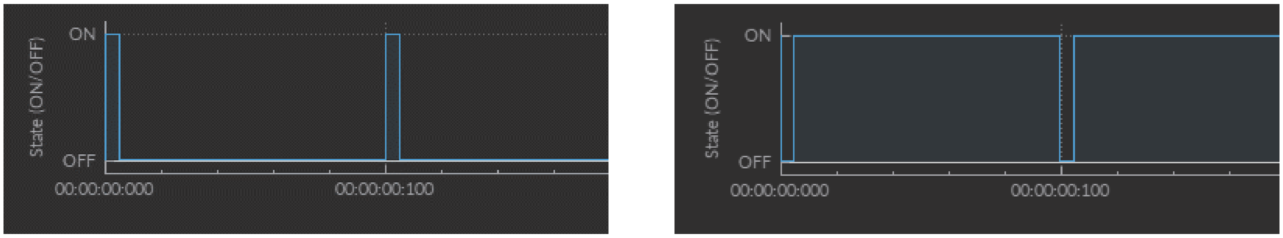
    - The **Continuous wave (CW)** Mode (Fig. 14.19a);
    - The **Square (TTL)** Mode (Fig. 14.19b);
    - The **Input** mode receives a signal that are either 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the BFPD (See Section 14.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.



(a) *Continuous* (b) *Square*

Figure 14.19: *Channel Options - Output Modes*

3. The **Inverted Output** checkbox (Fig 14.18, 3), when enabled, will convert every 0 to 1 and 1 to 0, such as in Fig. 14.20.



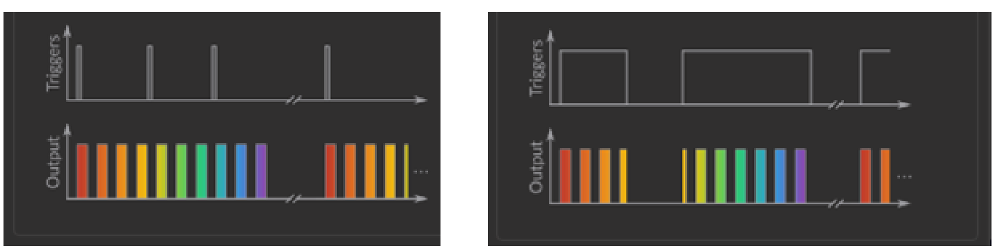
(a) *Disabled* (b) *Enabled*

Figure 14.20: *Inverted Output*

Trigger Options:

4. The **Source** trigger option (Fig 14.18, 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 14.18, 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. 14.21a), 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. 14.21b), 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***



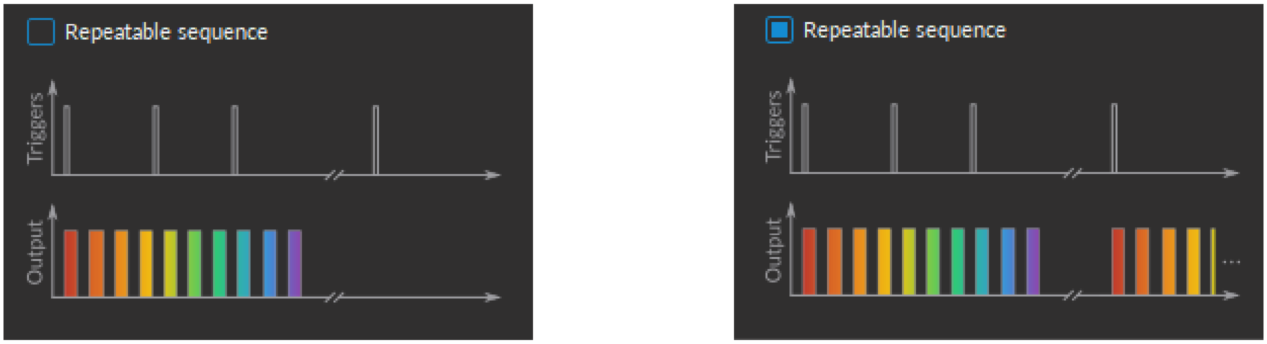
(a) *Trigger* (b) *Gate*

Figure 14.21: *Trigger Options Modes*

6. The **Mode** (Fig 14.18, 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 14.18, 7), when enabled, will run the sequence when additional TTL pulses are received (Fig. 14.22).



(a) *Disabled* (b) *Enabled*

Figure 14.22: *Repeatable sequence*

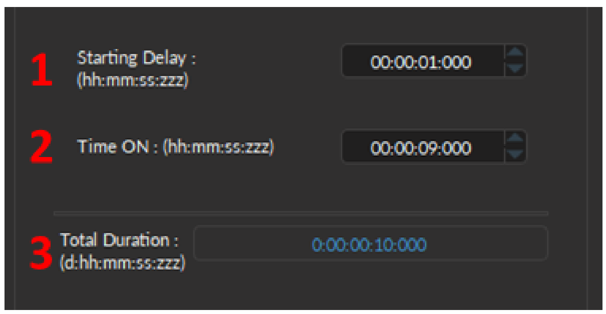
14.4.4.2 Sequence Options & Preview

The **Sequence options** section (Fig. 14.23a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 14.23b) 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**.

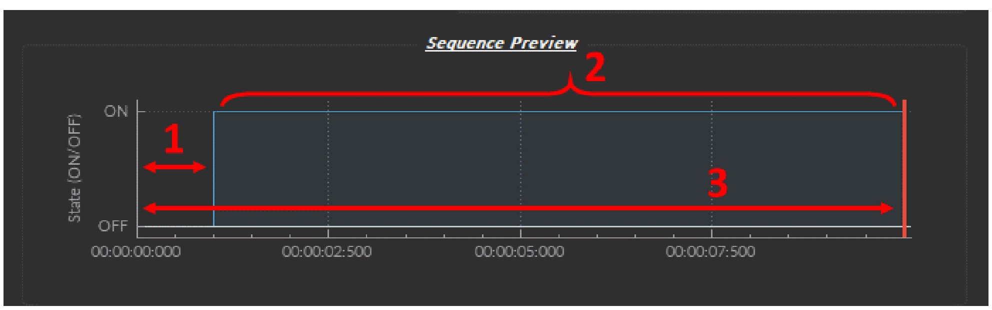
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 14.18), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 14.19a) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

    1. The **Starting Delay** (Fig 14.23, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Time ON** (Fig 14.23, 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 14.23, 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*



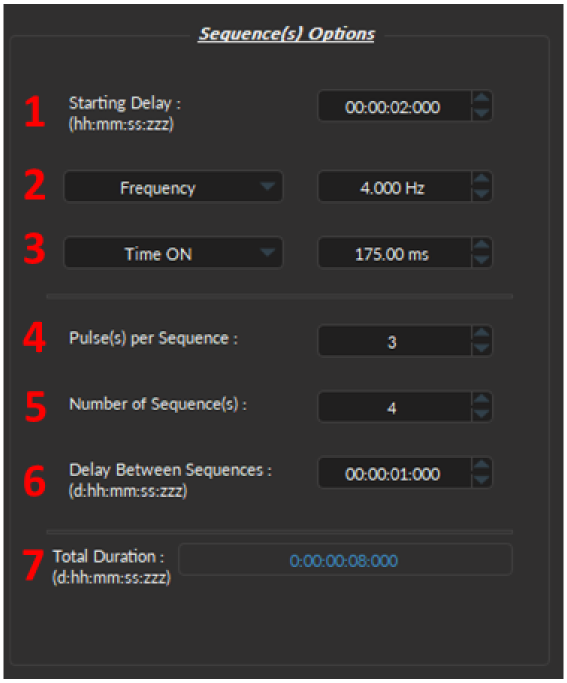
(b) *Sequence Preview*

Figure 14.23: *Channel(s) configuration window, Digital I/O - CW Mode*

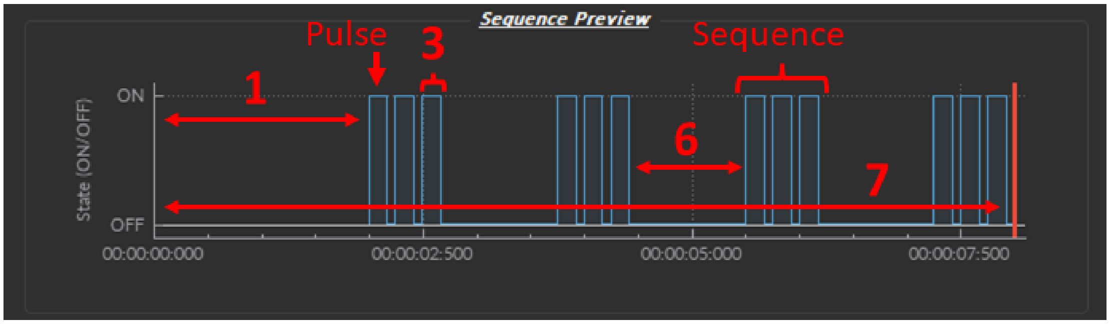
- The **Square** channel mode (Fig. 14.19b) allows the creation of a square TTL pulse sequence. The elements included in the Sequence Option box are as follows (Fig. 14.23, 1-3):

    1. The **Starting Delay** (Fig 14.24, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Frequency** (Fig. 14.24, 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. 14.24, 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. 14.24, 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. 14.24, 5) sets the number of times that the sequence will be repeated.
6. The **Delay between sequences** (Fig. 14.24, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).
7. The **Total Duration** (Fig 14.24, 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 14.24: *Channel(s) configuration window, Digital I/O - Square Mode*

14.4.5 Camera Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for their measurement.

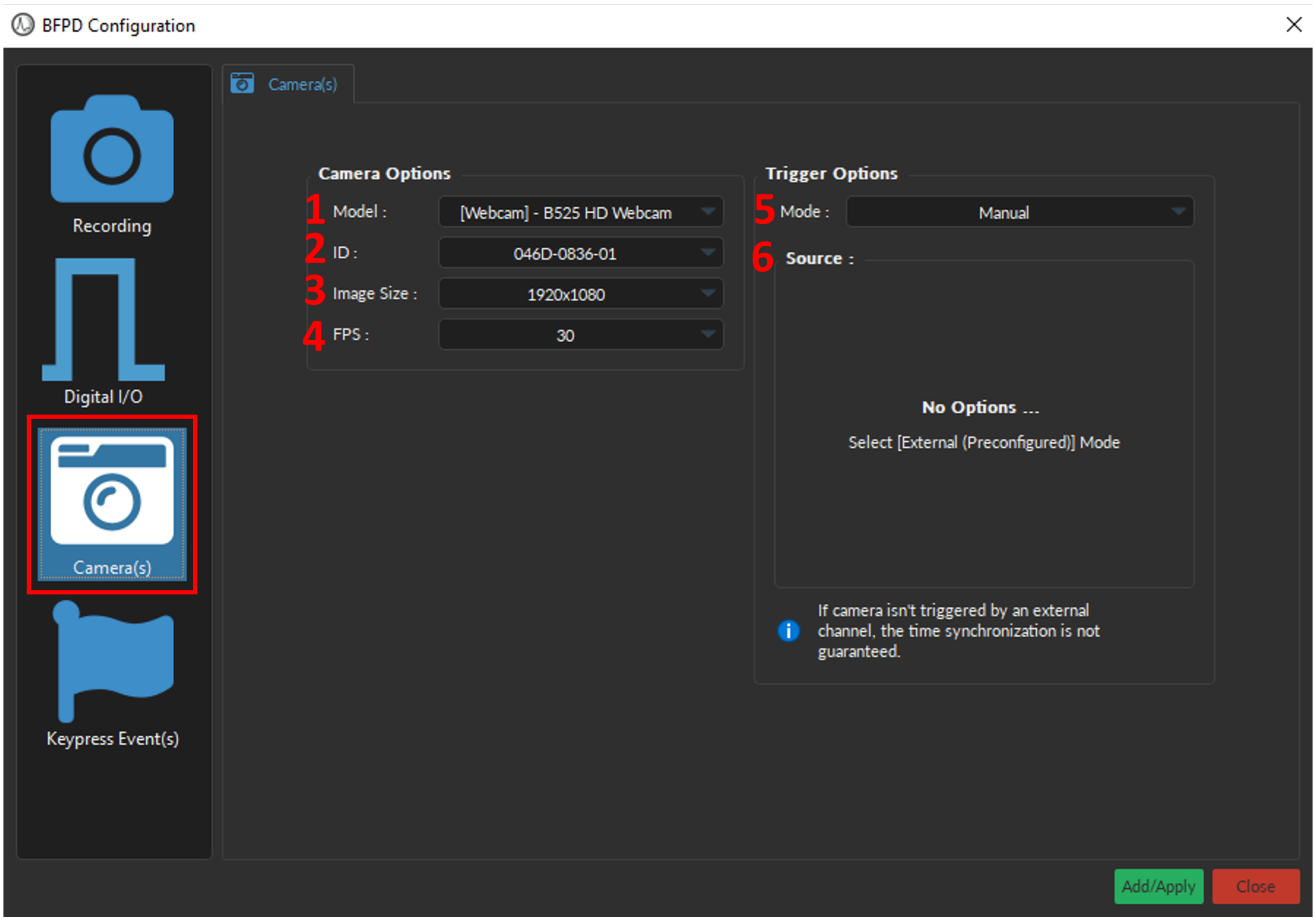


Figure 14.25: *Channel(s) configuration window, Camera*

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

Camera Options:

1. The **Model** (Fig. 14.25, 1) allows you to select the camera of choice based on the type of camera.
2. **ID** drop-down list (Fig. 14.25, 2) is used to select a camera based on its unique ID. The ID is beneficial when multiple cameras of the same model are required for the experiment.
3. The **Image Size** (Fig. 14.25, 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. 14.25, 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. 14.25, 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 *DifferenceMasterStart-ToFirstImage* attribute, located in *.doric* file under the **Web Camera ID** folder (Fig. 14.26). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

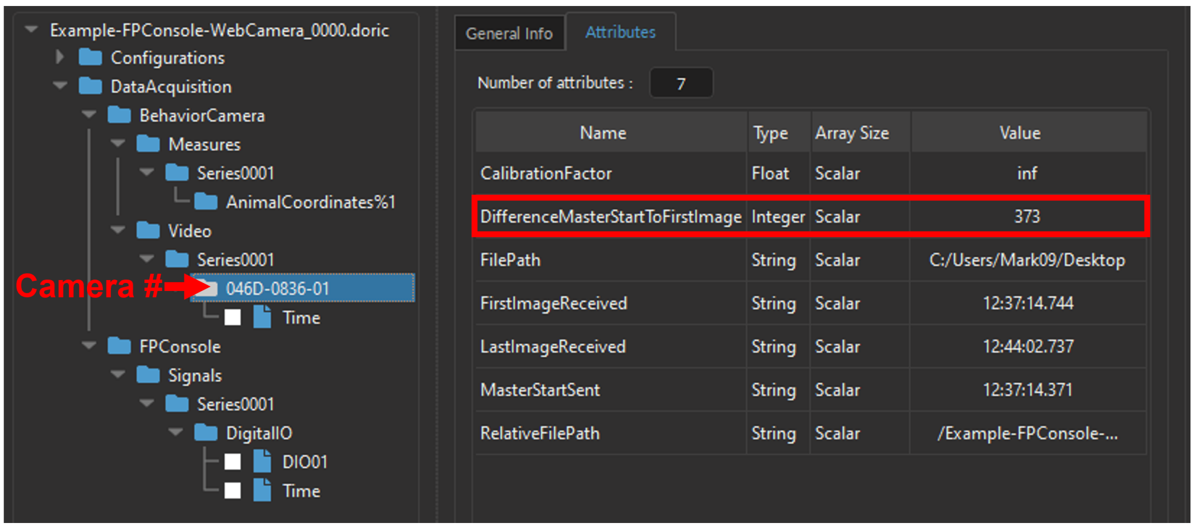


Figure 14.26: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **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. 14.25, 6 & Fig. 14.27) 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. 14.27). For a detailed description of each Digital I/O parameter see Section 14.4.4. Briefly, key parameters include:

   a) The **Channel** (Fig. 14.27, 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. 14.27, 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. 14.27, 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. 14.27, d) is by default 50%. The frame will be taken at the start of each square pulse.

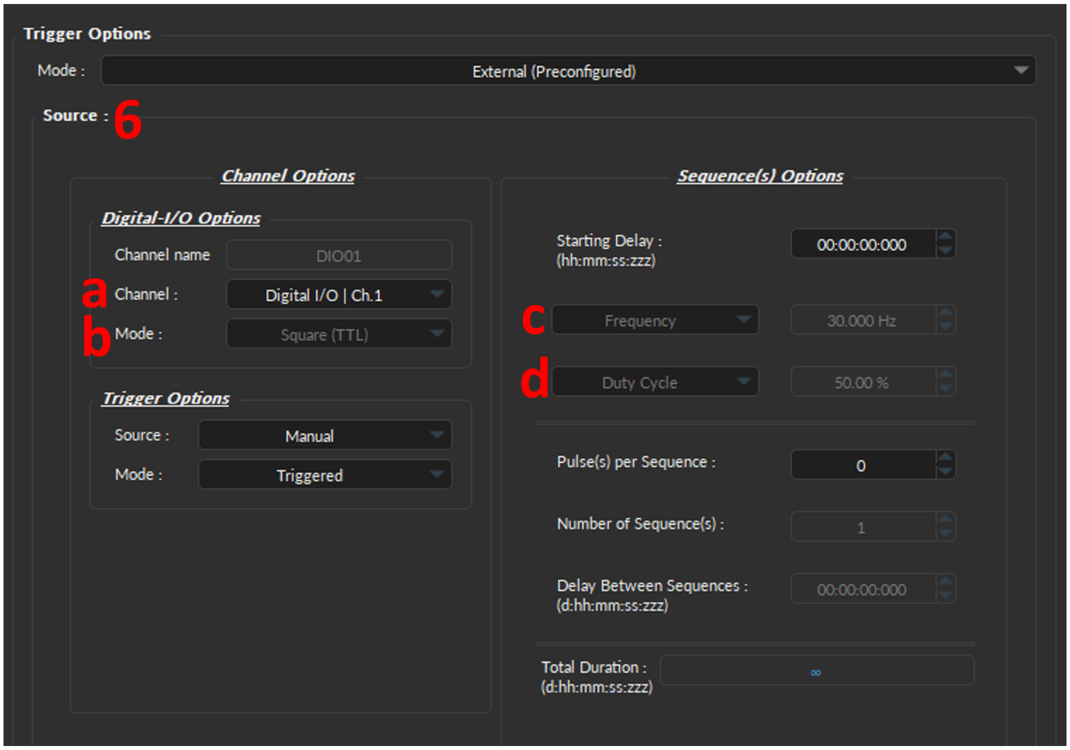


Figure 14.27: *Channel(s) configuration window, Camera - External (Preconfigured)*

14.4.6 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. 14.28, left). To remove a KeyPress, use - button (Fig. 14.28, 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. 14.28).

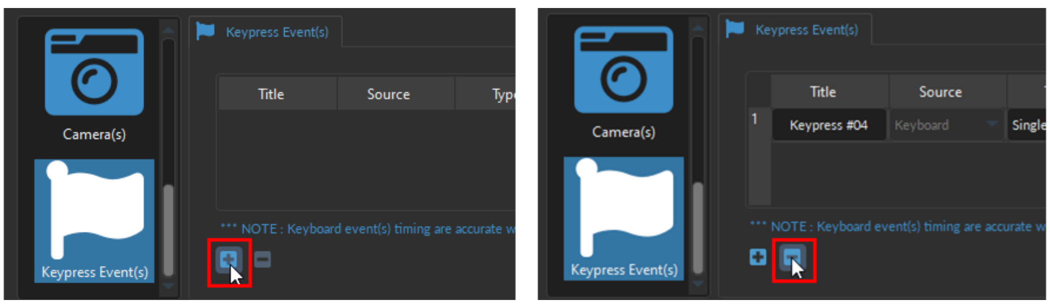


Figure 14.28: *Adding and Removing Keypress Events*

To edit a pre-existing **Keypress Event** Channel, select the left button (Fig. 14.29) in the **Acquisition View**.

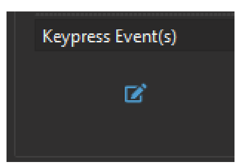
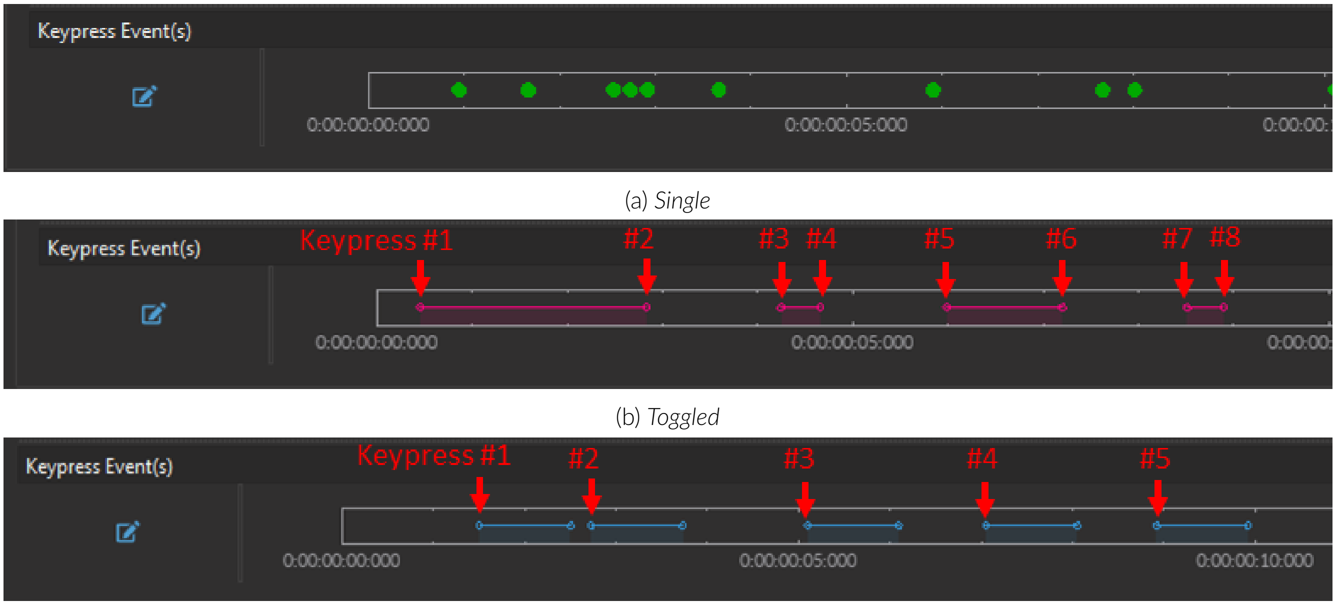


Figure 14.29: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 14.31:

1. The **Title** (Fig. 14.31, 1) allows you to give a name for the Keypress event.
2. The **Source** (Fig. 14.31, 2) is by default *Keyboard*.
3. Three **Types** of Keypress Event(s) (Fig. 14.31, 3) can be specified with the drop-down list:
    - **Single** - Records single event at the touch of a key (Fig. 14.30a).
    - **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. 14.30b).
    - **Timed** - Records an event for a predetermined duration of time (Fig. 14.30c). 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 14.30: *Three types of Keypress Event(s)*

4. The **Duration** (Fig. 14.31, 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. 14.31, 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. 14.31, 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. 14.31, column 6).
7. The **Information** column (Fig. 14.31, 7) provides space to make notes or write a short description of the Keypress Event.

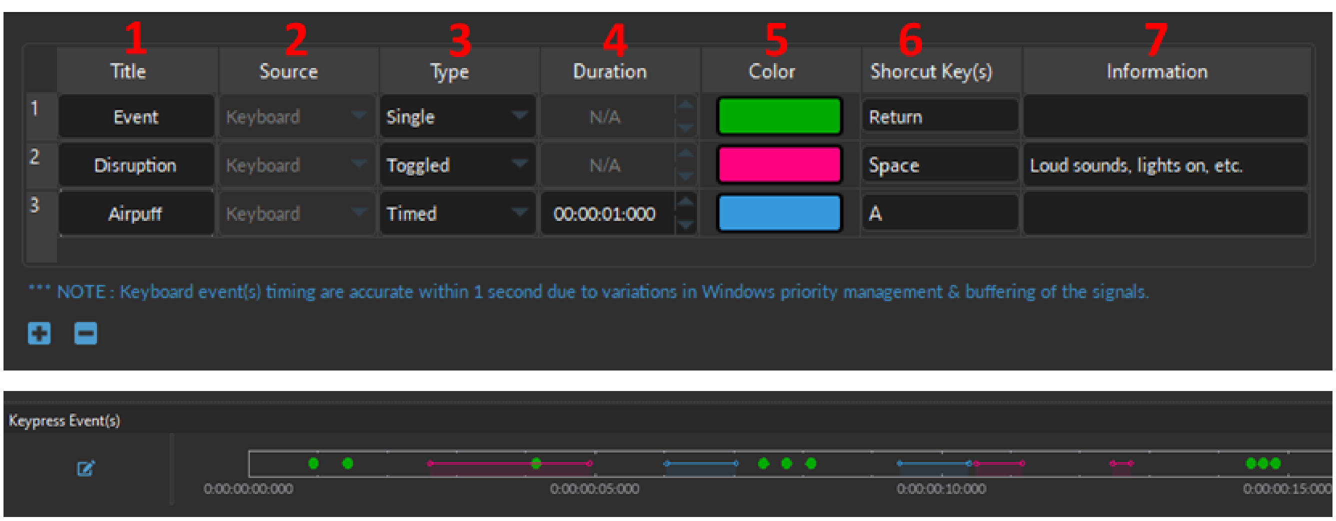


Figure 14.31: *Channel(s) configuration window, KeyPress Event(s)*

14.5 Acquisition View

The **Acquisition View** (Fig. 14.32) 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. 14.32, 1) - Section 14.5.1;
2. The **Camera View** (Fig. 14.32, 2) - Section 14.5.2;
3. The **ROI(s) View** (Fig. 14.32, 3) - Section 14.5.3.

Figure 14.32: *Acquisition View*

14.5.1 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. 14.33, 1), and (2) a **Graph** (Fig. 14.33, 2).

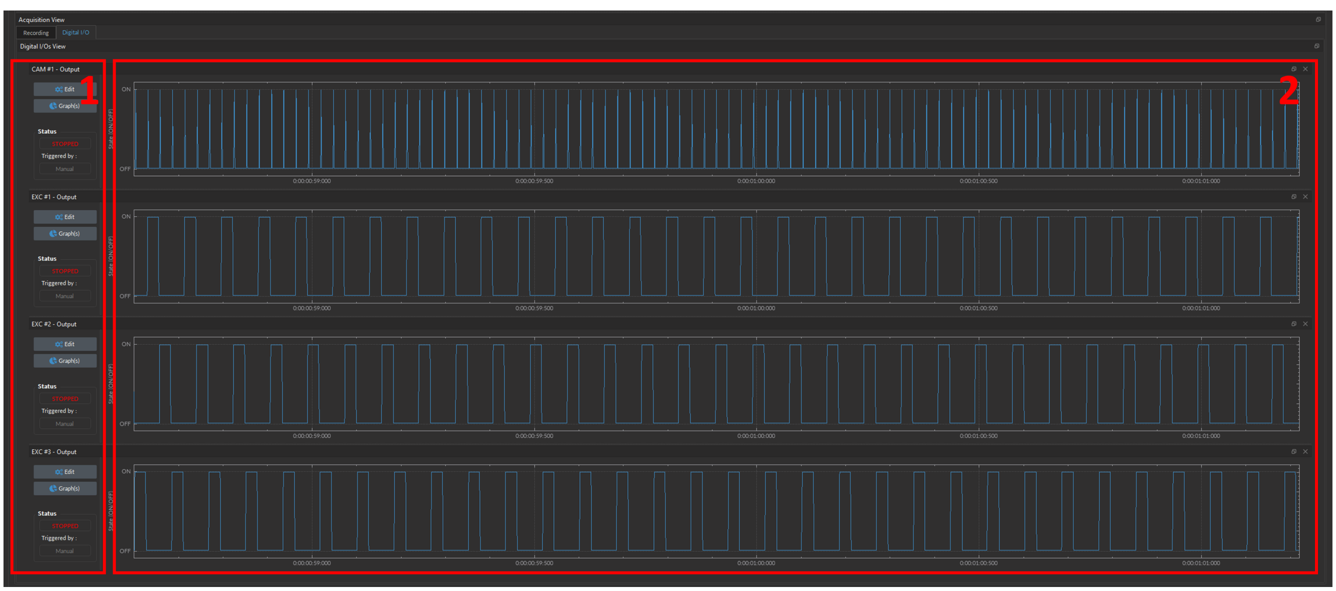


Figure 14.33: *Digital I/O(s) View*

14.5.1.1 DIO Control Box

The **Control box** of each channel allows users to track the status and edit the graph trace or the channel parameters.

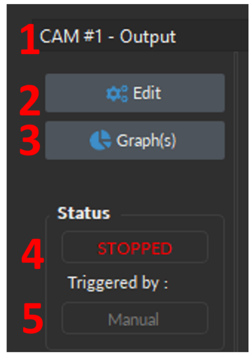


Figure 14.34: *Digital I/O View, Control box*

The following elements are contained within the **Control Box** of every Digitial channel (Fig. 14.34):

1. The **Channel name** (Fig. 14.34, 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. 14.34, 2) opens the **Channel Configuration** window, where the preset digital outputs can be modified (Fig. 14.35). For details on individual parameters, see Section 14.4.4.

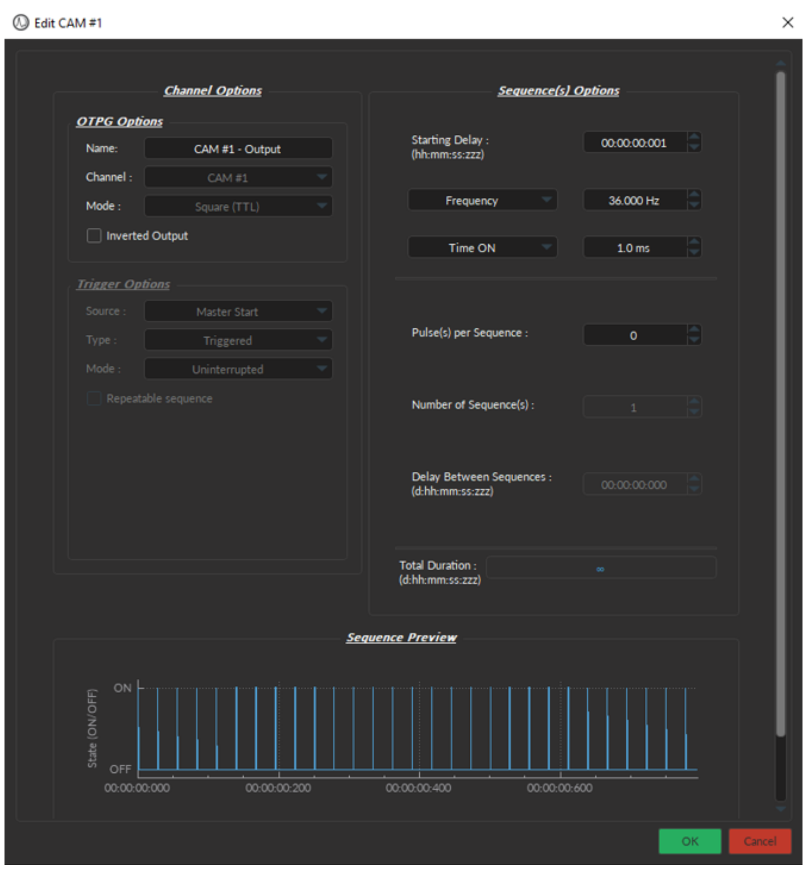


Figure 14.35: *Edit Preset CAM or EXC configuration*

3. The **Graph(s)** (Fig. 14.34, 3) button opens the **Graph Options** window (Fig. 14.36) 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:

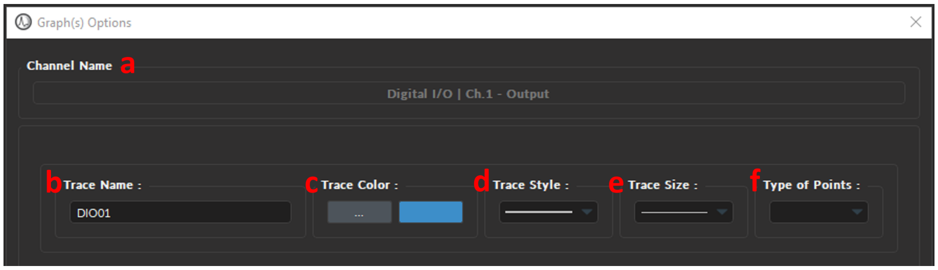


Figure 14.36: *Graph(s) Options Window*

a) The **Channel Name** (Fig. 14.36, 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. 14.36, 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. 14.36, 3) opens the **Color Select** window (Fig. 14.37), 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. 14.36, 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. 14.36, 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. 14.36, 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.

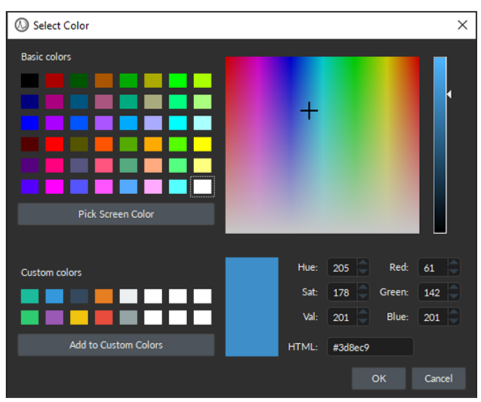


Figure 14.37: *Select Color Window*

4. The **Status** bar (Fig. 14.34, 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 14.3.2.1, no. 3).

5. The **Triggered by:** (Fig. 14.34, 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.

14.5.1.2 DIO Graph

The **Digital I/O** traces are displayed in the **Graph** box (Fig. 14.33, 2). Each channel graph includes the following components:

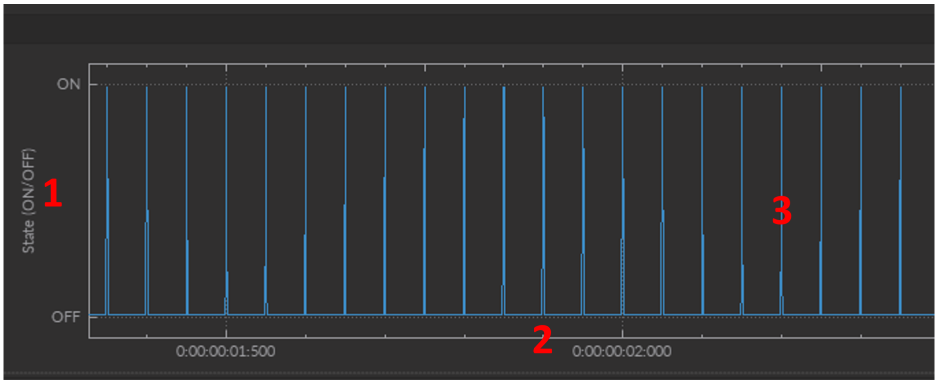


Figure 14.38: *Digital I/O(s) View - Graph*

1. The **Y-axis** (Fig. 14.38, 1) displays the Digital **State** of the channel, which can be either ON (1) or OFF (0).
2. The **X-axis** (Fig. 14.38, 2) displays the time in d:hh:mm:ss:zzz.
3. The **Trace** (Fig. 14.38, 3) can be edited by selecting the **Graph** button in Section 14.5.1.1, no. 3.

While Section 14.5.1.1, 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. 14.39) 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. 14.39) 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 14.39: *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. 14.40). 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.

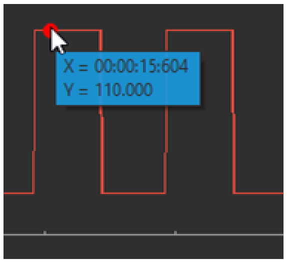


Figure 14.40: *Acquisition View - Instant values*

14.5.2 Camera View

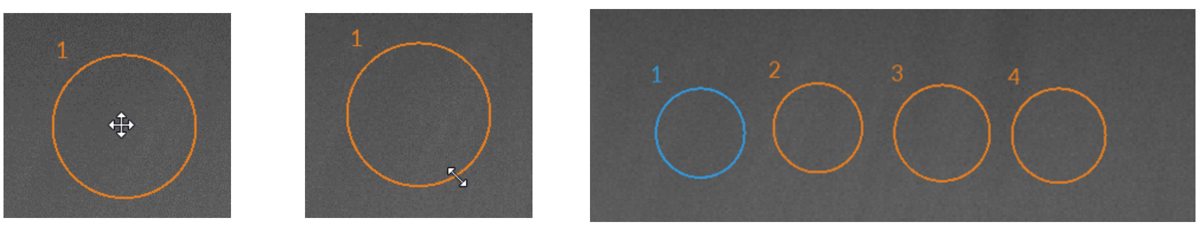
The **Camera View** displays the live video feed from the CMOS **Sensor(s)**. This view contains the following components:

Figure 14.41: *Camera View*

1. The **CAM # EXC #** tab (Fig. 14.41, 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. 14.15, 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. 14.45).
   - **Delete individual ROI** - Select a ROI (as detailed above) and press the **Delete** key on the Keyboard. To delete all ROIs, see Section 14.3.3, no. 1.
   - **Displace ROI** - Select the ROI and hove above the center of the ROI until a *Move* icon (Fig. 14.42a) 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. 14.42b) 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. 14.42c). This selection allows multi-ROI deletion or displacement.



(a) *Move ROI* (b) *Resize ROI* (c) *Multi-select*

Figure 14.42: *Edit ROI(s)*

3. The **Image Size** (Fig. 14.41, 3) displays the image resolution, set in the **Configuration Window** (as in Fig. 14.15, 2).

4. The **Effective Sampling Rate** (Fig. 14.41, 4) the value set in Section 14.4.3, no. 1c.

14.5.3 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**:

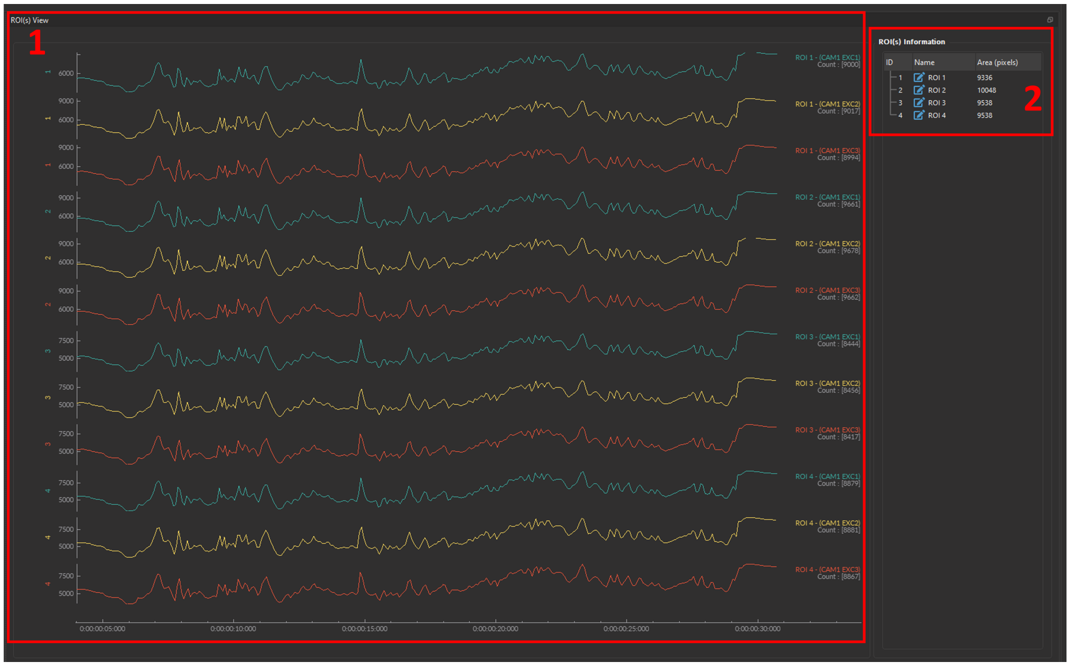


Figure 14.43: *ROI(s) View*

1. The **ROI(s) signal graph** (Fig. 14.43, 1) displays the raw signal trace for each ROI(s).

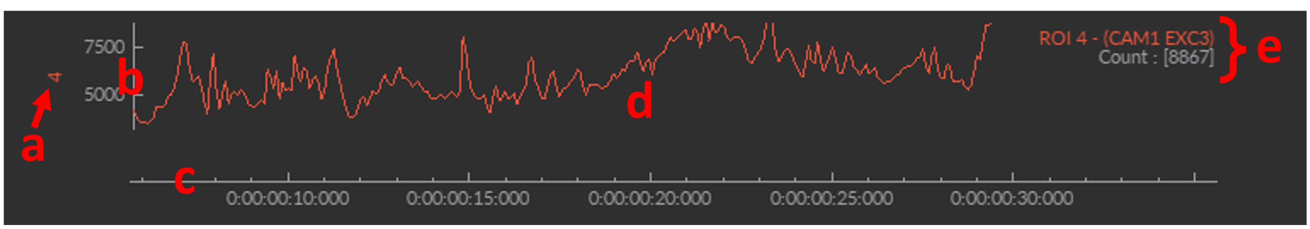


Figure 14.44: *ROI(s) View, Graph*

   a) The **ROI(s) ID** (Fig. 14.44, a) specifies which ROI the signal graph belongs to. The graphs are displayed in order of ROI created.

   b) The **y-axis** (Fig. 14.44, b) represents the mean signal intensity of the ROI, which is unit-less.

   c) The **x-axis** (Fig. 14.44, c) represents the time in d:hh:mm:ss:zzz.

   d) The **Trace** (Fig. 14.44, 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. 14.44, e)

      - *ROI label* - displays the ROI **Name** (specified within the **Name** column of **ROI(s) Information** tab; Fig., 14.45, b), followed by the **Sensor Name** in parenthesis (which can be specified in Fig. 14.15, 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. 14.43, 2) displays a table with ROI basic data, including:

   a) *ID* (Fig. 14.45, a) displays the number associated with ROI.

b) *Name* (Fig. 14.45, b) displays the label associated with the ROI. Double-click on the text-box to rename the ROI.

c) *Area* (Fig. 14.45, c) displays the number of pixels that fill the perimeter of the ROI.

d) *Edit* button (Fig. 14.45, d) will highlight in orange the corresponding ROI in the **Camera View**. To edit or delete the selected ROI, see section 14.5.2, no. 2.

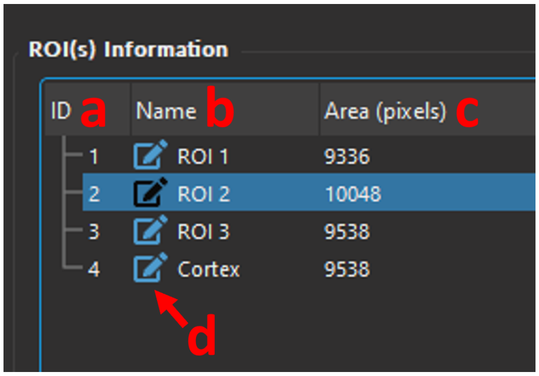


Figure 14.45: *ROI(s) View, ROI(s) Information tab*

15

Behavior & Bundle Photometry Console (BBC300)

The Behavior & Bundle Photometry Console module controls the *Bundle-imaging Fiber Photometry (BFMC)* and the new *Bundle-imaging Fiber with Targeted Optogenetics (BFTO)* systems. 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.

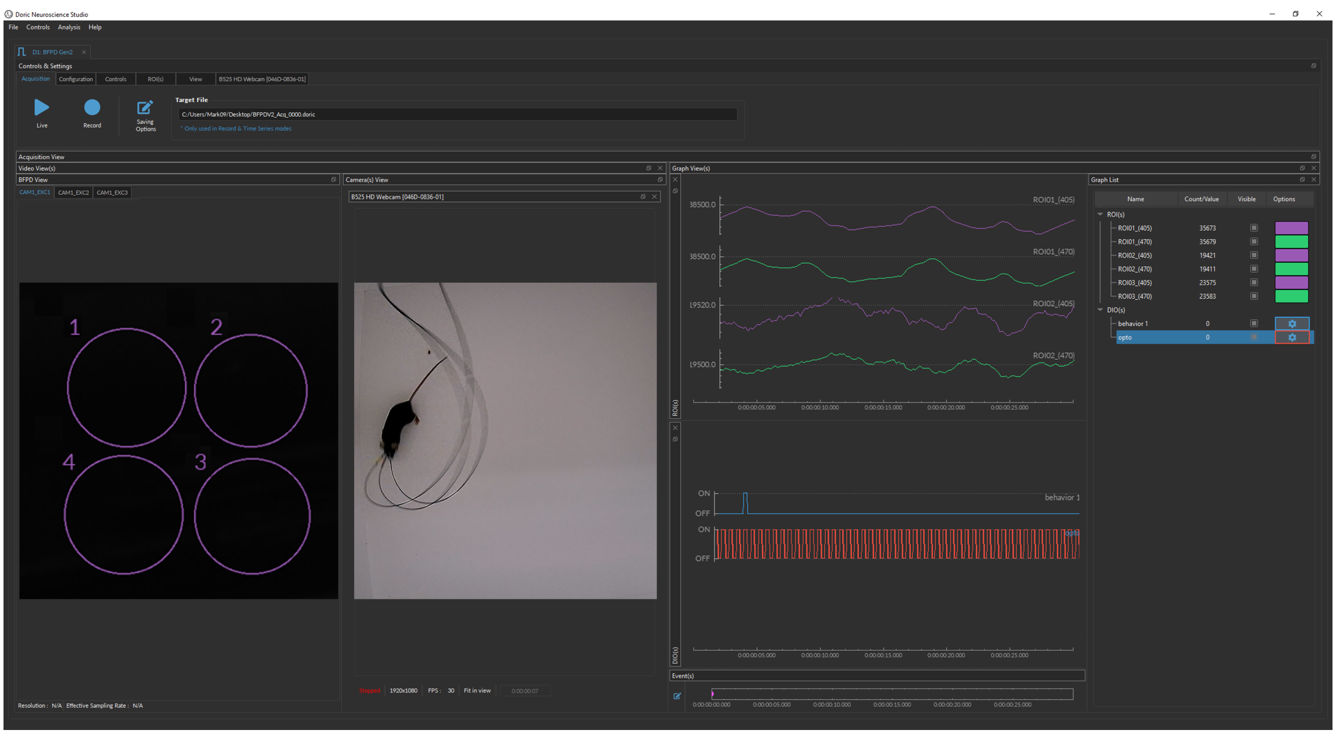


Figure 15.1: *BBC300 interface*

15.1 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 with the USB cable (as in Fig. 15.2).

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. 15.2).

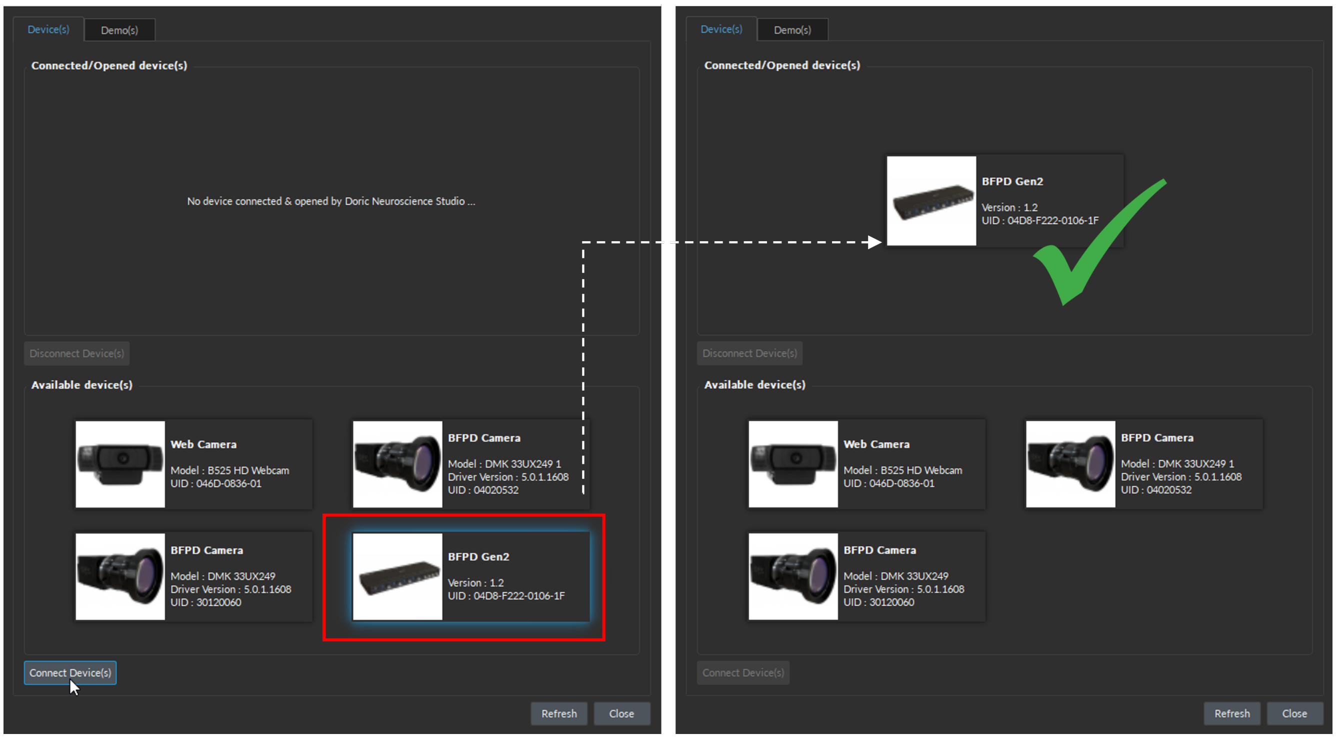


Figure 15.2: *Double click on the device of choice to connect it to DNS*

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. 15.3) or use the hot key: *Ctrl+N*.

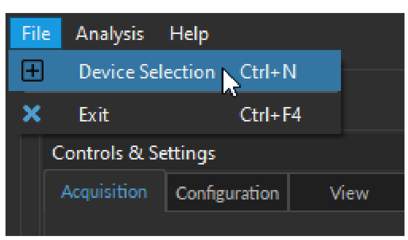


Figure 15.3: *Open Device Selection Window*

15.2 Overview

The **BBC300** interface is split into two sections (Fig. 15.4):

1. **Control and settings tabs** (Section 15.3) are used to manage different parameters and settings of the software (Acquisition, Configuration, ROI, and View).

2. **Acquisition view** (Section 15.5) displays the input and output traces for visualization.

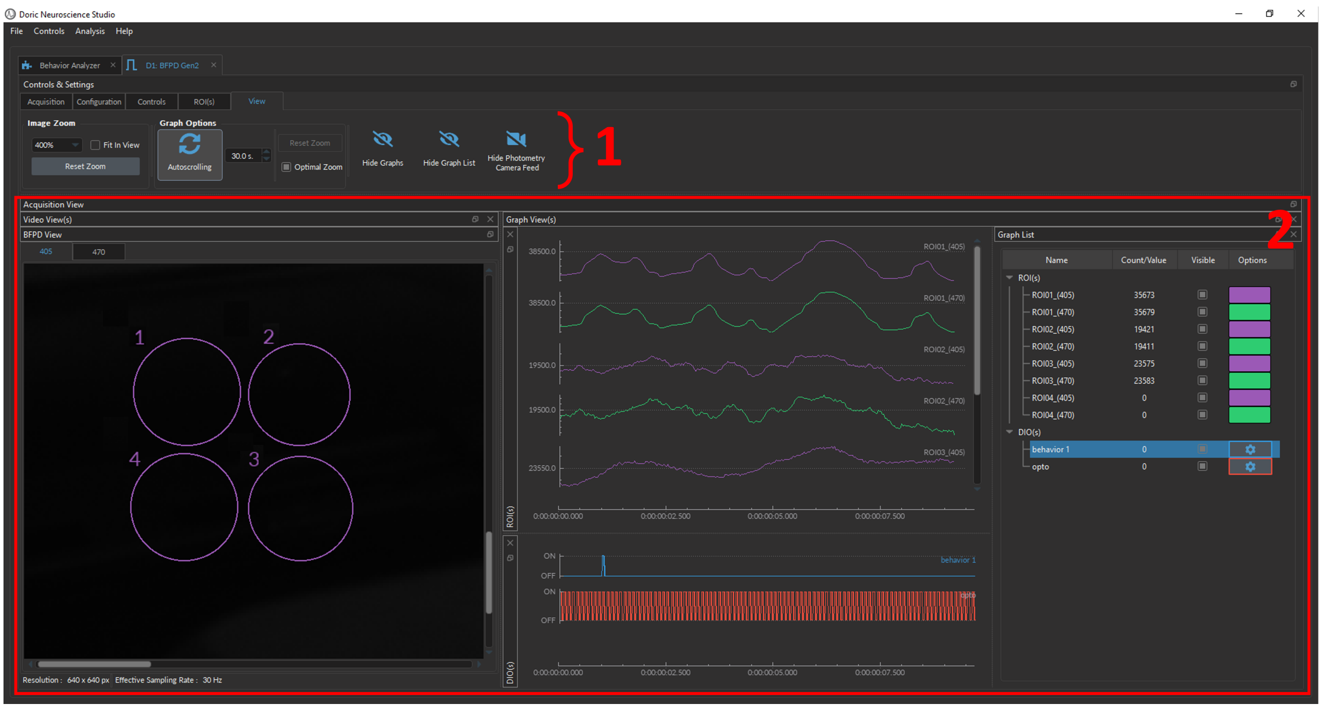


Figure 15.4: *BBC300 interface divisions*

15.3 Control and Settings tabs

The **Control and settings tabs** (Fig. 15.5) are used to manage the different parts of the software and are split into four separate tabs, each of which is detailed in the following sections:

1. *Acquisition* tab - Section 15.3.1;

2. *Configuration* tab - Section 15.3.2;

3. *Controls* tab - Section 15.3.3

4. *ROI(s)* tab - Section 15.3.4;

5. *View* tab - Section 15.3.5.

Figure 15.5: *Control & Settings*

15.3.1 Acquisition Tab

The **Acquisition** tab is used to start a live/recording session and set the Saving Options. The **Live** and **Record** buttons will not function if channels have yet to be set up. See section 15.4 to configure channels for recording.

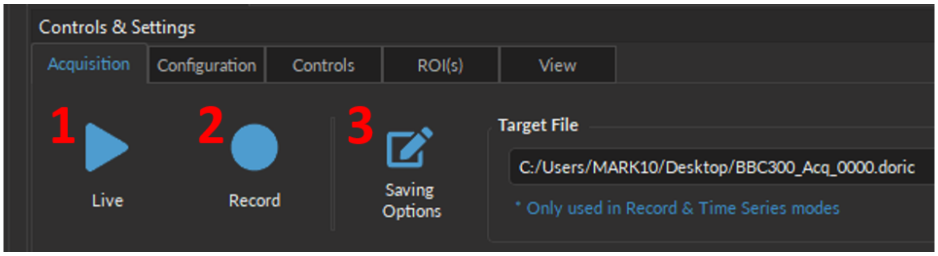


Figure 15.6: *Acquisition Tab*

1. The **Live** button (Fig. 15.6, 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. 15.6, 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. 15.6, 3) button opens the **Saving Options** window (Fig. 15.7). See section 15.3.1.1 for more details.
4. The **Target File** (Fig. 15.6, 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.

15.3.1.1 Saving Options

The **Saving Options** 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. 15.6, 3).

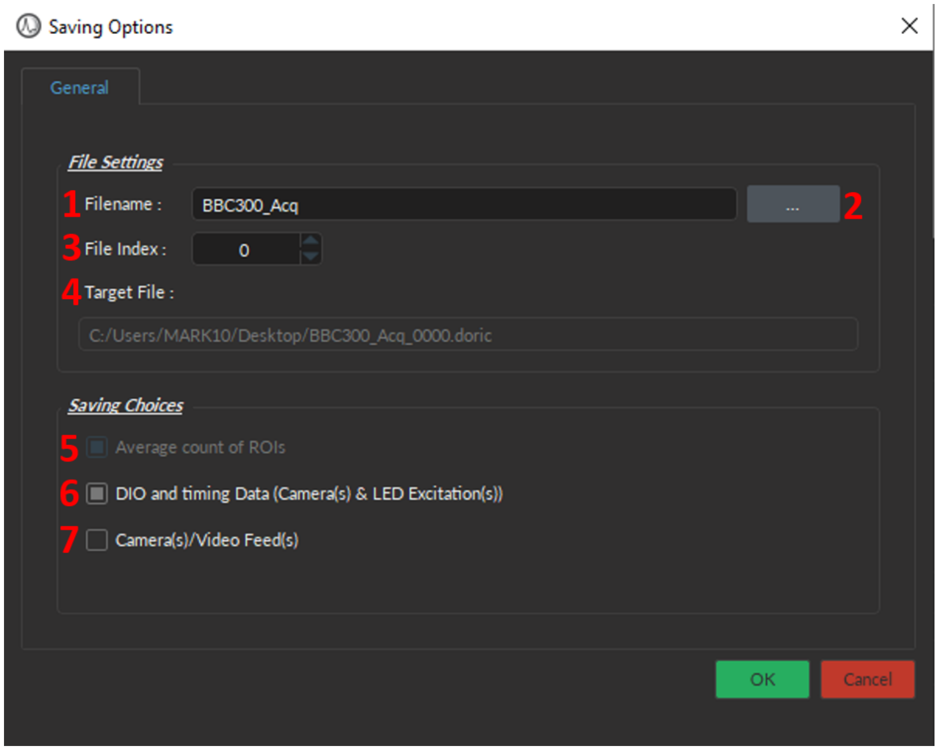


Figure 15.7: *Saving Options Window*

1. The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 15.7, 1).

2. The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 15.7, 2). Note that the file will be saved as a **.doric** file, 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.

3. The **File Index** (Fig. 15.7, 3) 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.

4. The **Target File** (Fig. 15.7, 4) 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:

5. The **Average count of ROI(s)** checkbox (Fig. 15.7, 5) saves the mean pixels intensity of each defined region of interest. This setting cannot be disabled.

6. The **DIO and timing Data (Camera(s) & LED Excitation(s))** checkbox (Fig. 15.7, 6), if enabled, will save the digital TTL outputs of the Camera(s) & LED Excitation(s). This value is by default enabled.

7. The **Camera(s) / Video Feed(s)** text-box (Fig. 15.7, 7), if enabled, will save the raw image stacks collected by the CMOS sensor(s). Note that selecting this option will generate large files.

15.3.2 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.

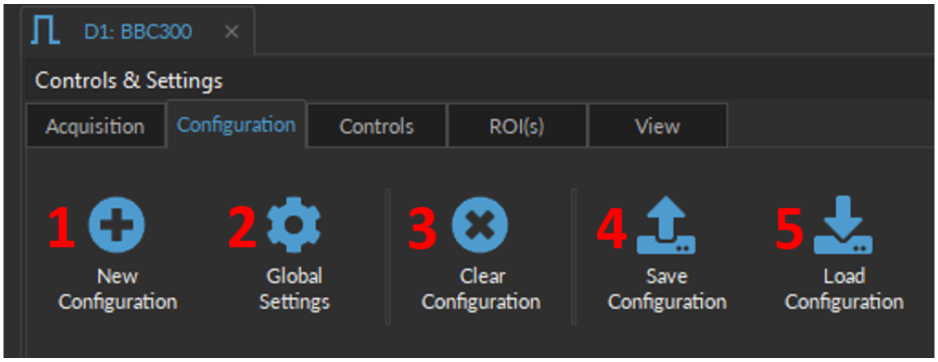


Figure 15.8: *Configuration Tab*

1. The **New Configuration** button (Fig. 15.8, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 15.4. Table 15.1 describes different types of channels available, their use cases, and their individual sections.
2. The **Global Settings** (Fig. 15.8, 2) opens the **Global Options** window in Fig. 15.9, where user can set the acquisition sampling rate and specify the master trigger options. See Sections 15.3.2.1 for more details.
3. The **Clear configuration** button (Fig. 15.8, 3) resets the acquisition view and all other parameters set. Any configurations not saved will be lost.
4. The **Save configuration** button (Fig. 15.8, 4) allows a BBC300 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. 15.8, 5) imports a pre-configured **.doric** file into the module.

15.3.2.1 Global Settings

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

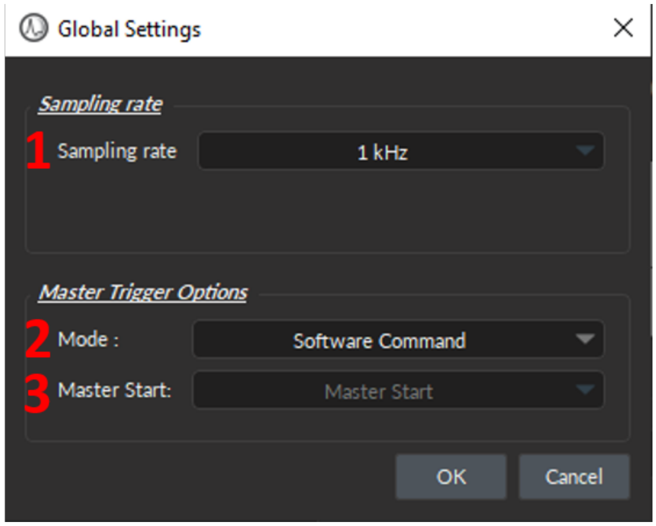


Figure 15.9: *Global Options Window*

1. The **DIO sampling rate** (Fig. 15.9, 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 and 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 **BFPD View** (Fig. 15.40, no. 4).

2. The **Mode** (Fig. 15.9, 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. 15.6, 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. 15.10a). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig. 15.10b) pops up. See section 15.3.2.2 for more details.

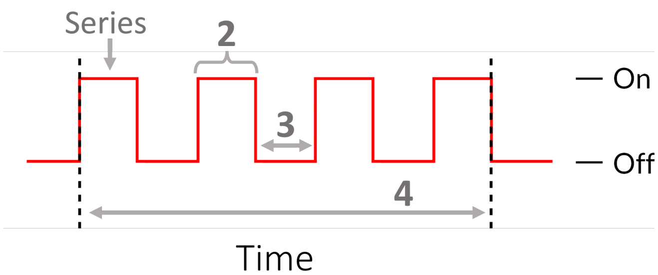
3. The **Master Start** (Fig. 15.9, 3) defines the source that will automatically start the recording. This source can either be:

   - *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.***

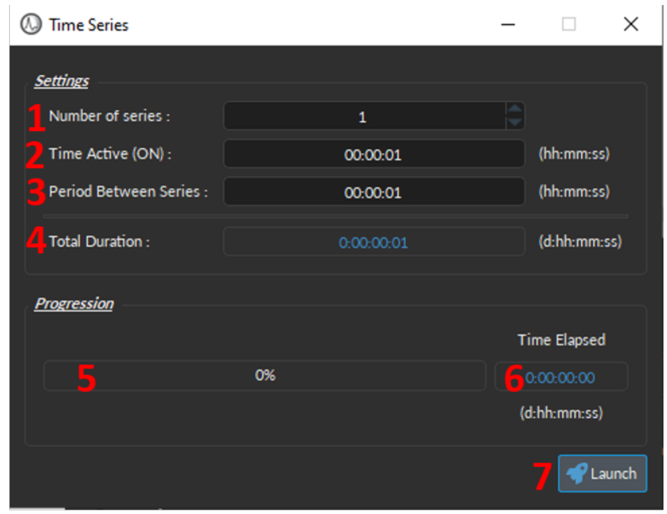
15.3.2.2 Time Series

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. 15.10b) can be opened by clicking on the **Record** button (Fig. 15.6, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 15.9, 2). Every **Time series** sequence is automatically saved to the same *.doric* file defined in **Saving Options** (Section 15.3.1.1).



(a) *Time Series Acquisition Timing Diagram*



(b) *Time Series Window*

Figure 15.10: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 15.10b) sets the following parameters:

1. The **Number of series** (Fig. 15.10b, 1) defines the amount of times the series is repeated.
2. The **Time Active (ON)** (Fig. 15.10b, 2) defines the duration of the series.
3. The **Period Between Series** (Fig. 15.10b, 3) defines the amount of time between each series, if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 15.10b, 4) displays the total amount of time that the timeseries recording will take place.
5. The **Progression bar** (Fig. 15.10b, 5) indicates the progression of the timeseries (in %).
6. The **Time Elapsed** counter (Fig. 15.10b, 6) indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** button (Fig. 15.10b, 7) start the series. While the series is active, it is impossible to add channels or change the configuration, though **View** settings can be modified.

15.3.3 Controls Tab

The **Controls** tab contains the parameters related to bundle photometry hardware (such as the excitations LEDs and the CMOS camera(s)) and directly affects the quality of the collected data. Parameters that change the interface visualization (but not the actual data) can be found in the **ROI(s)** tab and **View** tab (Sections 15.3.4 & 15.3.5).

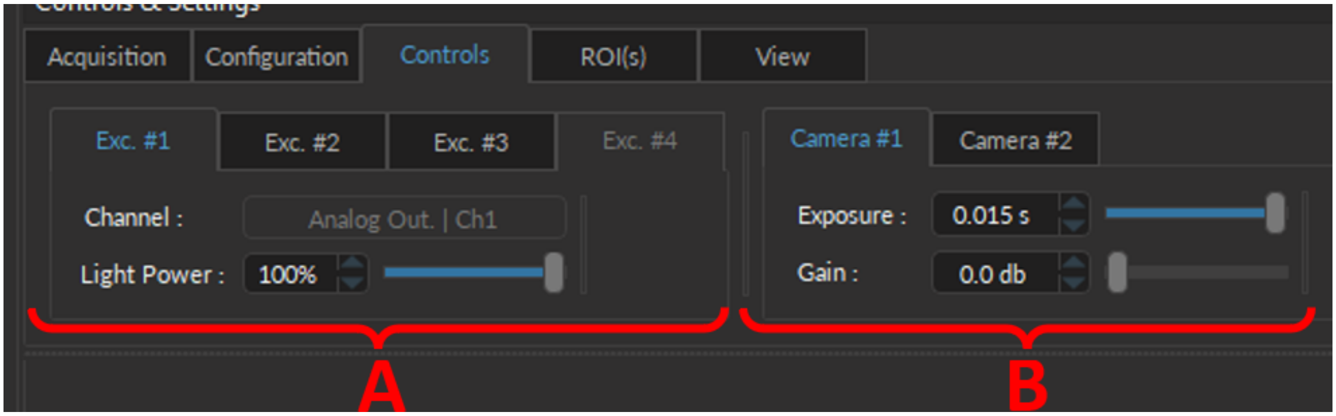


Figure 15.11: *Controls Tab*

The **Controls** section is split into two subsections (Fig. 15.11):

1. The **EXC # tabs** (Fig. 15.11, A) controls the strength of the LED excitation(s) required for fiber photometry.

   - The **Channel** specifies which of the *BBC300* **LED Output** ports are associated with the excitation LED (Fig. 15.12, Exc).

Figure 15.12: *Exc. & Camera Ports #*

   NOTE: When using the *BBC300* for a system with *BFMC* or *Rotary BFMC*, the **LED Output** ports will be connected to an *LED Driver*, while if using it with a *BFTO system*, the **LED Output** ports will be connected directly to the *IE, E1, and E2* ports of the *BFTO* (no *LED Driver* is required).

   - The **Light Power** specifies the percentage of output power of each excitation *LED*, where 100% is the maximum current of the *LED*. Note that the mapping between **Light Power %** and the actual measured power (in microwatts) will differ between *LEDs* and should be determined independently using a power meter.

2. The **Camera # tab(s)** (Fig. 15.11, B) controls the acquisition parameters of each *BFMC* or *BFTO* camera.

   - The **Exposure** adjusts the length of time that the CMOS sensor collects light from the sample. There are trade-offs between exposure time, image brightness, and phototoxicity. However, prior to the experiment, we recommend maximizing the camera **Exposure**. If the signal is too strong and/or the camera is saturated, reduce the excitation **Light power** before reducing the **Exposure** to minimize fluorophore bleaching.
   - The **Gain** sets the electronic amplification of the signal after collection, in logarithmic decibels (dB). Note that increasing the gain will simultaneously increase both the signal and noise (including electronic noise), reducing the signal-to-noise ratio. We recommend setting the gain at 0 dB unless signals are very weak.

- The **X and Y offsets** adjusts the horizontal and vertical position of *Camera#2* relative to the position of *Camera #1*. This adjustment allows the overlap of the image of both cameras to superimpose the **ROI(s)**. As such, the **X and Y offset** is only available for *Camera #2*.

15.3.4 ROI(s) Tab

The **ROI(s) Tab** (Fig. 15.13) contains parameters to save, load, clear, or edit bundle fiber photometry ROI(s).

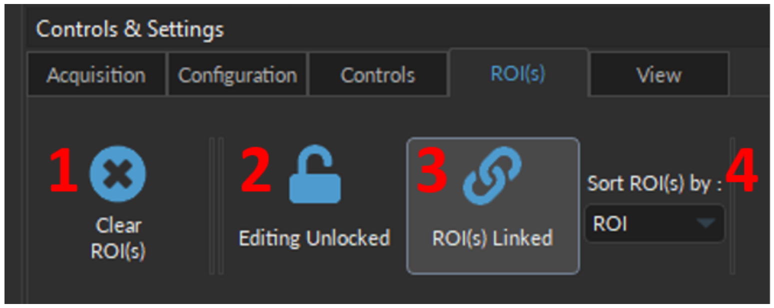
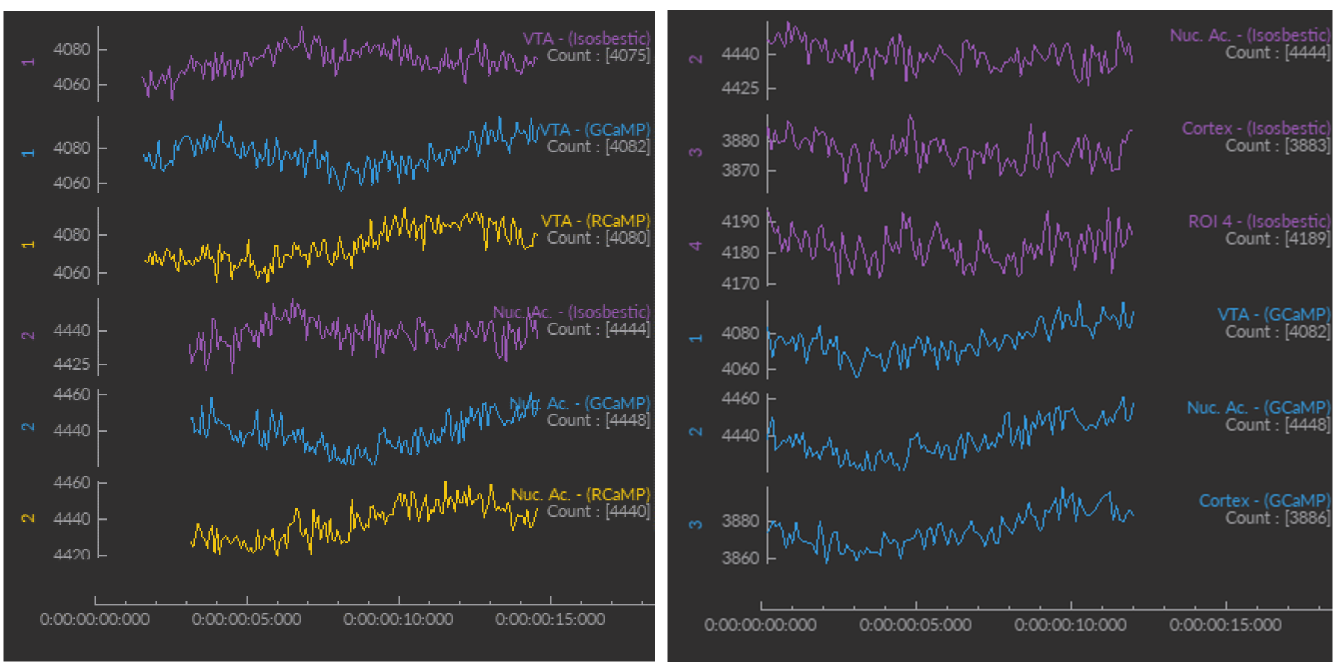


Figure 15.13: *ROI(s) Tab*

The **ROI(s)** parameters are as follows:

1. The **Clear ROI(s)** button (Fig. 15.13, 1) deletes all drawn regions of interest (ROI) within the **BFPD View**. Note that unless the ROI(s) were previously saved, these ROI(s) cannot be recuperated.
2. The **Editing Unlocked** button (Fig. 15.13, 2), when enabled, prevents new ROI(s) from being drawn, but does not prevent moving or reshaping a selected ROI (see section 15.5.1).
3. The **ROI(s) Linked** button (Fig. 15.13, 3) automatically redraws identical ROI(s) in the other CAM# excitation tabs (Fig. 15.40, 1) within the **BFPD View**. **Unlinking** previously linked ROI(s) deletes the ROI(s) from the **BFPD 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.
4. The **Sort ROI(s) by:** drop-down list allows users to organize the order of the traces within the **Graph View** by either their *ROI* (Fig. 15.14a) or by their *Excitation* type (Fig. 15.14b).



(a) *Sort by ROI*

(b) *Sort by EXC*

Figure 15.14: *Sort Graph View options*

TIP 1: We suggest drawing one ROI for each optical fiber, plus one outside to monitor the background.

15.3.5 View Tab

The **View Tab** (Fig. 15.15) is used to modify the presentation of graphs in the **Acquisition view**.

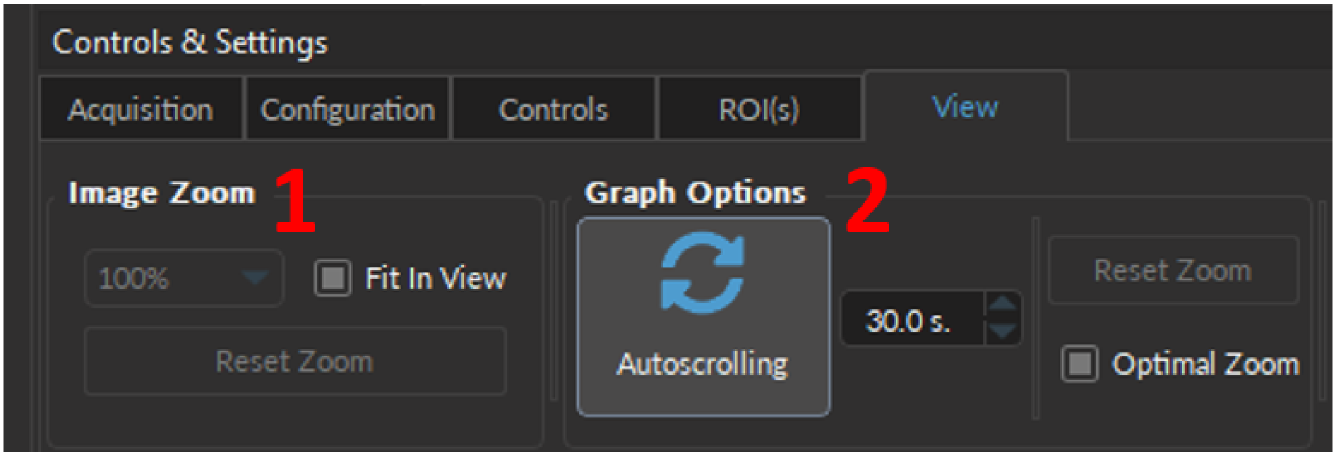


Figure 15.15: *View Tab*

The **View** parameters are as follows:

1. The **Image Zoom** (Fig. 15.15, 1) 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 **BFPD View** window.
   - The **Rest Zoom** button returns the zoom factor to 100%.
2. The **Graph Options** (Fig. 15.15, 2) includes the following parameters which modify the **Graph View**:
   - The **Autoscrolling** button, when selected, automatically sets the graphs to scroll as new data appears.
   - The **Autoscrolling range** sets the graph zoom to the value of choice, specified in the text-box.
   - The **Reset Zoom** button readjusts the graph zoom to the default value.
   - 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.

15.4 BBC300 Configurations

To create a new channel select the **New Configuration** button, which can be found under the **Configuration** tab (Fig. 15.8, 1). This opens the **BBC300 Configuration** window (Fig. 15.16), where users can select the type of channel.

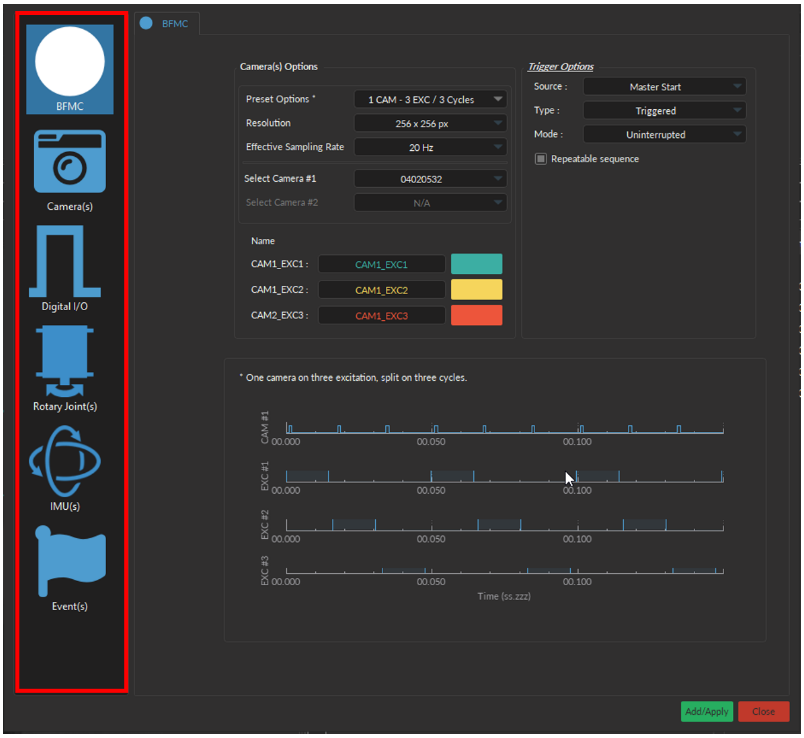
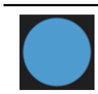


Figure 15.16: *Channel(s) configuration window, select channel type*

Table 15.1 details the types of inputs and output that are compatible with the BBC300, their use cases and gives quick access to their sections.

Table 15.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	BFMC	To collect the fluorescence signal of *BFMC* from branching or bundle-fibers (multiple sites simultaneously).	15.4.1
	Digital I/O	For input and output of TTL signals	15.4.2
	Camera(s)	To collect video for behaviour experiments	15.4.3
	Rotary Joint	Records the device's rotation to control for motor noise or compute animal motion.	15.4.4
	IMU(s)	Inertial Measurement Unit, measures changes in acceleration, head movements and other parameters linked to subject displacement	Coming Soon
	Event(s)	To manually flag events time-locked to the current recording using customized keys	15.4.5

15.4.1 BFMC Channel

The **BFMC** 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.

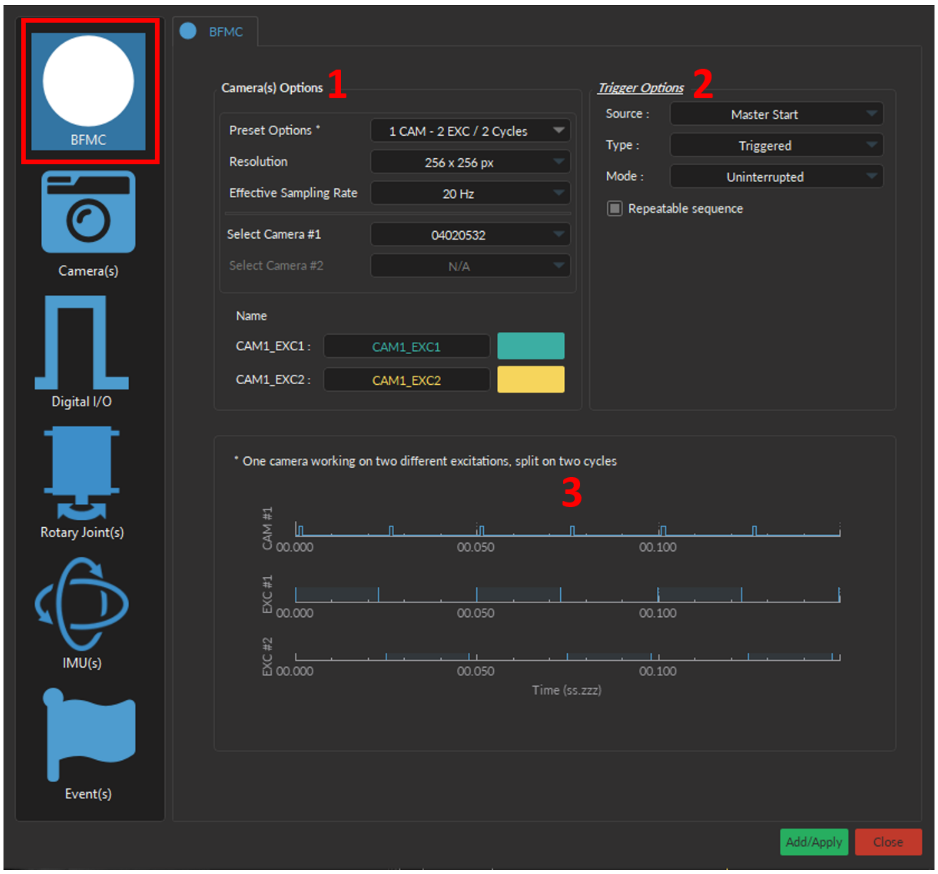


Figure 15.17: *Channel(s) configuration window, BFMC*

The **BFMC** channel is divided into three sections (Fig. 15.17):

1. The **Camera(s) Options** (Fig. 15.18) defines which preset option to use and allows users to select the proper camera and camera parameters for the recording, such as:

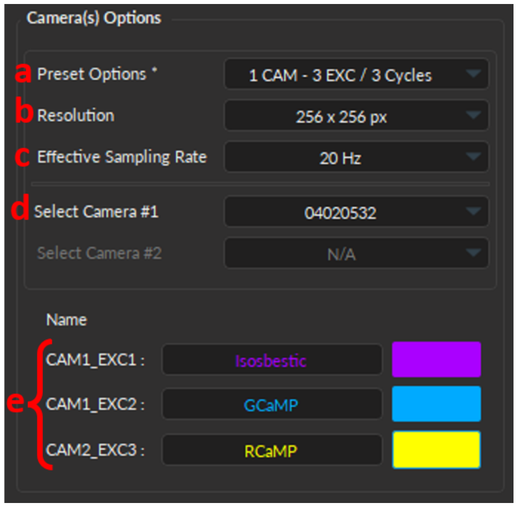


Figure 15.18: *BFMC Channel, Camera Options*

a) The **Preset Options** drop-down (Fig. 15.18, a) contains six pre-configured options which are listed in Table 15.2, along with their use cases.

b) The **Resolution** drop-down (Fig. 15.18, 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. 15.18, 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 EXC:* 3 Hz - 60 Hz
- *2 EXC:* 3 Hz - 30 Hz
- *3 EXC:* 3 Hz - 12 Hz

d) The **Select Camera #** drop-down (Fig. 15.18, 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. 15.18, 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 **Trigger Options** (Fig. 15.19)

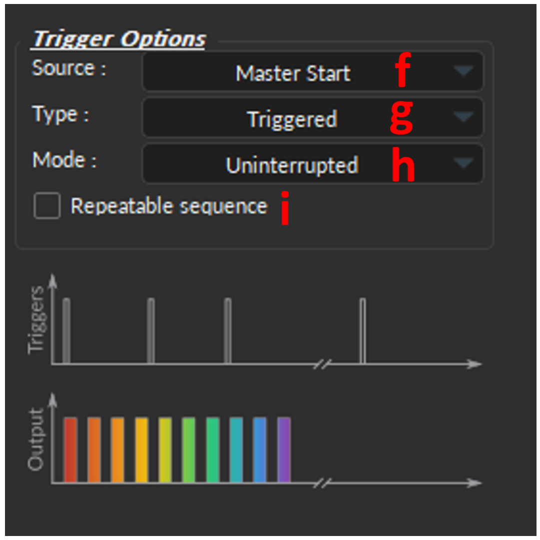
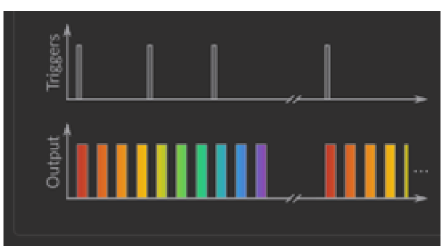


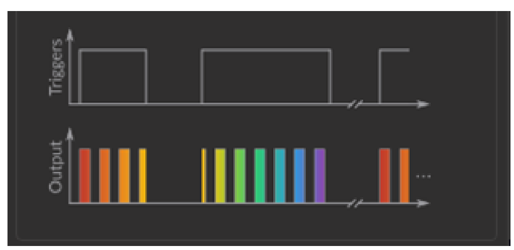
Figure 15.19: *BFMC, Trigger Options*

f) The **Source** trigger option (Fig. 15.19, f) 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.

g) The **Type** (Fig. 15.19, g) defines how the trigger activates a sequence. This includes input sequences, which can be triggered/gated by an outside source.
   - In **Triggered** mode (Fig. 15.20a), 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.



(a) *Trigger*



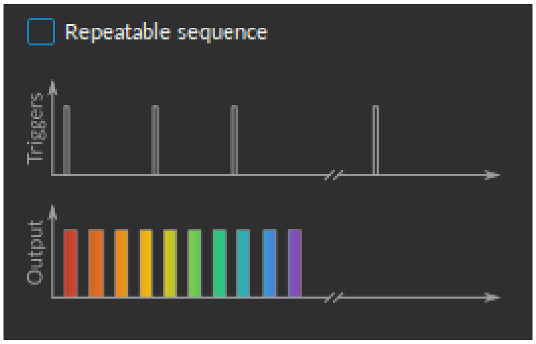
(b) *Gate*

Figure 15.20: *Trigger Options Modes*

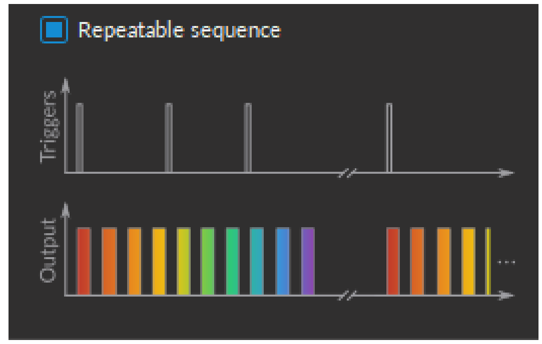
h) The **Mode** (Fig. 15.19, h) defines how the sequence will run if a second TTL pulse is received before the sequence ends. This includes input sequences, which can be triggered/gated by an outside source. Four options are available:
   - The **Uninterrupted** mode - Ignores the additional TTL input 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.

i) The **Repeatable sequence** checkbox (Fig. 15.19, i), when enabled, will run the sequence when additional TTL pulses are received (Fig. 15.21).



(a) *Disabled*



(b) *Enabled*

Figure 15.21: *Repeatable sequence*

3. The **Sequence Preview** (Fig. 15.22) displays the TTL output pulses for the **CAM** and **EXC** that will be used during the recording.

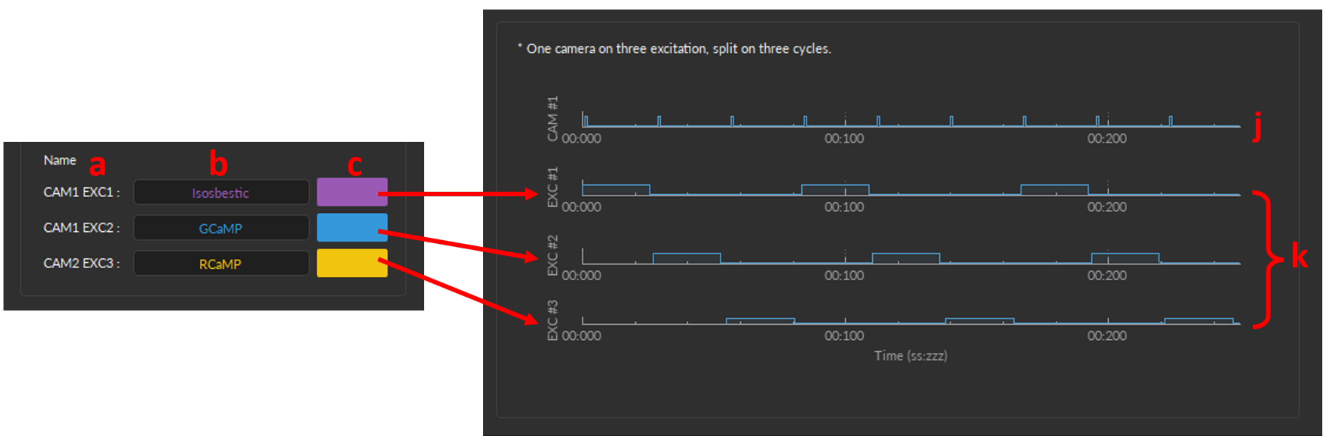


Figure 15.22: *Recording Channel - Preview digital Camera and Excitations outputs*

j) 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.

k) The **EXC#** displays a preview of the digital output signal that drives the specific LED excitation.

Table 15.2: *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***.**

[1]Series of events that occur during one measurement.

15.4.2 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. 15.23): (1) the **Channel Options** (Section 15.4.2.1), (2) the **Sequence(s) Options** & (3) **Sequence Preview** (both treated in Section 15.4.2.2).

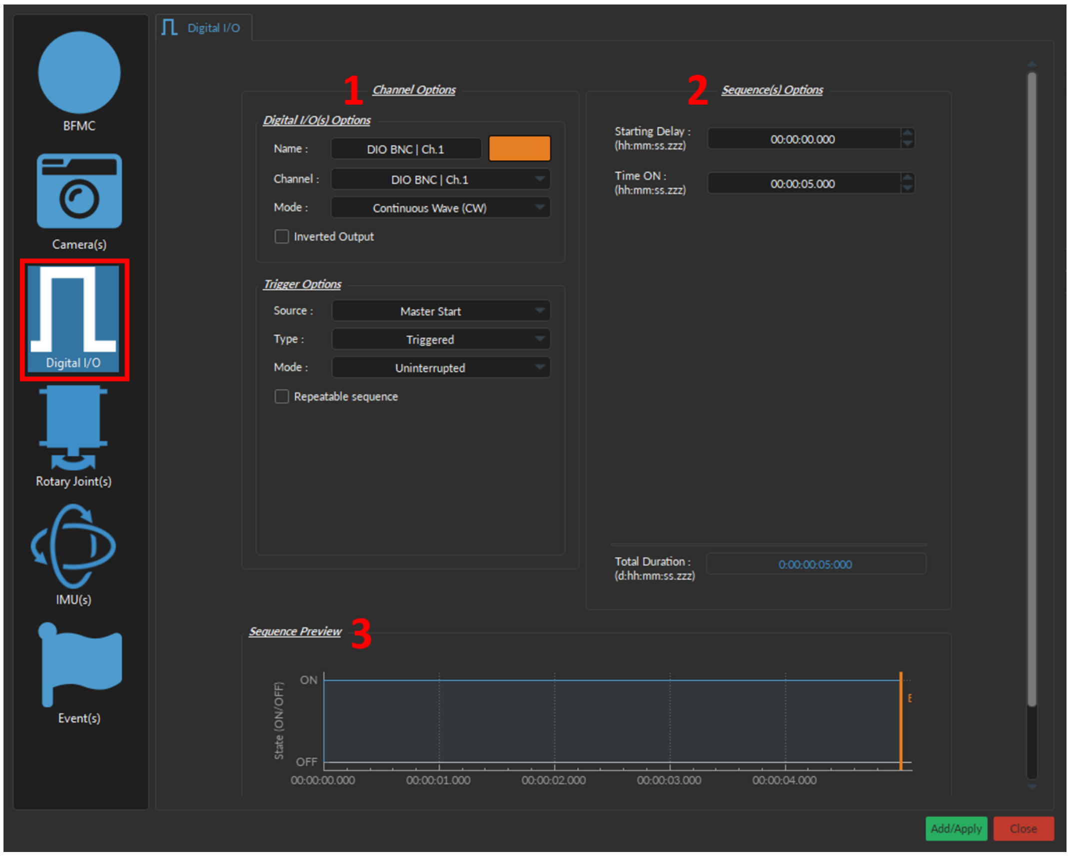


Figure 15.23: *Channel(s) configuration window, Digital I/O*

15.4.2.1 Channel Options

The **Channel Options** defines the channel, source and mode of the digital signal, through **Digital I/O Options** and **Trigger Options**.

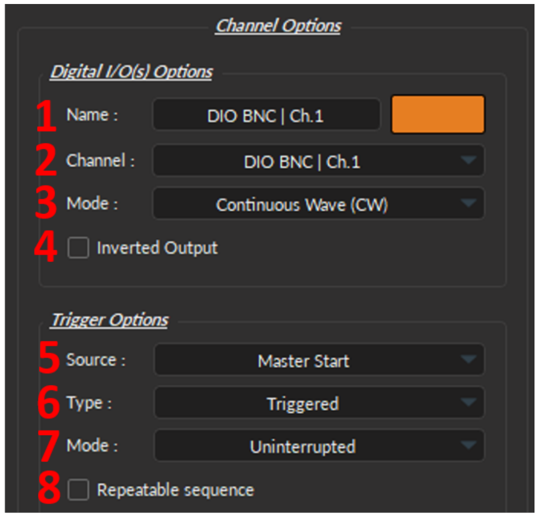


Figure 15.24: *Digital I/O, Channel Options*

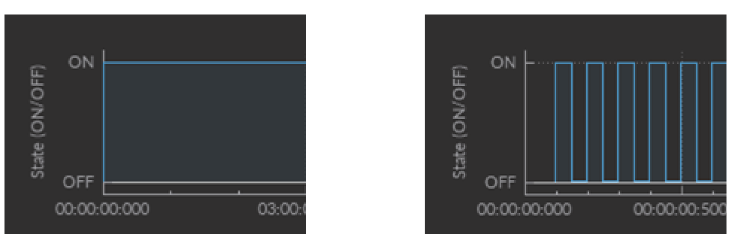
Digital I/O Options:

1. The **Channel Name** textbox (Fig. 15.24, 1) is used to give the new channel a more intuitive / experiment-relevant label. The **Channel Name** is used in the **Graph List** to identify the channel. And the **Color Selector** specifies the trace color used for the channel in the **Graph View**.

2. The **Channel** (Fig. 15.24, 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 BBC300 corresponds to the same number of the digital channel within the software.

3. The **Mode** (Fig. 15.24, 3) identifies the type of signal sent (for output channels) or the way the signal is measured (for input channels). Three modes are available:

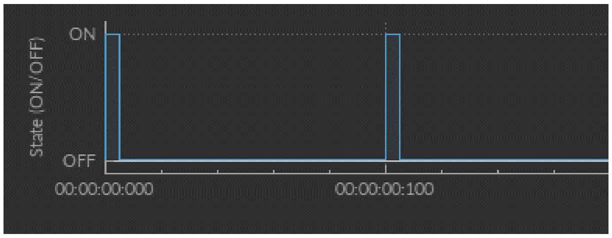
   - The **Continuous wave (CW)** Mode (Fig. 15.25a);
   - The **Square (TTL)** Mode (Fig. 15.25b);
   - The **Input** mode receives a signal that is either 0 (**Off**) or 1 (**On**). The channel can then be used as a trigger source for all the other channels of the BBC300 (See Section 15.3.2.1). No **Sequence Options** or **Sequence Previews** are available for this mode.



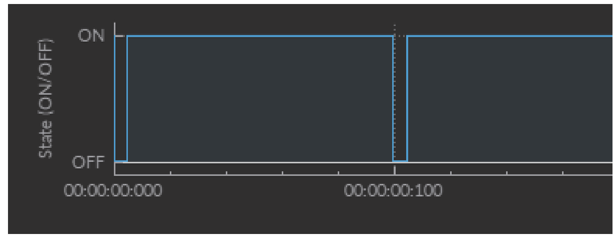
(a) *Continuous* (b) *Square*

Figure 15.25: *Channel Options - Output Modes*

4. The **Inverted Output** checkbox (Fig. 15.24, 4), when enabled, will convert every 0 to 1 and 1 to 0, such as in Fig. 15.26.



(a) *Disabled*

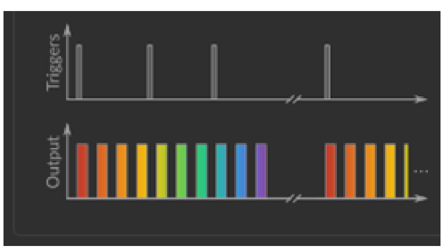


(b) *Enabled*

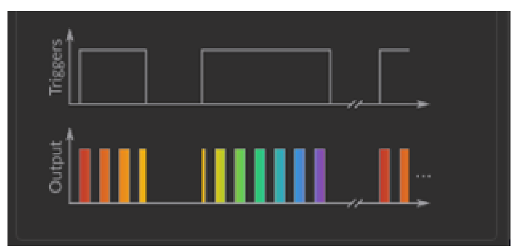
Figure 15.26: *Inverted Output*

Trigger Options:

5. The **Source** trigger option (Fig. 15.24, 5) 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.
6. The **Type** (Fig. 15.24, 6) defines how the trigger activates a sequence. This includes input sequences, which can be triggered/gated by an outside source.
   - In **Triggered** mode (Fig. 15.27a), the sequence is started manually 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. 15.27b), 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*



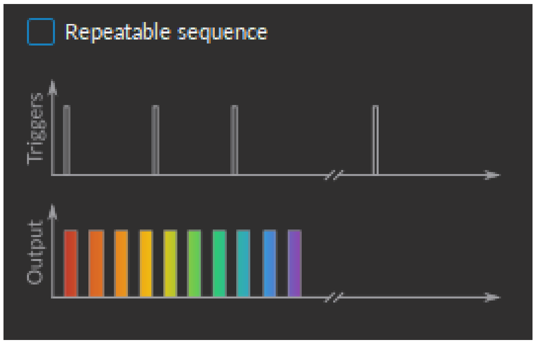
(b) *Gate*

Figure 15.27: *Trigger Options Modes*

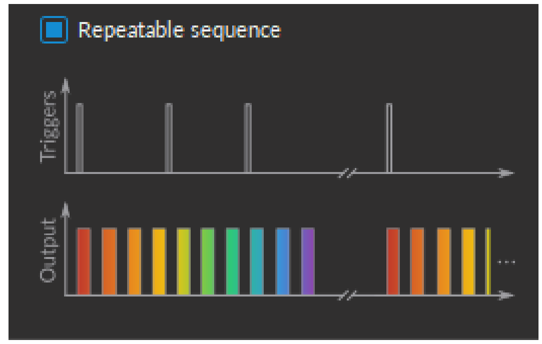
7. The **Mode** (Fig. 15.24, 7) defines how the sequence will run if a second TTL pulse is received before the sequence ends. This includes input sequences, which can be triggered/gated by an outside source. Four options are available:
   - The **Uninterrupted** mode - Ignores the additional TTL input 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.

8. The **Repeatable sequence** checkbox (Fig. 15.24, 8), when enabled, will run the sequence when additional TTL pulses are received (Fig. 15.28).



(a) *Disabled*



(b) *Enabled*

Figure 15.28: *Repeatable sequence*

15.4.2.2 Sequence Options & Preview

The **Sequence options** section (Fig. 15.29a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 15.29b) 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**.

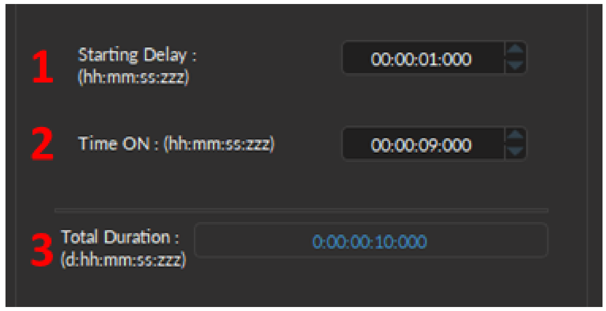
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 15.24), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 15.25a) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

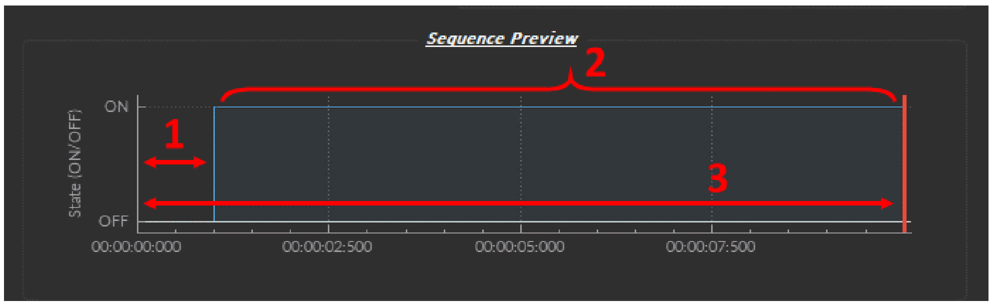
  1. The **Starting Delay** (Fig. 15.29, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Time ON** (Fig. 15.29, 2) defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse sequence is stopped manually.
  3. The **Total Duration** (Fig. 15.29, 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**.

- The **Square** channel mode (Fig. 15.25b) allows the creation of a square TTL pulse sequence. The elements included in the Sequence Option box are as follows (Fig. 15.30, 1-3):

  1. The **Starting Delay** (Fig. 15.30, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
  2. The **Frequency** (Fig. 15.30, 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. 15.30, 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. 15.30, 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. 15.30, 5) sets the number of times that the sequence will be repeated.



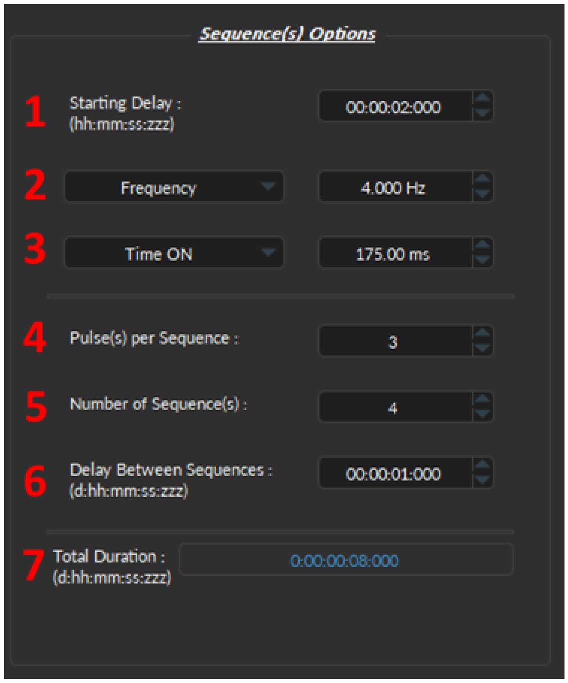
(a) *Sequence Options*



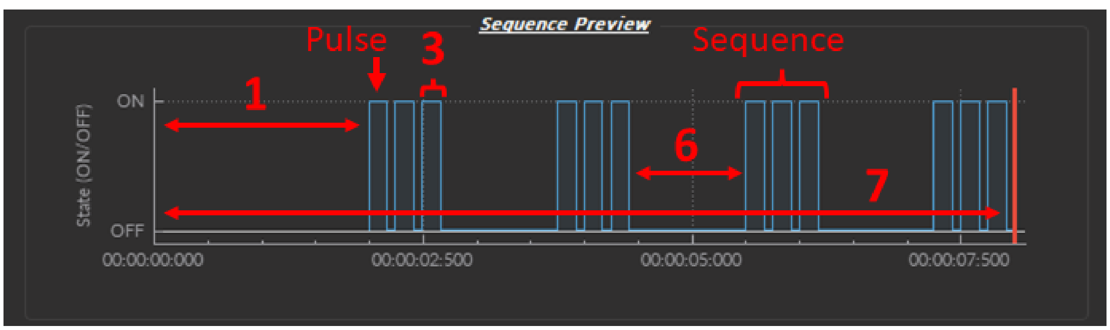
(b) *Sequence Preview*

Figure 15.29: *Channel(s) configuration window, Digital I/O - CW Mode*

6. The **Delay between sequences** (Fig. 15.30, 6) sets the amount of time separating any two sequences (excluding the **Starting Delay**).
7. The **Total Duration** (Fig. 15.30, 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 15.30: *Channel(s) configuration window, Digital I/O - Square Mode*

15.4.3 Camera(s) Channel

It is natural to pair Doric neural recordings with behaviors. Many behaviors, especially freely moving behaviors, require camera inputs for their measurement.

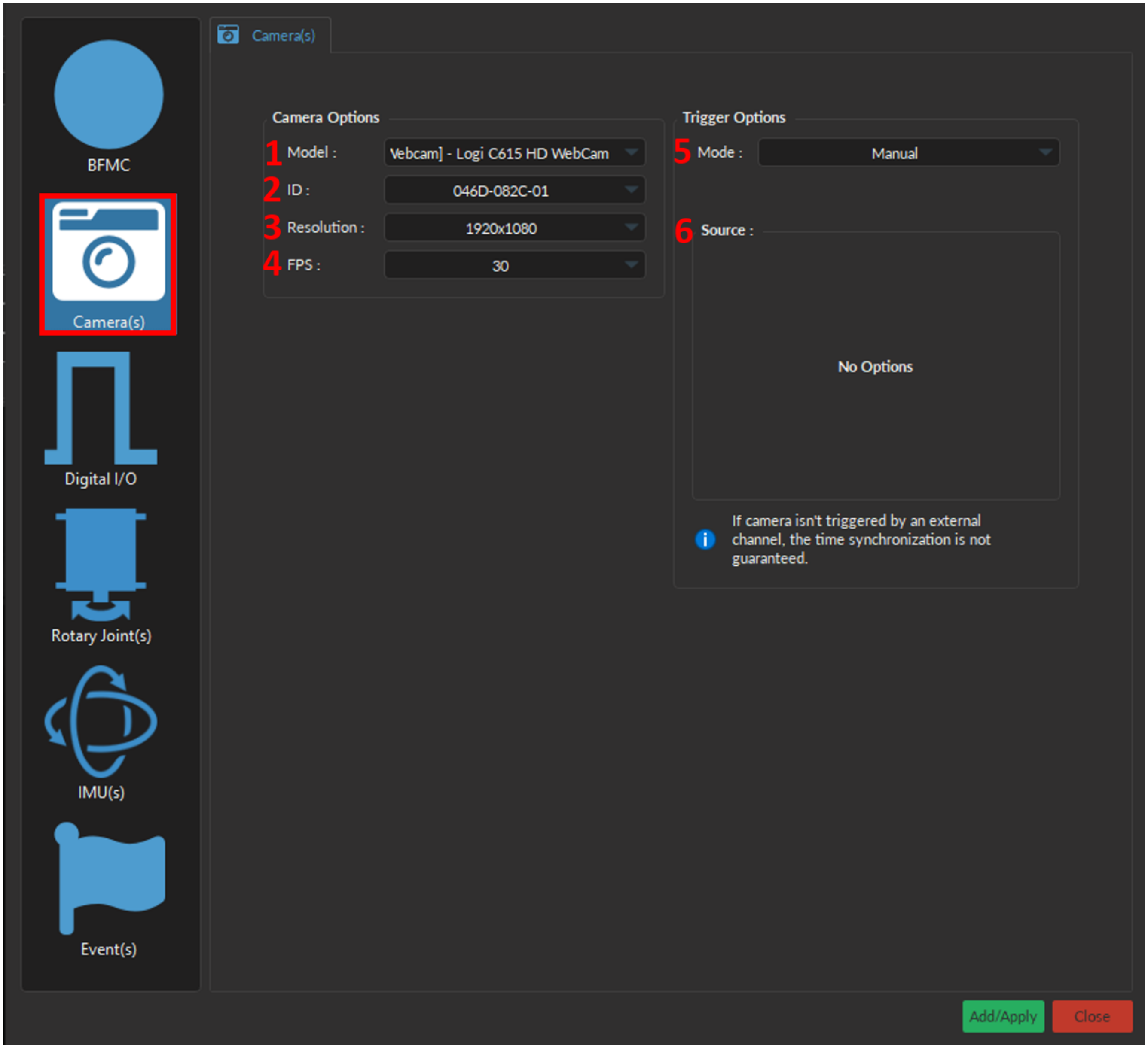


Figure 15.31: *Channel(s) configuration window, Camera(s)*

WARNING:
A camera cannot be used for BOTH **BBC300** and **Camera(s)** modules.
When creating a Camera Channel, if *No available camera detected...*, disconnect the camera in the **Device Selection** window to close the extra module.

Camera Options:

1. The **Model** (Fig. 15.31, 1) allows you to select the camera of choice based on the type of camera.
2. **ID** drop-down list (Fig. 15.31, 2) is used to select a camera based on its unique ID. The ID is beneficial when multiple cameras of the same model are required for the experiment.

3. The **Resolution** (Fig. 15.31, 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. 15.31, 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. 15.31, 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 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.*** A delay of arounf 1 second 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. 15.32). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

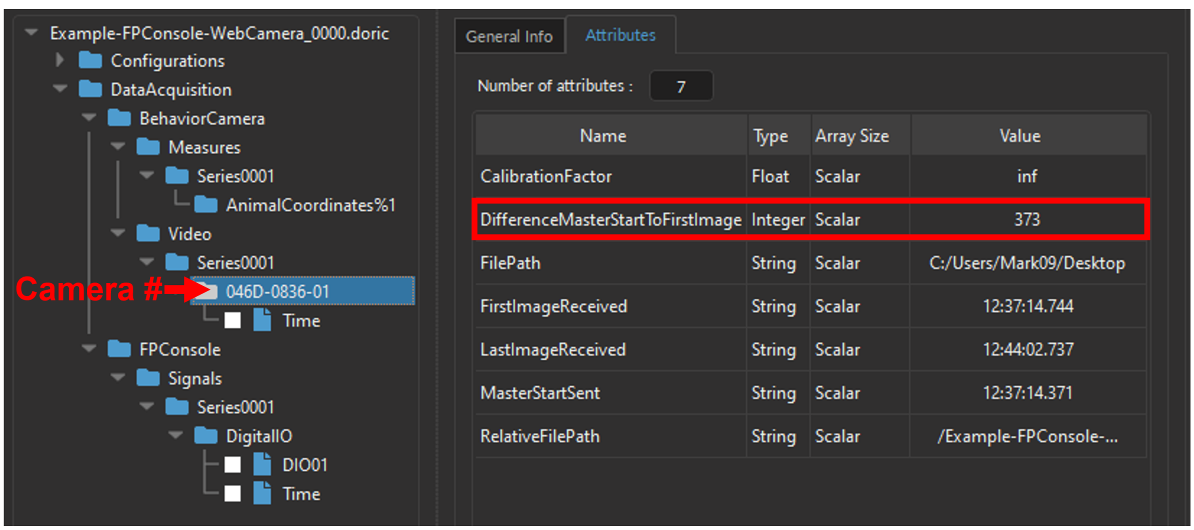


Figure 15.32: *Doric File Viewer, Web Camera Attributes - Video Alignment Variable*

- **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. 15.31, 6 & Fig. 15.33) 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. 15.33). For a detailed description of each Digital I/O parameter see Section 15.4.2. Briefly, key parameters include:

a) The **Channel** (Fig. 15.33, a) corresponds to the physical Digital I/O channel number on the BBC300 that is connected to the trigger cable of the *Doric Behavior Camera*.

b) The **Mode** (Fig. 15.33, 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. 15.33, 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. 15.33, d) is by default 50%. The frame will be taken at the start of each square pulse.

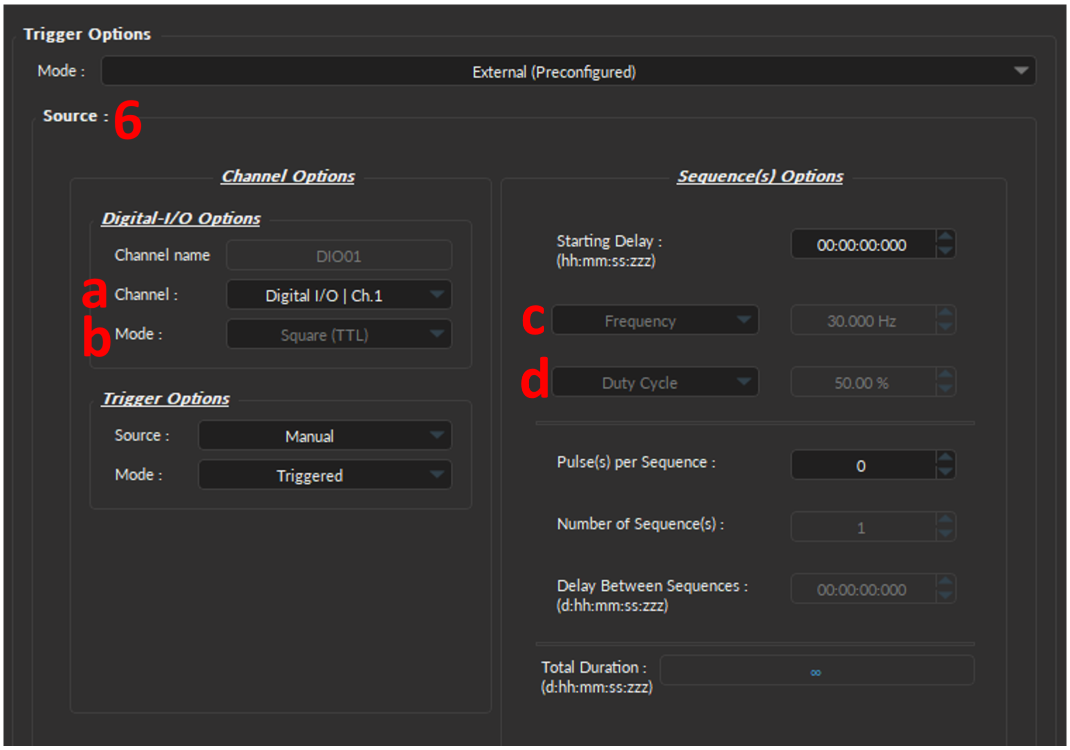


Figure 15.33: *Channel(s) configuration window, Camera - External (Preconfigured)*

15.4.4 Rotary Joint(s) Channel

Adding a **Rotary Joint(s)** channel allows an easy way to control and record when the device's motor is turned on. This can be especially important when even small noise can impact experiments. Once the channel is added, a new **Assisted Rotary Joint** tab is automatically added in the **Control & Settings** of the interface.

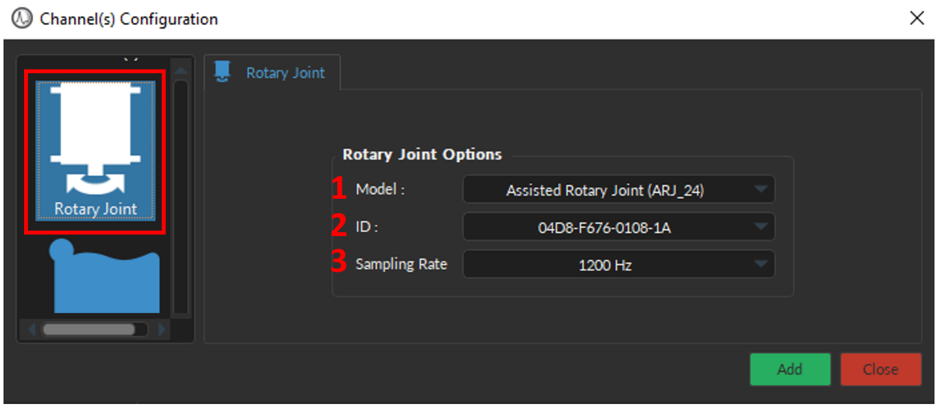


Figure 15.34: *Rotary Joint channel*

The following rotary joint options must be specified when adding the channel:

1. The **Model** (Fig. 15.34, 1) displays the type of rotary joint available. Assisted rotary joints (specifically *ARJ24*), when connected to the computer, will be automatically detected. Compatible devices include:
   - Assisted 1x1 Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 1x2 Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 2x2 Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 1x1 Pigtailed Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 2x2 Pigtailed Fiber-optic & Electric Rotary Joints - 24 contacts
2. The **ID** (Fig. 15.34, 2) displays the serial number of the rotary joint in question, which identifies the proper device when more than one rotary joint of the same model is connected to the computer.
3. The **Sampling Rate** (Fig. 15.34, 3) specifies the number of data points per second that are saved in the *.doric* file. The **Sampling Rate** can range between 10 Hz - 1200 Hz.

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

To add a new **Event**, select the + sign at the bottom of the window (Fig. 15.35, left). To remove an Event, use the - button (Fig. 15.35, 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. 15.35).

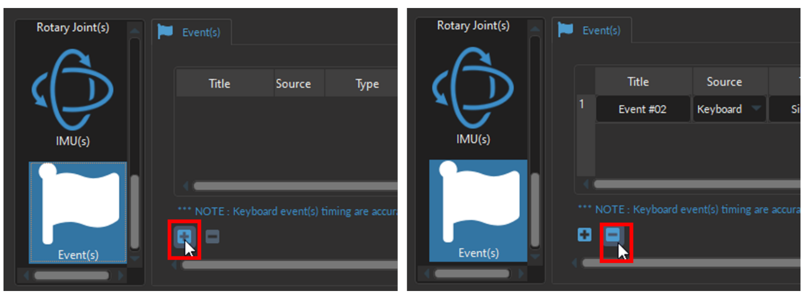


Figure 15.35: *Adding and Removing Events*

To edit a pre-existing **Event** Channel, select the left button (Fig. 15.36) in the **Acquisition View**.

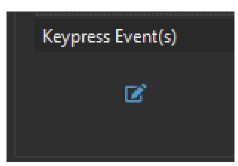


Figure 15.36: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 15.37:

1. The **Title** (Fig. 15.37, 1) allows you to give a name for the Keypress event.
2. The **Source** (Fig. 15.37, 2) is by default *Keyboard*. However, when a DIO channel was added to the configuration and set in *Input Mode*, it is possible to select this DIO as being the source of the created Event.
3. Three **Types** of Event(s) (Fig. 15.37, 3) can be specified with the drop-down list:
   - **Single** - Records single event at the touch of a key (Fig. 15.38a).
   - **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. 15.38b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 15.38c). Every keypress is a new event, with the start of the event occurring when the key was depressed.

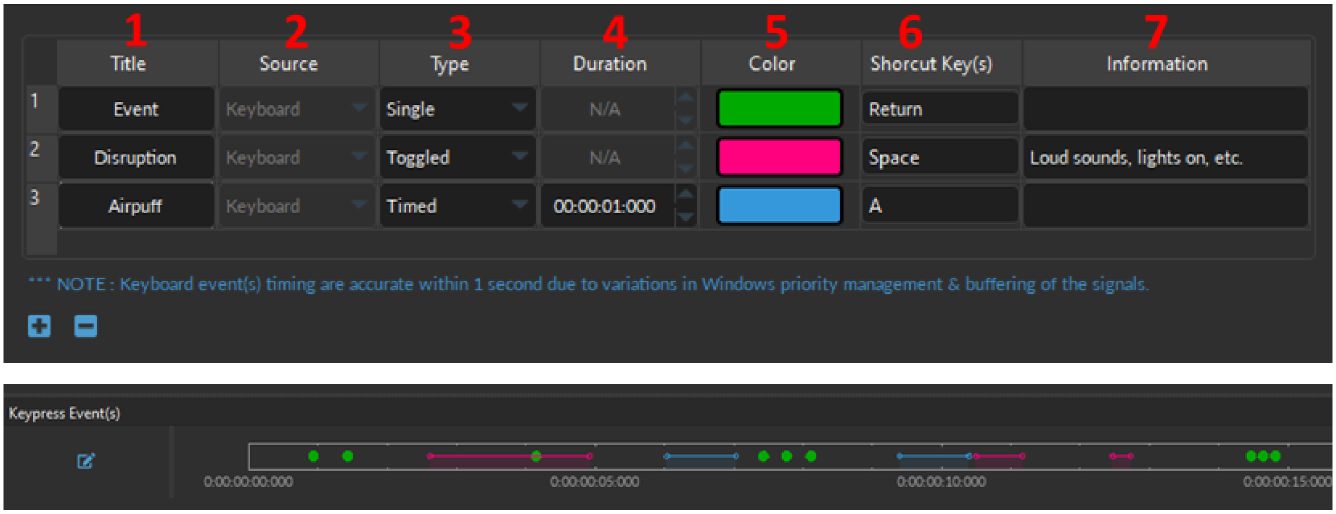
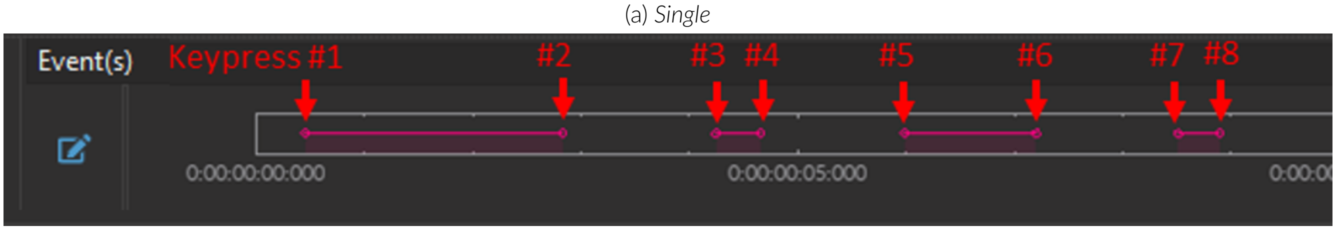


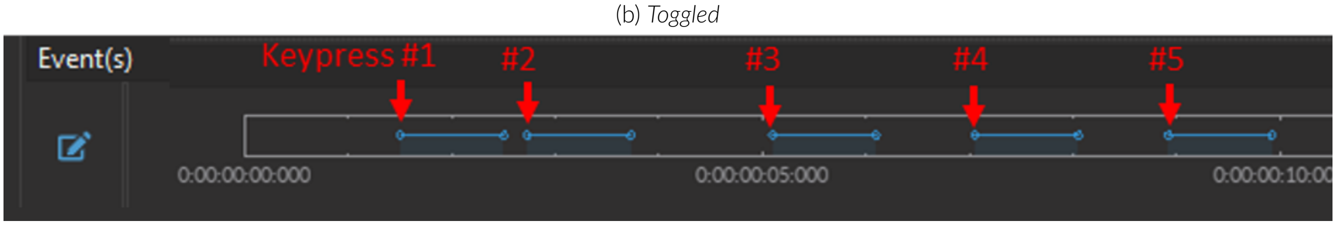
Figure 15.37: *Channel(s) configuration window, Event(s)*

4. The **Duration** (Fig. 15.37, 4) is only used for the **Timed** Keypress type to specify the predetermined amount of time an Event will span. The duration is set in hh:mm:ss:zzz.
5. Select the **Color** (Fig. 15.37, 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. 15.37, 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. 15.37, column 6).
7. The **Information** column (Fig. 15.37, 7) provides space to make notes or write a short description of the Event.

(a) *Single*



(b) *Toggled*



(c) *Timed*

Figure 15.38: *Three types of Event(s)*

15.5 Acquisition View

The **Acquisition View** (Fig. 15.39) is split into separate divisions, each of which visualizes different types of data in the following sections:

1. The **BFPD View** (Fig. 15.39, 1) - Section 15.5.1;
2. The **Graph View** (Fig. 15.39, 2) - Section 15.5.2;
3. The **Graph List** (Fig. 15.39, 3) - Section 15.5.3.

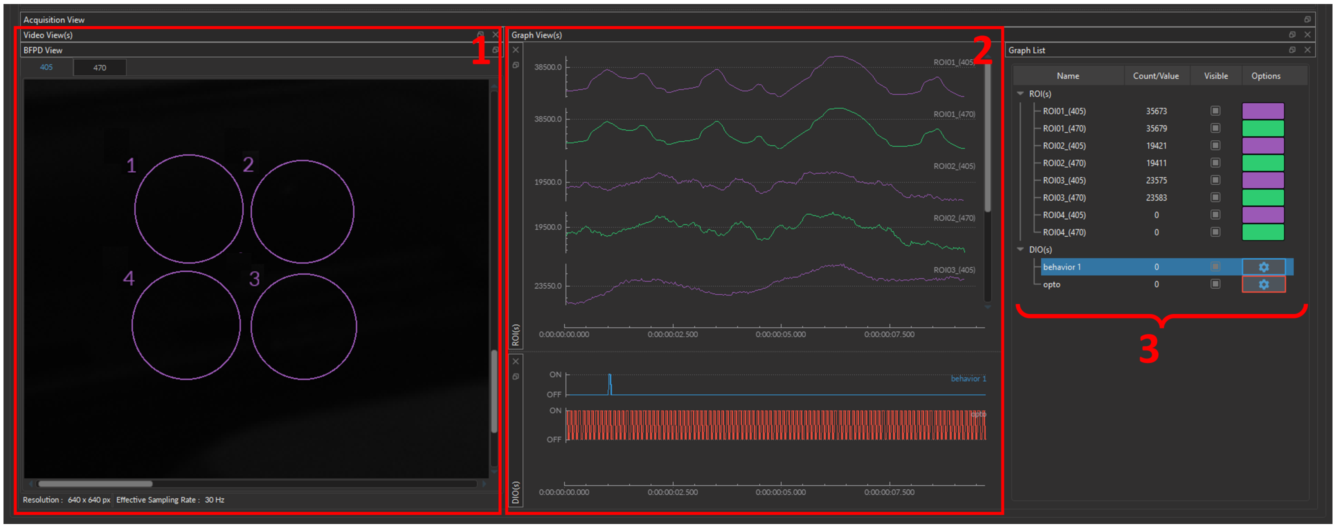


Figure 15.39: *Acquisition View*

15.5.1 BBC300 View

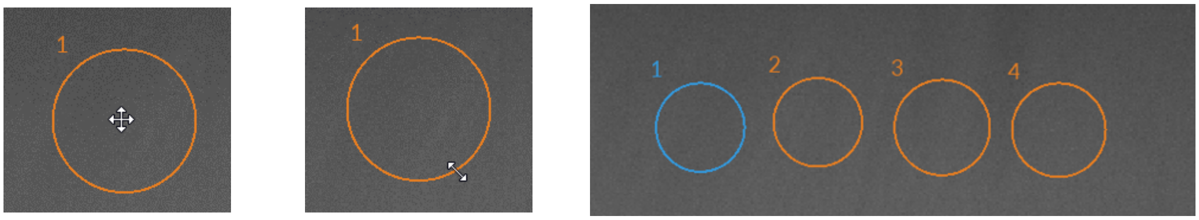
The **BBC300 View** displays the live video feed from the CMOS **Sensor(s)**. This view contains the following components:

Figure 15.40: *BFPD View*

1. The **CAM # EXC #** tab (Fig. 15.40, 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. 15.18, e).

2. The **Sensor Feed** (Fig. 15.40, 2) 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 **Graph List** tab.
   - **Delete individual ROI** - Select a ROI (as detailed above) and press the **Delete** key on the Keyboard. To delete all ROIs, see Section 15.3.4, no. 1.
   - **Displace ROI** - Select the ROI and hove above the center of the ROI until a *Move* icon (Fig. 15.41a) 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. 15.41b) 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. 15.41c). This selection allows multi-ROI deletion or displacement.



(a) *Move ROI* (b) *Resize ROI* (c) *Multi-select*

Figure 15.41: *Edit ROI(s)*

3. The **Resolution** (Fig. 15.40, 3) displays the image resolution, set in the **Configuration Window** (as in Fig. 15.18, b).
4. The **Effective Sampling Rate** (Fig. 15.40, 4) displays the value set in Section 15.4.1, no. 1c.

15.5.2 Graph View

The **Graph View** displays the ROI traces calculated by averaging the pixel intensity value within each ROI, and the DIO channels used to trigger camera(s), synchronize behavior measures, and control optogenetic stimulation(s).

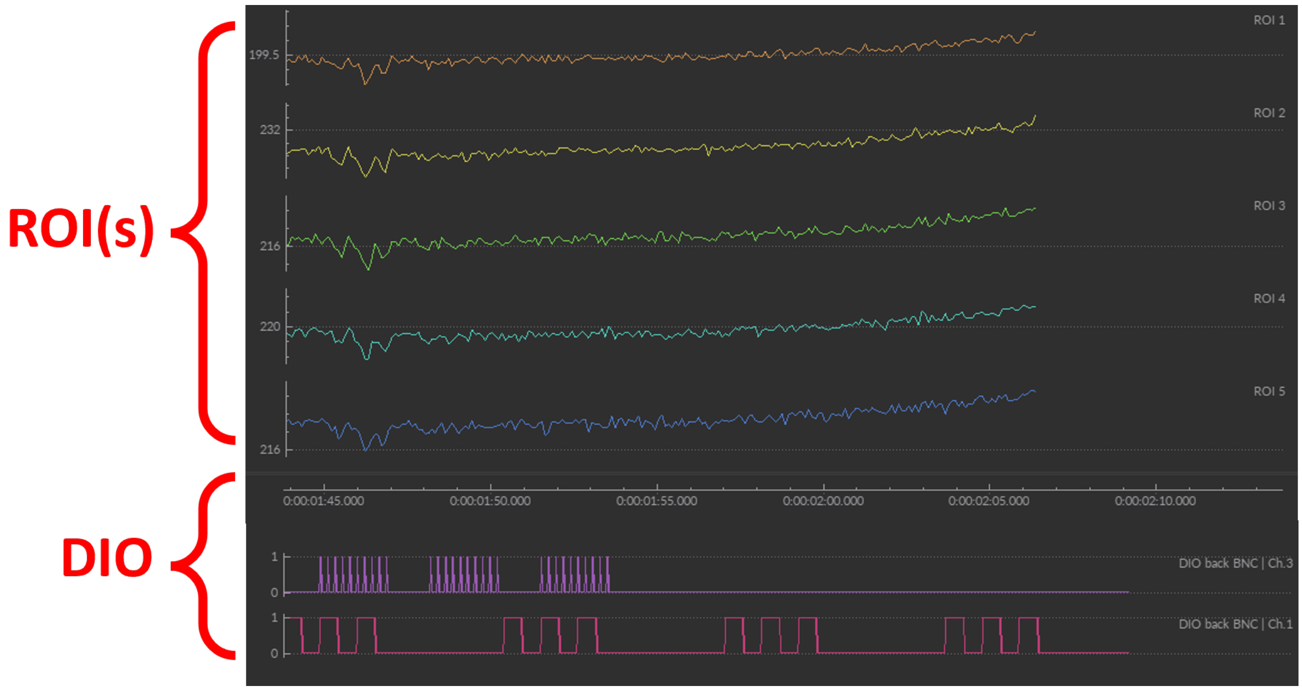


Figure 15.42: *Graph View*

The order of individual graphs can be modified (within the channel types) by *right clicking* one of the graphs in the **Graph View**. This opens a small pop-up menu (Fig. 15.43), that includes the following options:

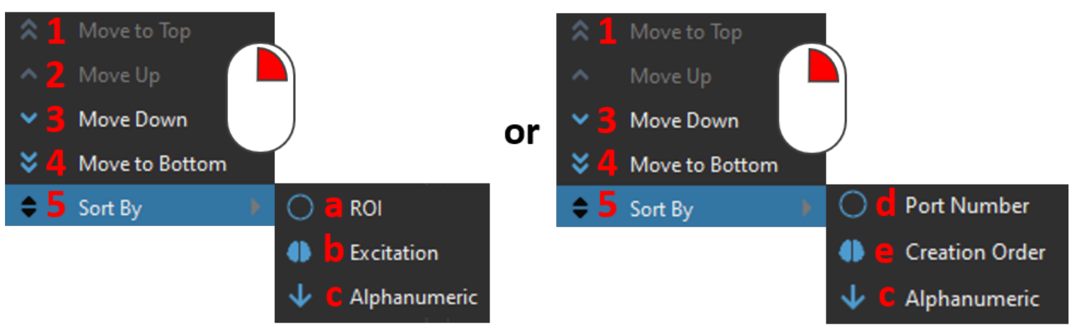


Figure 15.43: *Graph View, Arrange graphs order*

1. The **Move to Top** option (Fig. 15.43, 1) sets the selected graph as the first one of its channel type within **Graph View**.
2. The **Move Up** option (Fig. 15.43, 2) shifts the selected graph towards the top by one graph, but only within its channel type.
3. The **Move Down** option (Fig. 15.43, 3) shifts the selected graph towards the bottom by one graph, but only within its channel type.
4. The **Move to Bottom** option (Fig. 15.43, 4) sets the selected graph as the last one of its channel type within **Graph View**.
5. The **Sort By** option (Fig. 15.43, 5) rearranges the graph order based on:

a) The **ROI ID** option (Fig. 15.43, a) such that the first created ROI will be on top and the last created will be on the bottom.

b) The **Excitation** option (Fig. 15.43, b) such that all channel corresponding to the same excitation (for exemple Isosbestic, 470, 560) will be grouped together in their order of creation.

c) The **Alphanumeric** option (Fig. 15.43, c) such that the **UserName** of each channel will be ordered alphanumerically.

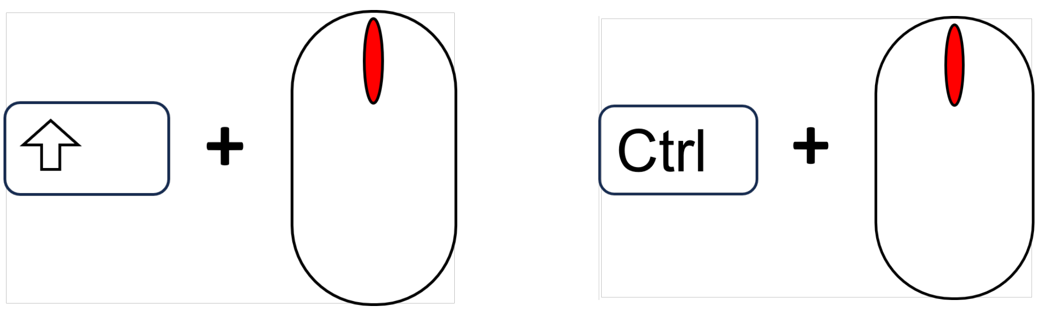
d) The **Port Number** option (Fig. 15.43, d), set a chronological order within each channel type (e.g AIN01, AIN02, AIN03, etc.).

e) The **Creation Order** option (Fig. 15.43, e) such that the first created channel will be on top and the last created channel will be on the bottom.

All channel types within the **Graph View** share a common **Time Axis** (Fig. 15.42). As such, when zooming in or out along the **Time axis** is updated the x-axis range, on all graphs simultaneously. However, while the time axis is shared, each graph has an individual y-axis. Analog channels (**Ain** and **Aout**) use continuous y-values and digital channels (**DIO**) use binary y-values (Fig. 15.42).

Graph HotKeys

There are several **Graph HotKeys** that can be used to quickly adjust the zoom factor in the **Graph View**, including:



(a) *X-axis zoom* (b) *Y-axis zoom*

Figure 15.44: *Zoom Shortcuts*

- **X-Axis Zoom** - *Shift + mouse wheel* (Fig. 15.44a) increases or decreases the range ONLY along the **Time axis** of all graphs within **Graph View**.
- **Y-Axis Zoom** - *Ctrl + mouse wheel* (Fig. 15.44b) to increases or decreases the range ONLY along the **Y-Axis** of the **Graph**.
- **Instantaneous point** - *Double clicking* (Fig. 15.45) on any element of the trace within a graph adds a red dot over that section. Mousing over the dot with the cursor displays the X- and Y-coordinates of the data point. These data points are strictly a visualization tool and their coordinates will not be saved with the rest of the data. To remove a created dot, double-click on it a second time.

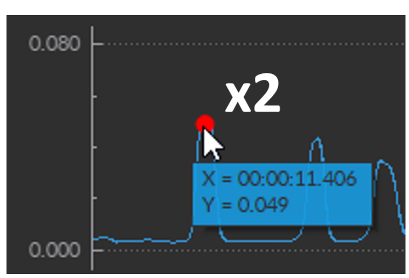


Figure 15.45: *Instantaneous Value*

15.5.3 Graph List

The **Graphs List** (Fig. 15.46) always accompanies the **Graph View**. The **Graphs List** (Fig. 15.46) contains a list of all the active channels organized by types: Digital In/Out (**Dio**) and Region-of-Interest(s) (**ROI(s)**), each of which is split in columns:

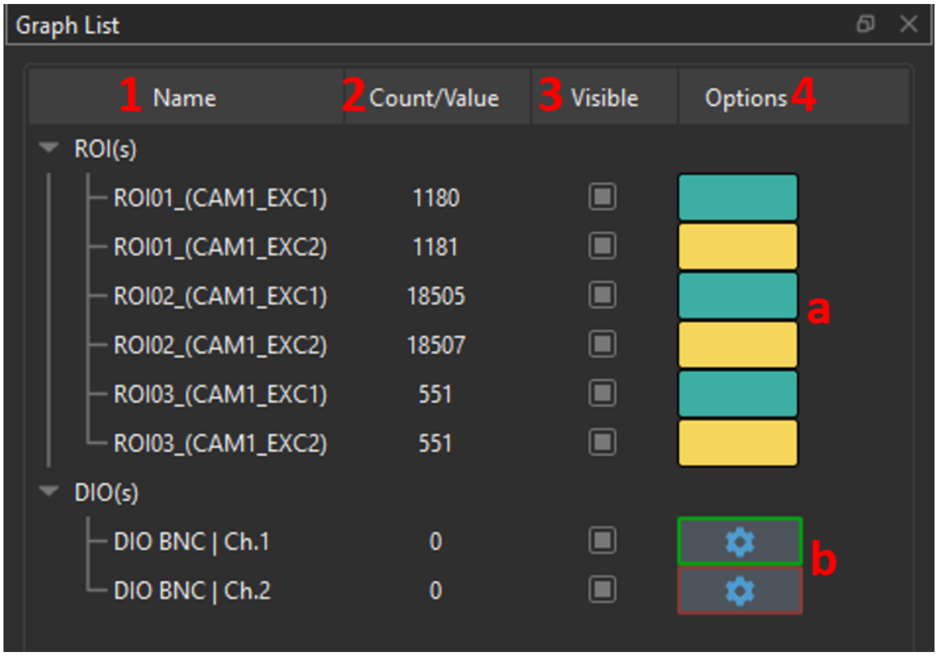


Figure 15.46: *Graph List*

1. The **Name** column (Fig. 15.46, 1) displays the **User Name** of the channel, as specified in either the **Channel List** (for regular channel-types) or in the **Live Processing** list (for channels generated from a pre-set configuration). To change the **Name** of an ROI, double click on its name and type any more intuitive ROI name based on your experimental design.

2. The **Count/Value** column (Fig. 15.46, 2) displays the most recent average fluorescence count in the designated ROI (arbitrary unit) Note that a maximum value of 65000 counts indicates a detector saturation. The **Count/Value** input is particularly useful to visualize live changes in fluorescent activity.

3. The **Visible** checkbox (Fig. 15.46, 3), when enabled, includes the selected graph(s) in the **Graph View**, while disabling the checkbox hides the graph(s).

4. The **Trace Colour buttons** (Fig. 15.46, a) in the **Options** column (Fig. 15.46, 4) opens the **Select Color** window (Fig. 15.47), where users can assign a unique/more intuitive color to each graph to easily identify and interpret the data.

5. The DIO channel(s) **Edit button** (Fig. 15.46, b) (one available per DIO channel added to the configuration) allows to edit the DIO channel parameters that were previously set.

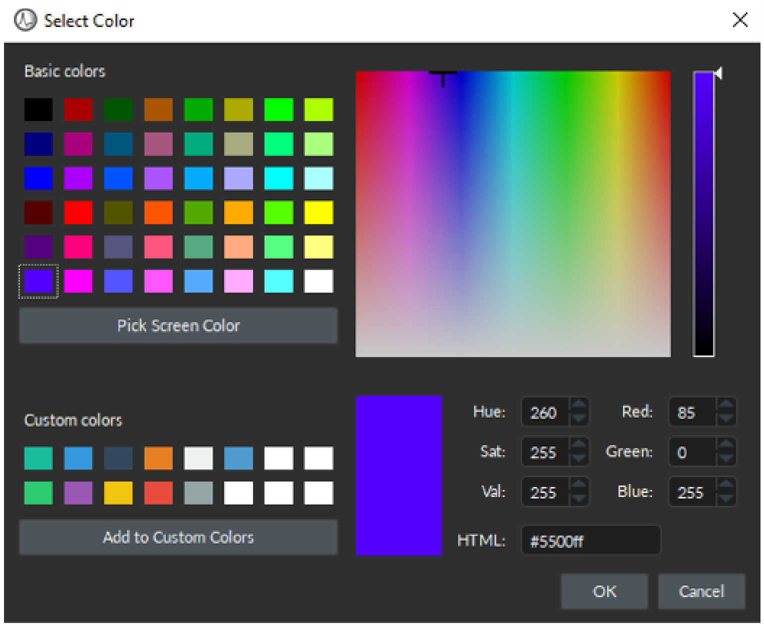


Figure 15.47: *Select Color Window*

15.5.3.1 Camera View

The **Camera View** is added to the **Acquistion View** whenever a **Camera Channel** is created (Section 15.4.3), and contains the following sections:

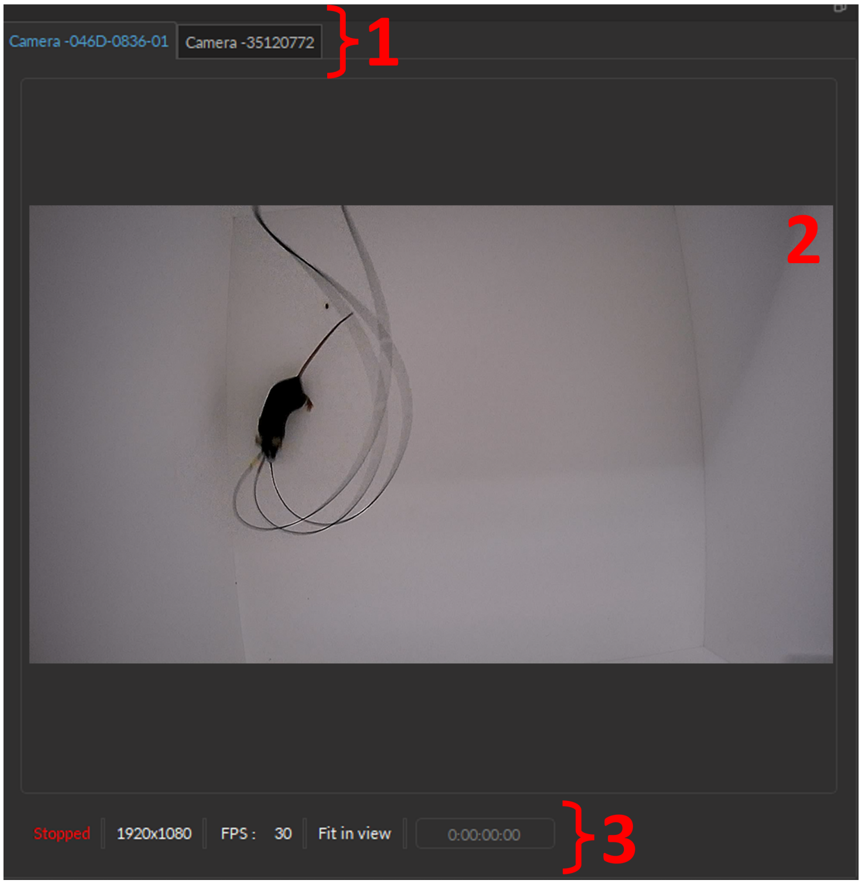


Figure 15.48: *Camera View*

1. The **Sensor Tabs** (Fig. 16.19, 1) allows users to select which **Camera Channel** (if there are more than one camera channels) to view since only one **Camera Feed** can be displayed at a single time.
2. The **Camera Feed** (Fig. 16.19, 2) displays the video footage of the selected **Camera Tab** (in blue). Toggle between the Tabs to switch to see the video feed from a different camera.
3. The **Live Monitoring Bar** (Fig. 16.19, 3) tracks the current parameters of the camera, including:

Figure 15.49: *Live Monitoring bar*

a) The **Status** (Fig. 16.20, a) displays whether the camera is **Stopped**, **Active** or **Waiting for image**.
b) The **Resolution** (Fig. 16.20, b) displays the current image size (in pixels x pixels).
c) The **FSP** (Fig. 16.20, c) displays the current number of frames per second used to record the behavior video.
d) The **Zoom** (Fig. 16.20, d) displays the current zoom value.
e) The **Time** (Fig. 16.20, f) displays the amount of time since the camera was turned on in *d:hh:mm:ss*.

16

Neuroscience Console 500

The **Neuroscience Console 500 (NC500)** is *Doric Lenses'* new data acquisition hardware that supports multiple modalities in parallel with additional ports, higher resolution, and faster sampling rate compared to the Doric *Fiber Photometry console*. It has been developed in parallel with a new version of the Doric Neuroscience Studio to manage multiple data sources and visualize various data streams in a user-friendly and efficient interface.

Figure 16.1: *Neuroscience Console 500*

The main functionalities of the *Neuroscience Console 500* software module are as follows:

- High-resolution analog voltage acquisition with pre-sets for lock-in (Section 16.7.1.3) and time-interleaved (Section 16.7.1.2) detection for multi-color, multi-site fiber photometry recordings
- Doric miniaturized fluorescence microscope recordings (Section 16.6.8)
- Doric electrophysiology recordings (Section 16.6.4)
- Multiple digital input and output (TTL) (Section 16.6.1) for synchronization and closed-loop experiments with external devices as behavior cameras, operant conditioning chambers, video tracking software, optogenetic light sources, etc.
- Keypress events (Section 16.6.7) for manual behavior stimuli/event tagging during recording
- Simultaneous visualization of behavior camera and neural signal recording (Section 16.6.5)

16.1 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 with the USB cable (as in Fig. 16.2).

To add a device, either highlight the device in blue and select the **Connect Device(s)**, or **double click** on the *NC500* icon. If there is no *NC500* icon, double-check that the two ends of the USB cable are correctly connected to both the console itself and the USB ports of the computer. Then click **Refresh**. When properly connected to the system, the *NC500* will appear in the *Connected/Opened device(s)* section of the Window (see the green checkmark in Fig. 16.2).

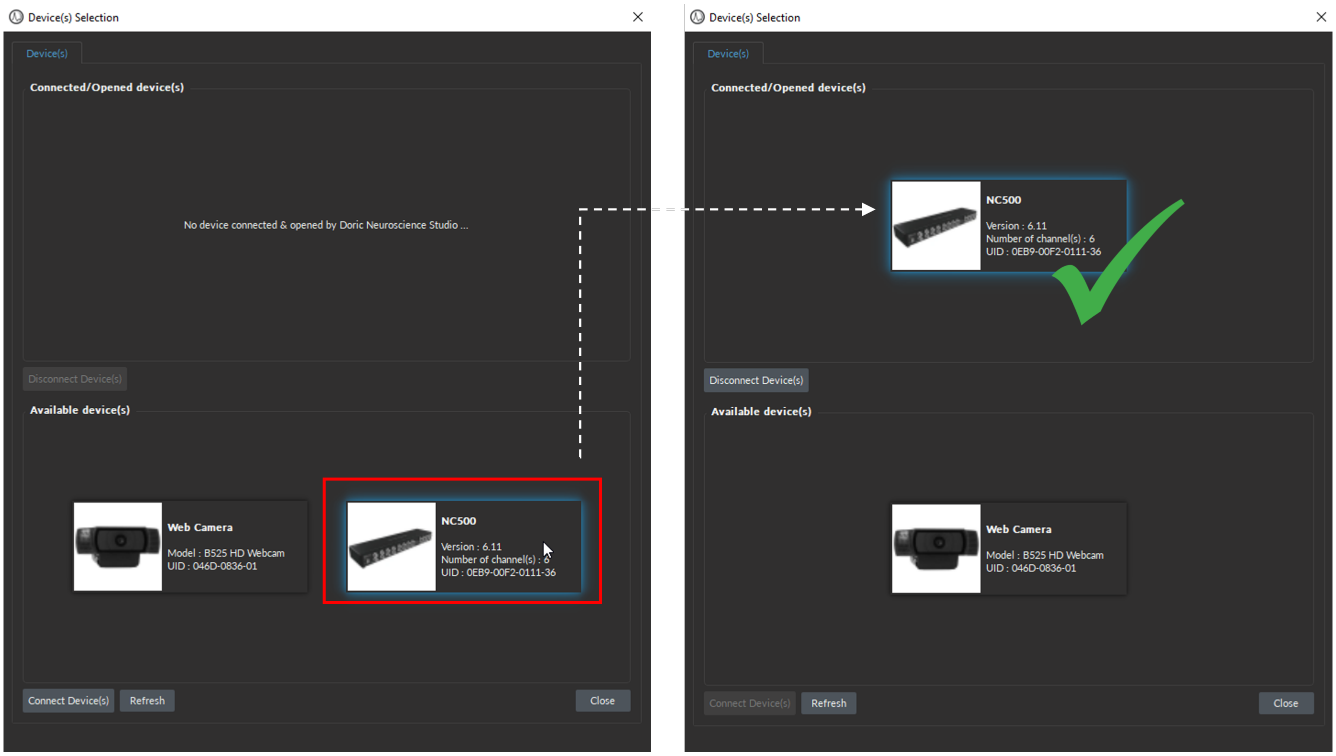


Figure 16.2: *Double click on the device of choice to connect it to DNS*

Manually opening the *Device(s) Selection* window:
To manually open the *Device(s) Selection* window, select *File*, then *Device Selection* (as per Fig. 16.3) or use the hot key: *Ctrl+N*.

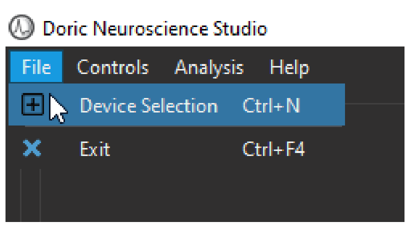


Figure 16.3: *Open Device Selection Window*

16.2 Overview

The **NC500** interface is split into two main sections (Fig. 16.4):

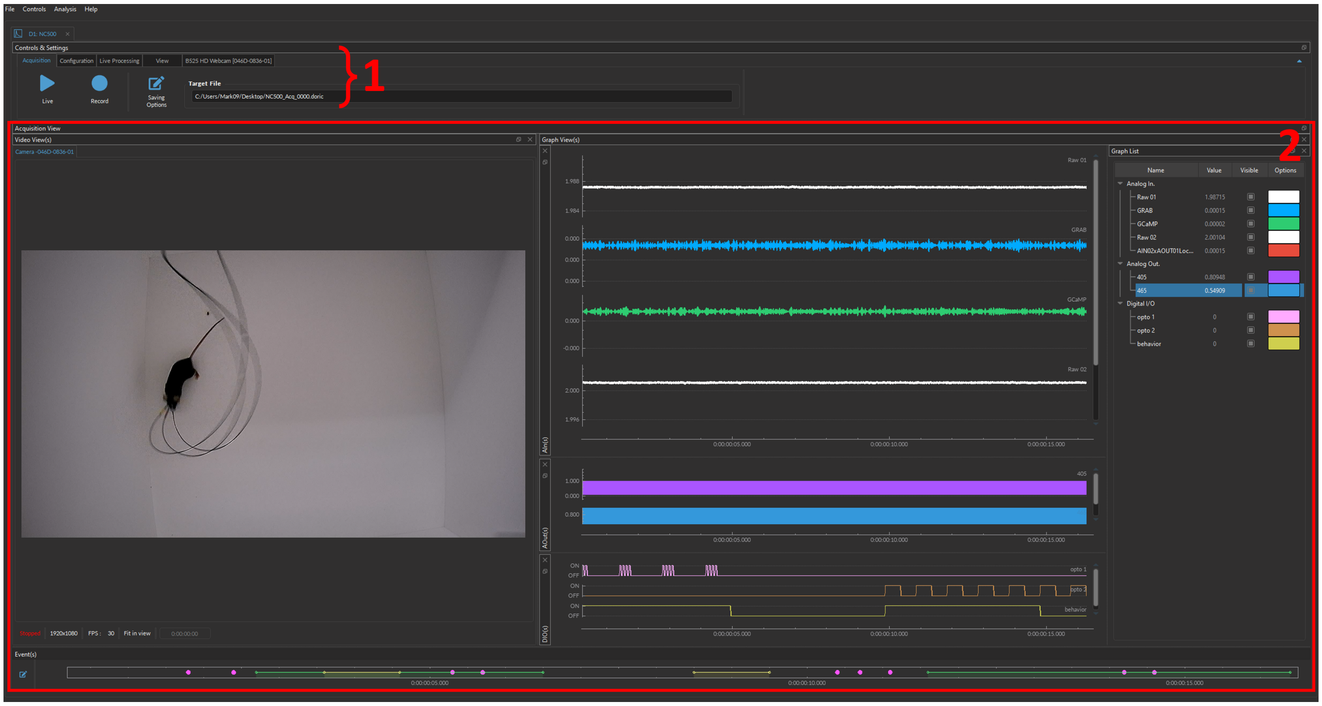


Figure 16.4: *NC500 interface*

1. The **Control & Settings** tabs (Fig. 16.4, 1) contains all the functions and tools of the *NC500* interface. The **Controls & Settings** are split into three tabs, which will be detailed in the following sections:

   a) The **Acquisition** tab (Fig. 16.5, a) contains recording-related tools.
      - Section 16.3

   b) The **Configuration** tab (Fig. 16.5, b) contains channel configuration-related tools. See **Table 16.1** for a complete list of available channel types, their use cases, and their individual User Manual Sections.
      - Section 16.5.1

   c) The **Live Processing** tab (Fig. 16.5, c) contains preset configurations, such as **Filters** (Section 16.7.1.1), **Interleaved** (Section 16.7.1.2), **Lock-In** (Section 16.7.1.3) and **Ephys** (Section 16.6.4).
      - Section 16.7.1

   d) The **View** tab (Fig. 16.5, d) contains visualization-related tools.
      - Section 16.10

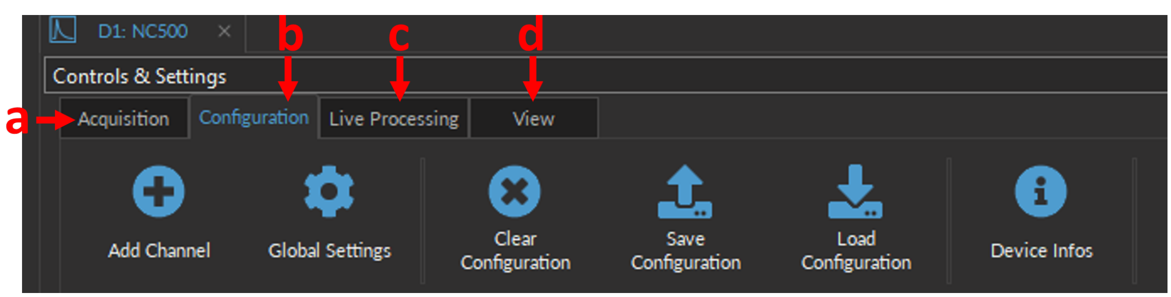


Figure 16.5: *Control & Setting Tabs*

Note: In special cases, additional tabs are added within the **Control & Settings** (Fig. 16.6), including:

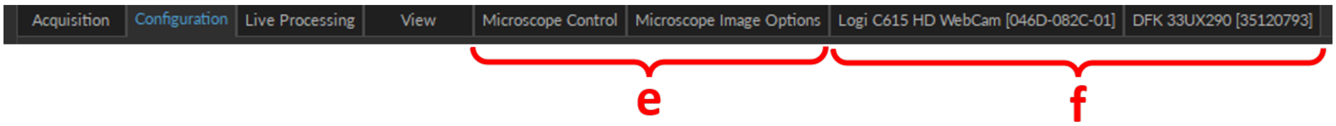


Figure 16.6: *Control & Setting Tabs, additional tabs*

e) The **Microscope Controls** & **Microscope Image Options** tabs (Fig. 16.6, e) are automatically added to the **Controls & Settings** once a **Microscope** channel is added (Section 16.6.8).
   - Section 16.8 - *Microscope Control*
   - Section 16.9 - *Microscope Image Options*

f) The **Camera** tab (Fig. 16.6, f) is automatically added to the **Controls & Settings** once a **Camera** channel is added (Section 16.6.5).
   - Section 16.11 - *Camera Imaging Options*

g) The **Rotary Joint** tab is automatically added to the **Controls & Settings** once a **Rotary Joint** channel is added (Section 16.6.6).
   - Section 16.12 - *Rotary Joint Options*

2. The **View** section (Fig. 16.4, 2) displays information corresponding to each **Control & Settings** tab:

   a) The **Acquisition View** (Fig. 16.4, 2) visualizes the input and output traces and/or images in real-time as data is collected, and can only be accessed by selecting the **Acquisition** tab.
      - Section 16.4

   b) The **Configuration View** displays all the channels that have already been added/configured, organized by channel types. Here, users can rename channels, select what data should be saved, or edit the configuration parameters.
      - Section 16.5.2

   c) **Live Processing** viewer displays the list of preset configurations selected by the user. Here, users can rename channels or edit the preset options.
      - Section 16.7.2

16.3 Acquisition Tab

The **Acquisition** tab contains the buttons to control the data acquisition process and the visualization of the **Acquisition View**. The **Live** and **Record** buttons will not function if channels have yet to be set up. See section 16.6 to configure channels for recording.

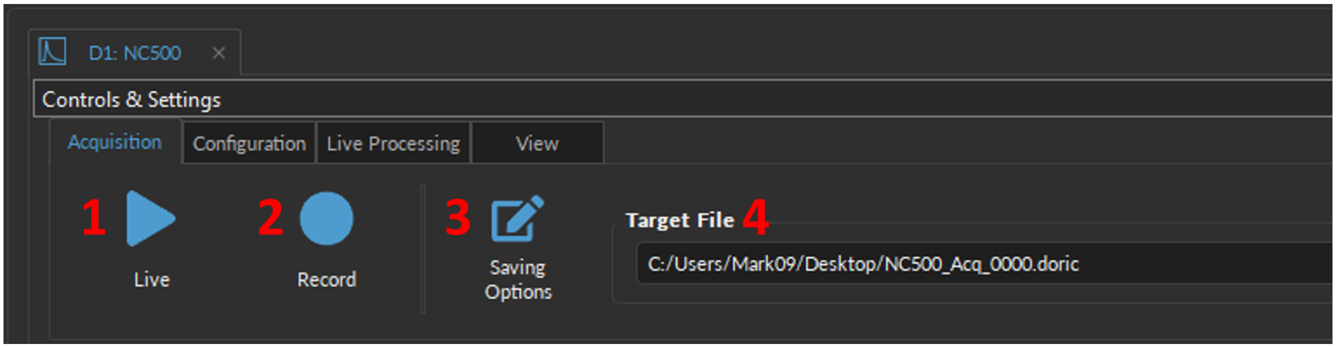


Figure 16.7: *Acquisition Tab*

The **Acquisition** Tab contains the following tools (Fig. 16.7):

1. The **Live** button (Fig. 16.7, 1) activates all configured channels from the **Configuration**. This mode does not save data and only keeps around 1 minute of data on screen. The **Live** mode is useful for quickly testing the recording software and ensuring the parameters are properly set.

2. The **Record** button (Fig. 16.7, 2) activates all configured channels from the **Configuration** and saves the recorded data in the *.doric* file (directory specified in Section 16.3, no. 3). However, modifying the **Master Trigger Options** (Section 16.5, no. 2b) from the default (*Software Command* mode) to any other options changes the function of the **Record** button. For instance, in the *Triggered* or *Gated* mode, the **Record** button sets the acquisition system to a listening state that waits for a trigger from a DIO channel to start the recording (Section 16.5, no. 2b, triggered & gated). Alternatively, when using the **TimeSeries** mode, the **Record** button opens the **TimeSeries** window (Fig. 16.26b) where users can set the timing parameters and launch the recording (Section 16.5, no. 2b, timeseries).

3. The **Saving Options** (Fig. 16.7, 3) button opens the **Saving Parameters** window (Fig. 16.8), which includes:

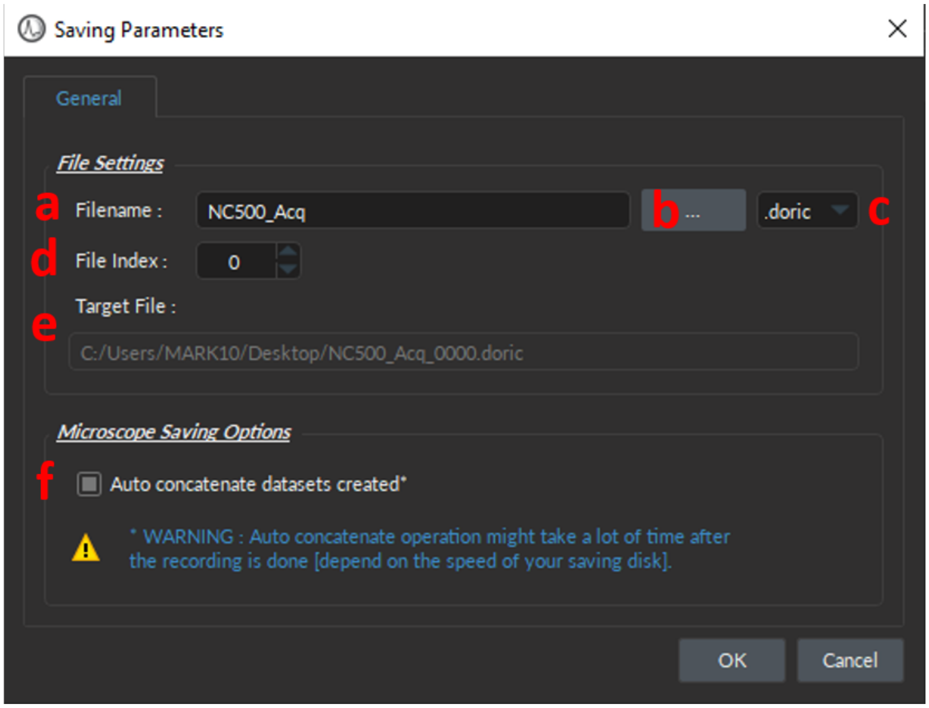


Figure 16.8: *Saving Parameters window*

a) The **Filename** text-box lets users specify the name of the data file that will be saved (Fig. 16.8, a).

b) The **[...]** button opens a File Explorer window where users can select the folder where the data will be saved (Fig. 16.8, b).

c) The **File format** (Fig. 16.8, c) is **.doric**[1], an HDF5-based format that supports metadata (signal, video, images, tables, parameters, etc.). However, while not recommended, it is possible to export a *.doric* file into .csv format through the **Doric File Editor** module (Chapter 4, Section 4.2.4).

d) The **File Index** (Fig. 16.8, d) 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 within the same path & filename during the same session. This prevents accidentally overwriting data.

e) The **Target File** (Fig. 16.8, e) displays the absolute path and filename where the data will be saved.

f) The **Auto concatenate datasets created** checkbox (Fig. 16.8, f), if checked, will automatically merge data files of larger size (above 10 Gb) that would be saved into individual files if the box is unchecked. For the latest case, any recording of more than 10 Gb in size will be saved accordingly into split files with the same name but a different File Index. Note that the Auto concatenate operation might take a lot of time after the recording is done to create a single file.

4. The **Target File** (Fig. 16.7, 4) displays the path and file name (from Section 16.3, no. 3e) where the data will be stored once the **Record** button is pressed. Select the **Saving Options** button (Fig. 16.7, 3) to change the path and filename.

[1] We provide Matlab, Python, and Octave codes to read **.doric** files HERE.

16.4 Acquisition View

The **Acquisition View** visualizes the data from active channels, including graphs, images, plots, and a list of the active channels. The **Acquisition View** was specifically designed to support simultaneous recordings of multiple devices (such as fiber photometry, single-cell fluorescent imaging (miniature microscopy), electrophysiology, optogenetics, and behavior camera recordings) at the same time and visualize the data in a single interface.

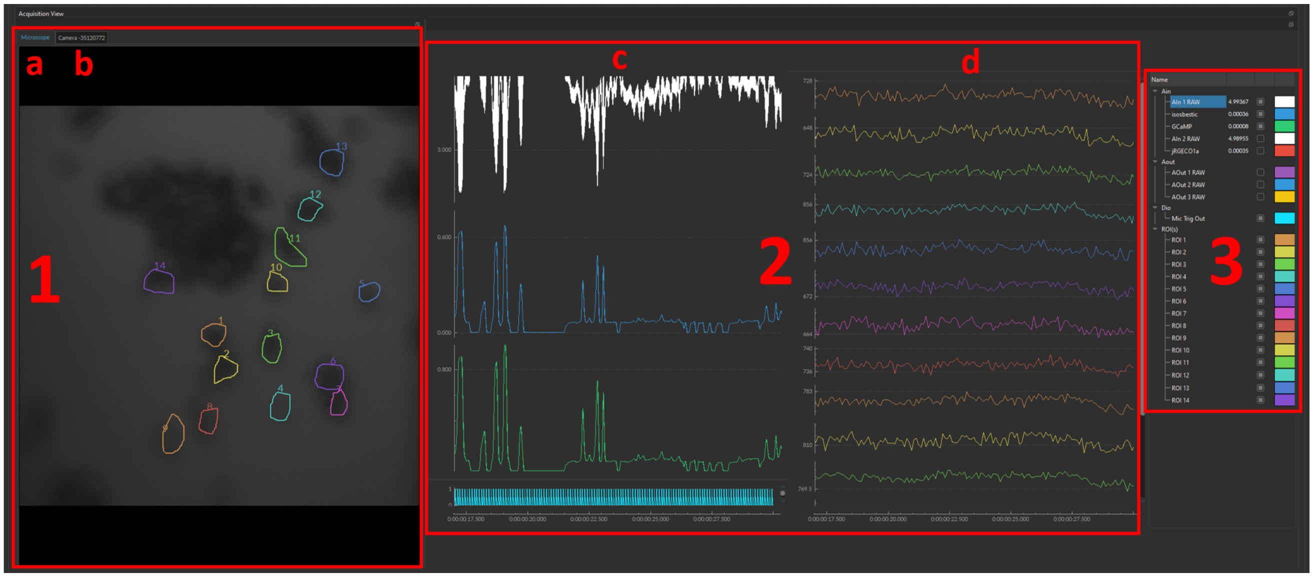


Figure 16.9: *Acquisition View*

Thus, depending on what type of channels are active, the interface can look drastically different. Nonetheless, there are a few common elements that are typically displayed in the **Acquisition View** (Fig. 16.9), which will be treated in the following sections:

1. The **Video View** (Fig. 16.9, 3) **only available upon addition of either* ***Camera*** *or* ***Microscope*** *channels**
   a) Microscope View (Fig. 16.9, a) - *Section 16.4.4*
   b) Camera View (Fig. 16.9, b) - *Section 16.4.3*
2. The **Graph View** (Fig. 16.9, 2) - *Section 16.4.1*.
   c) Analog & Digital Inputs and Outputs (Fig. 16.9, c)
   d) ROI(s) View (Fig. 16.9, d) - **only available upon* ***Microscope*** *channel addition**
3. The **Graph List** (Fig. 16.9, 3) - *Section 16.4.2*.

Organizing Acquisition View

You can rearrange the different **Acquisition View** elements around by first detaching the window with the icon (top right corner of each **View** window), then dragging the window along the interface to the desired spot, as demonstrated in Fig. 16.10. Double-clicking on the white bar atop the detached window will reintegrate it into the main interface in its default location.

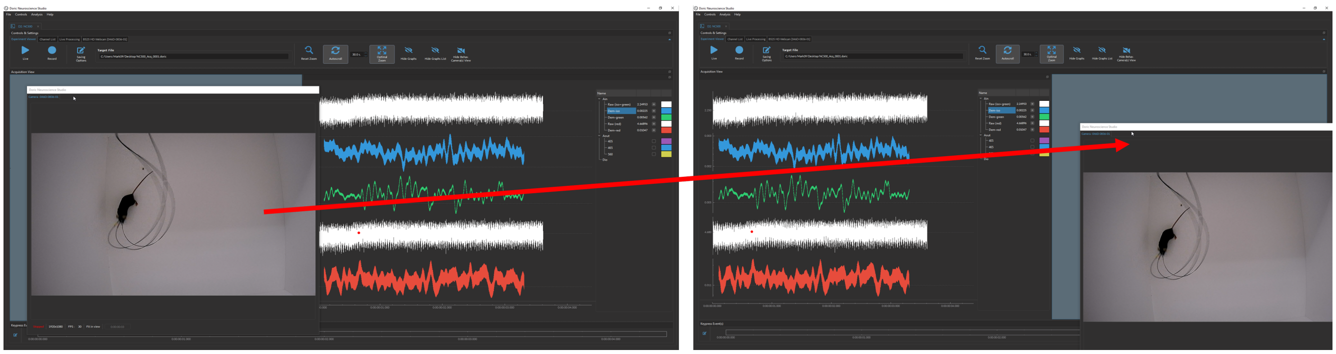


Figure 16.10: *Rearrange Acquisition View*

Enable/Disable a device view

Right-clicking one of the elements from the **Acquisition View** opens the drop-down menu in Fig. 16.11 where you can select/deselect the devices window to show in the **Acquisition View**.

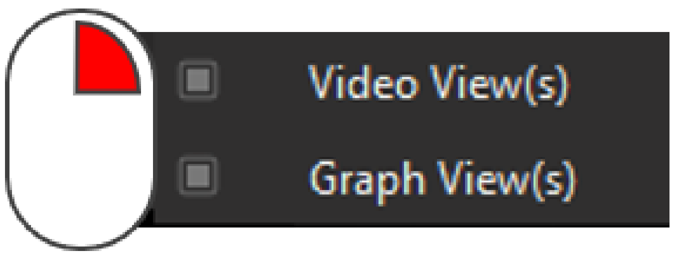


Figure 16.11: *Rearrange Acquisition View*

16.4.1 Graph View

The **Graph View** (Fig. 16.12) displays the enabled Analog In (**Ain**), Analog Out (**Aout**) and Digital In/Out (**DIO**) channels from **Graph List** (Fig. 16.17). If a **Microscope** channel is being used (Section 16.6.8), **ROI(s)** graphs will also be included in the **Graph View**.

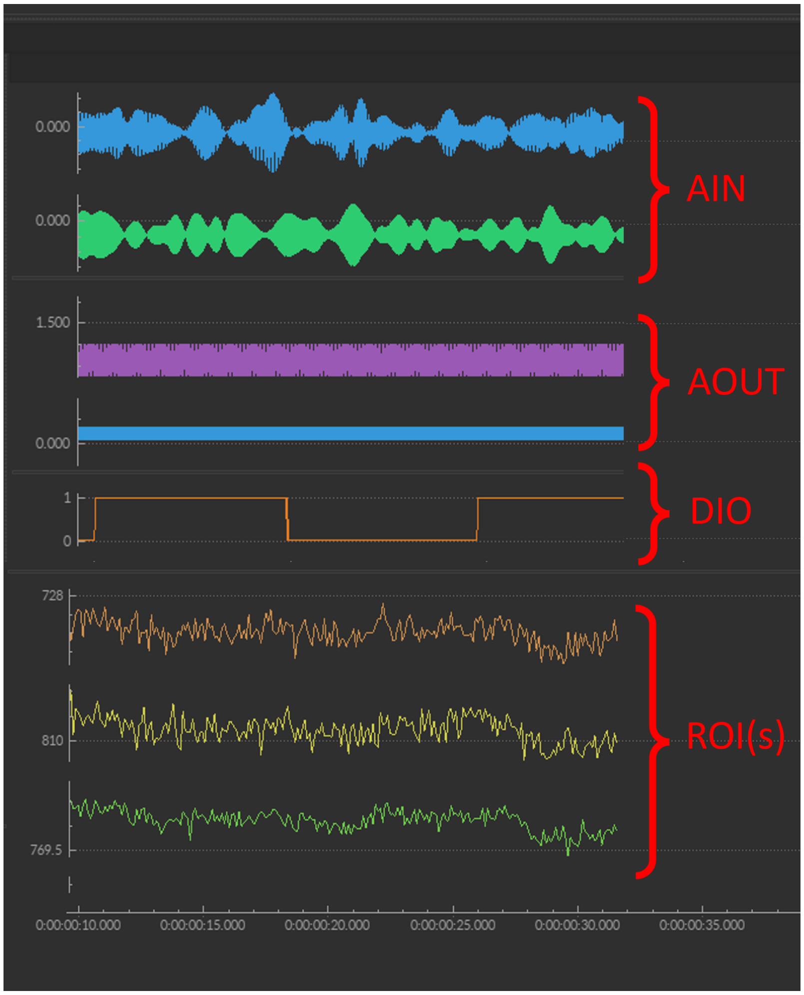


Figure 16.12: *Graph View*

The order of the individual graph can be modified (within the channel types) by *right clicking* one of the graphs in the **Graph View**. This opens a small pop-up menu (Fig. 16.13), that includes the following options:

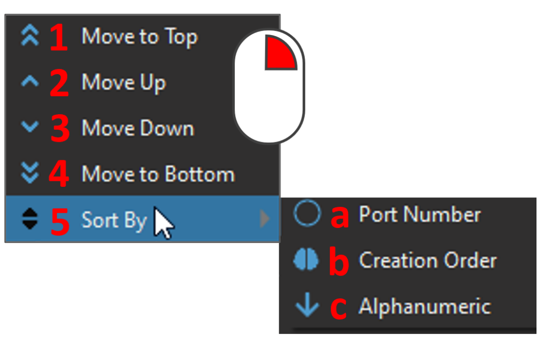


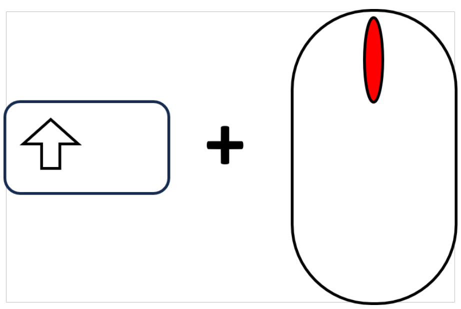
Figure 16.13: *Graph View, Arrange graph order*

1. The **Move to Top** option (Fig. 16.13, 1) sets the selected graph as the first one of its channel type within **Graph View**.
2. The **Move Up** option (Fig. 16.13, 2) shifts the selected graph towards the top by one graph, but only within its channel type.
3. The **Move Down** option (Fig. 16.13, 3) shifts the selected graph towards the bottom by one graph, but only within its channel type.
4. The **Move to Bottom** option (Fig. 16.13, 4) sets the selected graph as the last one of its channel type within **Graph View**.
5. The **Sort By** option (Fig. 16.13, 5) rearranges the graph order based on:
    a) The **Port Number** option (Fig. 16.13, a), set a chronological order within each channel type (.e.g AIN01, AIN02, AIN03, etc.).
    b) The **Creation Order** option (Fig. 16.13, b), such that the first created channel will be on top and the last created channel will be on the bottom.
    c) The **Alphanumeric** option (Fig. 16.13, c), such that the **UserName** of each channel will be ordered alphanumerically.

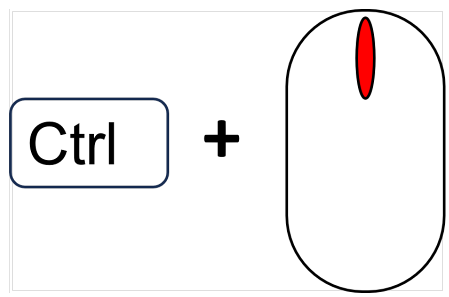
All channel types within the **Graph View** share a common **Time Axis** (Fig. 16.12). As such, when zooming in or out along the **Time axis** updates the x-axis range on all graphs simultaneously. However, while the time axis is shared, each graph has an individual y-axis. Analog channels (**Ain** and **Aout**) use continuous y-values and digital channels (**DIO**) using binary y-values (Fig. 16.12).

Graph HotKeys

There are several **Graph HotKeys** that can be used to quickly adjust the zoom factor in the **Graph View**, including:



(a) *X-axis zoom*



(b) *Y-axis zoom*

Figure 16.14: *Zoom Shortcuts*

- **X-Axis Zoom** - *Shift + mouse wheel* (Fig. 16.14a) increases or decreases the range ONLY along the Time axis of all graphs within **Graph View**.
- **Y-Axis Zoom** - *Ctrl + mouse wheel* (Fig. 16.14b) increases or decreases the range ONLY along the **Y-Axis** of the **Graph**.
- **Instantaneous point** - *Double clicking* (Fig. 16.15) on any element of the trace within a graph adds a red dot over that section. Mousing over the dot with the cursor displays the X- and Y-coordinates of the data point. These data points are strictly a visualization tool and their coordinates will not be saved with the rest of the data. To remove a created dot double-click on it a second time.

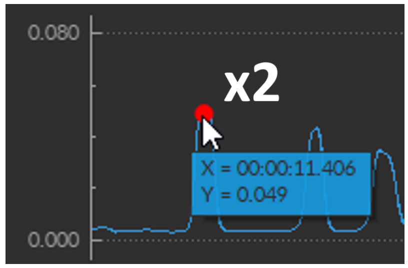


Figure 16.15: *Instantaneous Value*

16.4.2 Graph List

The **Graph List** (Fig. 16.9, 3) always accompanies the **Graph View** (Fig. 16.9, 2), but can be hidden/shown, as any of the different channels from the list, by right clicking on any of the channel name shown in the **Graph View(s)** (Fig. 16.16).

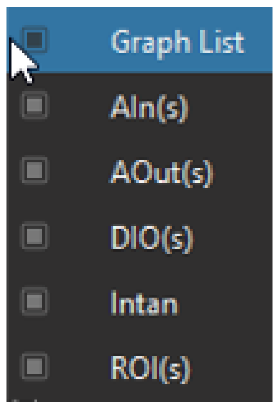
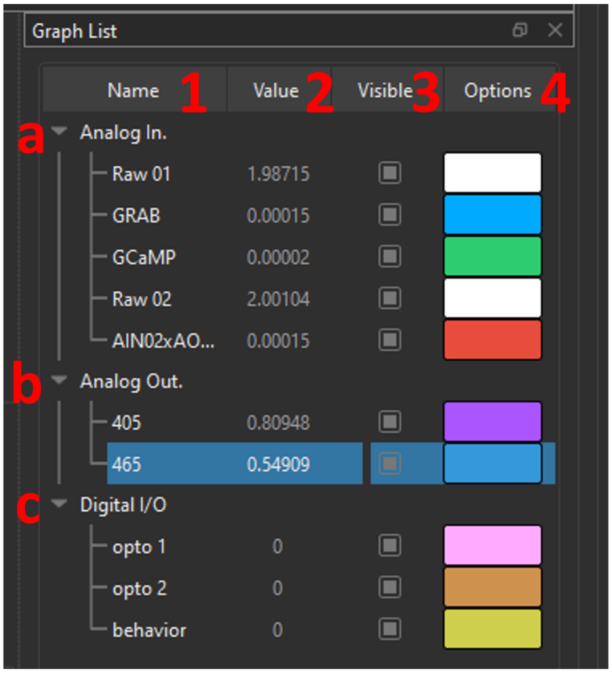


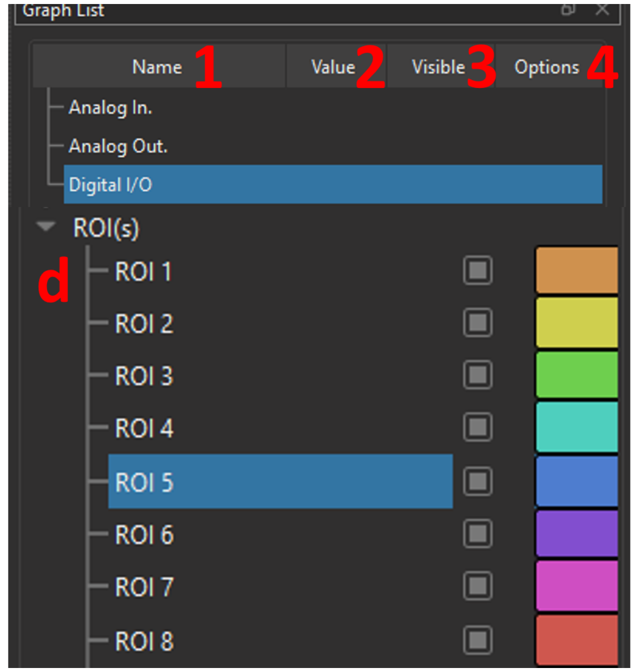
Figure 16.16: *Graph List*

The **Graph List** (Fig. 16.17) contains a list of all the active channels organized by types: Analog In (**Ain**), Analog Out (**Aout**), Digital Input/Output (**DIO**) and Region-of-Interest(s)[2] (**ROI(s)**), each of which is split into four columns:

[2]When **Microscope** channel is created; Section 16.6.8



(a) *Ain, Aout & Dio channels*

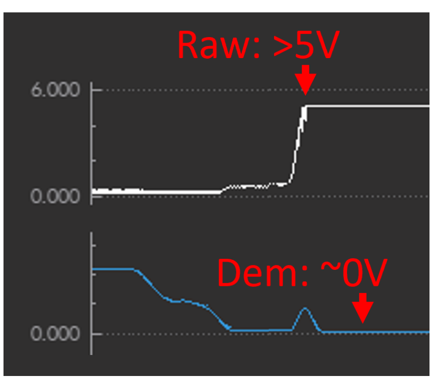


(b) *ROI(s)*

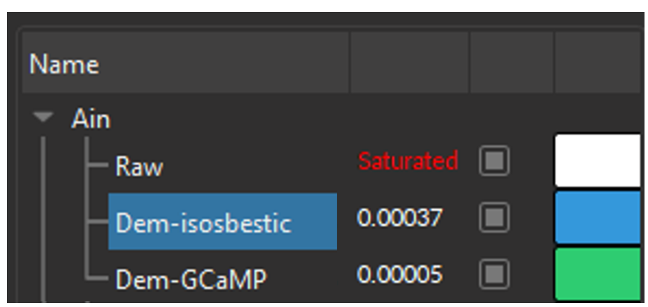
Figure 16.17: *Graph List*

1. The **Name** column (Fig. 16.17, 1) displays the **User Name** of the channel, as specified in either the **Configuration** (for regular channel-types) or in the **Live Processing** list (for channels generated from a pre-set configuration). To change the **Name** of a channel, see Section 16.5.2 and/or Section 16.7.2.

2. The **Value** column (Fig. 16.17, 2) displays the most recent y-value (in Voltage). **Only for* ***Analog Input*** *channels.** This input is particularly useful to determine if the detector is saturated. When the y-value is larger than 5V (or maximum value of the detector; such as 7.5V for Newport detector), the y-value displays **SATURATED** in red.



(a) *Graph*



(b) *Graph List*

Figure 16.18: *Detector saturation example*

3. The **Visible** checkbox (Fig. 16.17, 3), when enabled, includes the selected graph(s) in the **Graph View**, while disabling the checkbox hides the graph(s).

4. The **Trace Colour buttons** in the **Options** column (Fig. 16.17, 4) opens the **Select Color** window, where users can assign a unique/more intuitive color to each graph to easily identify and interpret the data.

16.4.3 Camera View

The **Camera View** is added to the **Acquistion View** whenever a **Camera Channel** is created (Fig. 16.55), and contains the following sections:

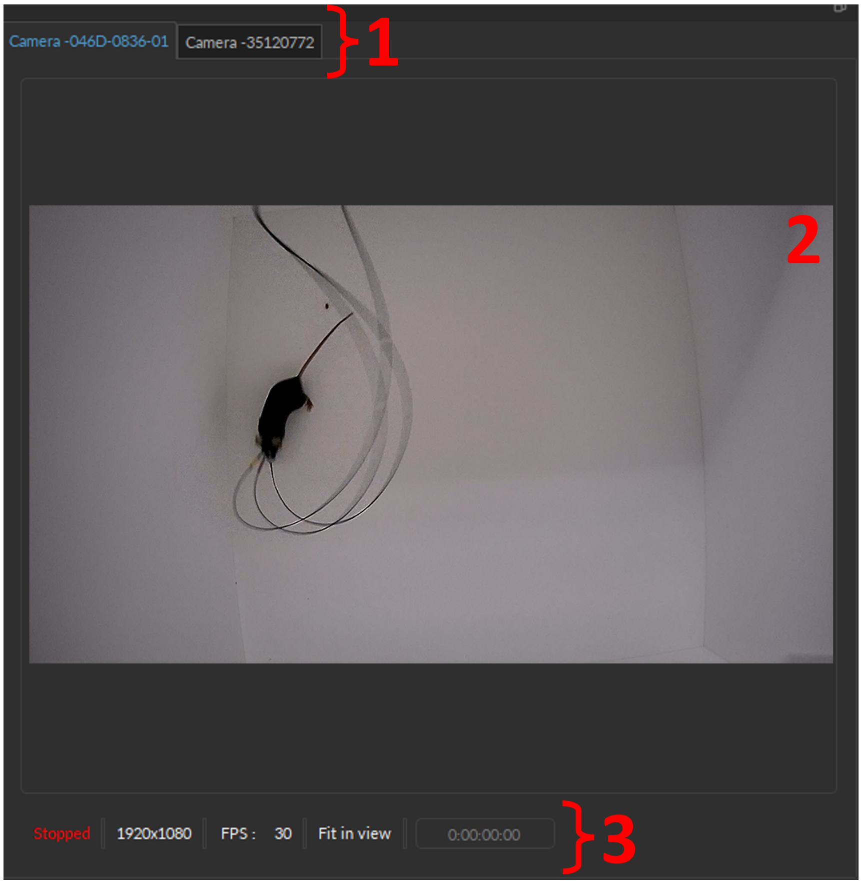


Figure 16.19: *Camera View*

1. The **Sensor Tabs** (Fig. 16.19, 1) allows users to select which **Camera Channel** (if there are more than one camera channels) to view since only one **Camera Feed** can be displayed at a single time.
2. The **Camera Feed** (Fig. 16.19, 2) displays the video footage of the selected **Camera Tab** (in blue). Toggle between the Tabs to see the video feed from a different camera.
3. The **Live Monitoring Bar** (Fig. 16.19, 3) tracks the current parameters of the camera, including:

Figure 16.20: *Live Monitoring bar*

a) The **Status** (Fig. 16.20, a) displays whether the camera is **Stopped**, **Active** or **Waiting for image**.
b) The **Resolution** (Fig. 16.20, b) displays the current image size (in pixels x pixels).
c) The **FSP** (Fig. 16.20, c) displays the current number of frames per second used to record the behavior video.
d) The **Zoom** (Fig. 16.20, d) displays the current zoom value.
e) The **Time** (Fig. 16.20, f) displays the amount of time since the camera was turned on in *d:hh:mm:ss*.

16.4.4 Microscope View

The **Microscope View** displays the live video feed from the microscope **Sensor**. This view includes the following:

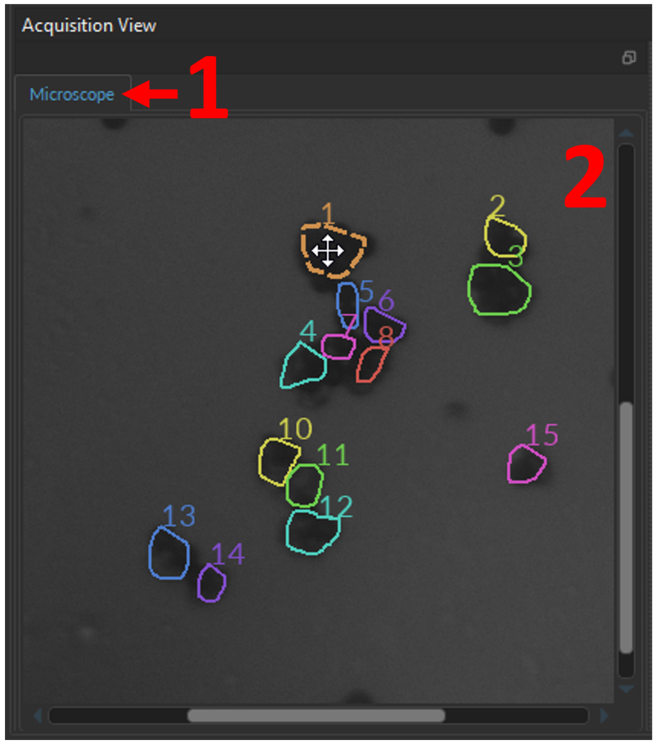


Figure 16.21: *Microscope View*

1. The **Sensor** tab (Fig. 16.21, 1) - Toggle between tabs (if multiple channels exist) to display the device's (microscope or camera) live images.
2. The **Microscope Feed** (Fig. 16.21, 2) - Displays the live microscope images where users can:
   - **Draw ROI** - Click the area within the **Microscope View** to outline the ROI over the area of interest. A maximum of 20 ROI(s) can be drawn. To change the shape type see Section 16.9, no. 6.
   - **Select ROI** - Click either the edge or within the ROI to select it. When selected, the ROI(s) outline becomes dotted and it automatically highlights the corresponding ROI(s) in the **Graph List** tab (Fig. 16.17b).
   - **Delete individual ROI** - Select an ROI (as detailed above) and press the *Delete* key on the Keyboard. To delete all ROIs, see Section 16.9, no. 5.
   - **Displace ROI** - Select the ROI and hover near the center of the ROI until a *Move* icon  appears. Click and drag the ROI to its new desired location.
   - **Resize ROI** - Select the ROI and hover above the outline of the ROI until a *Resize* icon 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 the outline of each selected ROIs becomes dotted (Fig. 16.22). This selection allows multi-ROIs deletion or displacement.

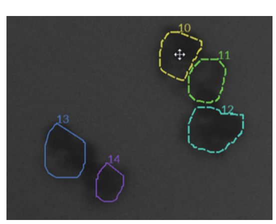


Figure 16.22: *Microscope View, multi-select*

16.5 Configuration Tab

The **Configuration** tab visualizes a list of all the configured channels, organized by channel type, where users can easily create, delete, rename, or change configuration parameters. The **Configuration** is split into two sections: (1) **Configuration Tab** that contains the related tools/ buttons (Section 16.5.1) and (2) **Configuration View** which displays the added channels (Section 16.5.2).

16.5.1 Configuration Tab

The **Configuration** tab contains buttons related to creating standard channel configuration. See Section 16.7 for preset configuration options.

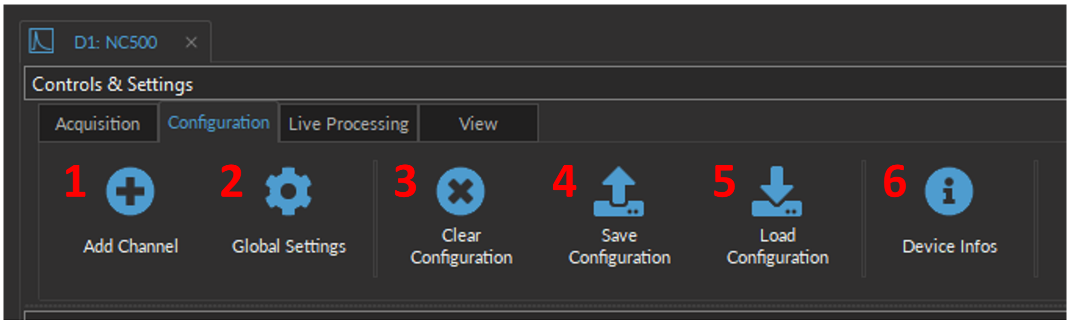


Figure 16.23: *Configuration Tab*

1. The **Add Channel** button (Fig. 16.23, 1) opens the **Channels configuration** window. How to *add* and *configure* a channel is detailed in Section 16.6.
2. The **Global Settings** (Fig. 16.23, 2) opens the **Global Options** window in Fig. 16.24, where the user can set the acquisition sampling rate and specify the master trigger options. Specifically:

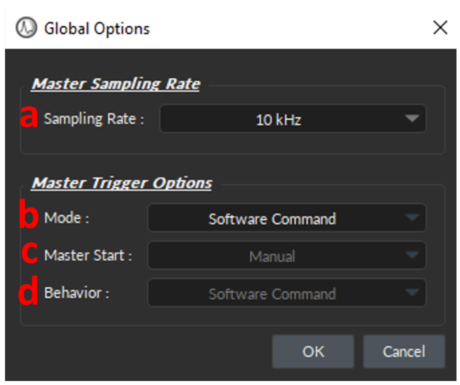


Figure 16.24: *Global Options Window*

a) The **Master Sampling Rate** (Fig. 16.24, a) sets the number of data points collected per second. Its value is set at 10kHz by default. See section 16.3, no. 3 to enable the *Decimation* and effectively reduce the saving sampling rate and restrict the data file size.

b) The **Mode** (Fig. 16.24, b) 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. 16.7, 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. 16.26). This mode works similarly to the *Sotware Command* mode, however, when the **Record** button is selected, the **Time Series Window** (Fig. 16.26b) pops up. See section 16.5.1.1 for more details.

c) The **Master Start** (Fig. 16.24, c) defines the source that automatically starts the recording. This source can either be:

- *Manual* - the user ultimately starts the recording session by clicking **Record** within the **Acquisition Tab** of *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.***

d) The **Behavior** (Fig. 16.24, d) defines the source of the **Master Trigger** for the **Camera Channels** (see Section 16.6.5).

3. The **Clear configuration** button (Fig. 16.23, 3) resets the acquisition view by removing all configured channels and parameters. Any un-saved configurations will be permanently lost.
4. The **Save configuration** button (Fig. 16.23, 4) allows a console configuration to be saved in the **.doric** format. This file preserves the channel configuration/parameters, the Acquisition View window organization, and any custom trace colors and names.
5. The **Load configuration** button (Fig. 16.23, 5) imports a pre-configured **.doric** file into the module.
6. The **Device Infos** button (Fig. 16.23, 6) opens a pop-up window (Fig. 16.25), which includes the following:

a) The **Device Name** (Fig. 16.25, a) refers to the name of the data acquisition hardware and will always be *NEUROSCIENCE CONSOLE 500*.

b) The **Item Number** (Fig. 16.25, b) is the Doric Lenses product identifier for the NC500.

c) The **Serial Number** (Fig. 16.25, c) is the unique code given to each NC500 unit.

d) The **Calibration Date** (Fig. 16.25, d) displays the date (yyyy-mm-dd) and time (hh:mm) of the most recent calibration process.

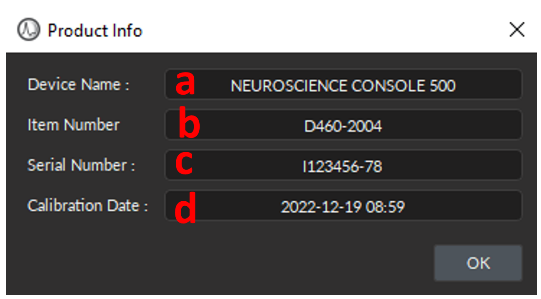
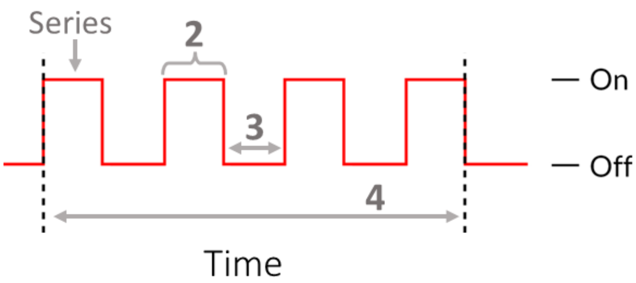


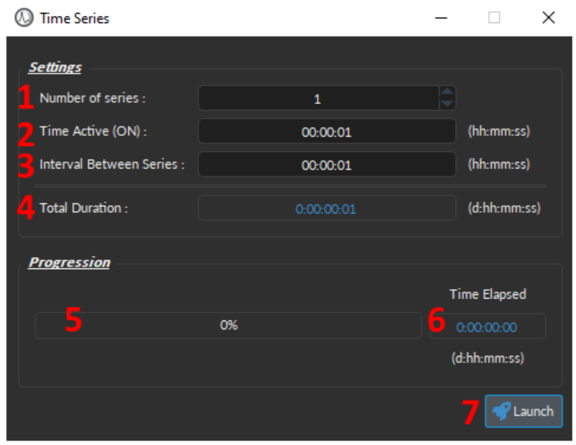
Figure 16.25: *Product Info Window*

16.5.1.1 Time Series

The **Time Series** Window (Fig. 16.26) can be opened by clicking on the **Record** button (Fig. 16.7, 2) when the **Master Trigger** is in **Time Series** mode in the **Global Settings** window (Fig. 16.24, 2). Every **Time Series** sequence is automatically saved to the *.doric* file defined in **Saving Options** (Section 16.3, no. 3).



(a) *Time Series Acquisition Timing Diagram*



(b) *Time Series Window*

Figure 16.26: *Time Series Mode can be set through Global Settings*

The **Time Series** window (Fig. 16.26) sets the following parameters:

1. The **Number of series** (Fig. 16.26, 1) defines the total number of time periods (*i.e.* serie, Fig. 16.26a) when the recording will be ON.
2. The **Time Active (ON)** (Fig. 16.26, 2) defines the duration of a serie.
3. The **Interval Between Series** (Fig. 16.26, 3) defines the amount of time between each series if the **Number of series** is greater than 1.
4. The **Total Duration** (Fig. 16.26, 4) displays the total amount of time that the time series recording will take.
5. The **Progression** bar (Fig. 16.26, 5) indicates the progression of the time series (in %).
6. The **Time Elapsed** (Fig. 16.26, 6) counter indicates the amount of time that has already passed in d:hh:mm:ss.
7. The **Launch** (Fig. 16.26, 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.

16.5.2 Configuration View

The **Configuration View** (Fig. 16.27) displays all channels created using the **Add Channel** button (Fig. 16.5.1, 1), in addition to channels/dependencies created after using a **Live Processes**. See Table 16.1 and Table 16.3 for a complete list of **Channels** / **Live Processes** that can be used with the NC500.

The **Configuration View** is organized into sections, each corresponding to a different channel type (Fig. 16.27):

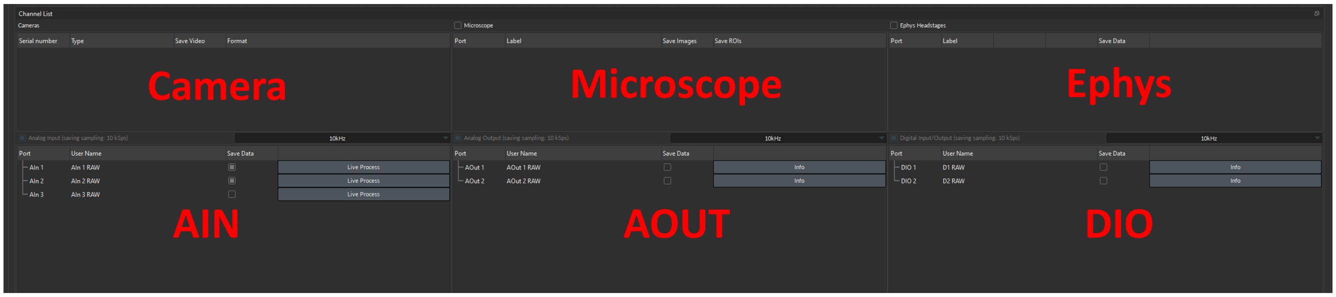


Figure 16.27: *Configuration Overview*

Each section is further divided into a table, where the rows are the individual channels and the columns are channel-specific information, as follows (Fig. 16.28):

1. The **Activate Channel** check-box (Fig. 16.28, 1) is only available for **Microscope** and **Ephys**-type channels. When disabled, no data will be collected from the disabled port, even if a channel is added to the **Configuration** and is present in the **Acquisition View** (graphs will be empty during **Live** and **Record** modes).

2. The **Sampling Rate** (Fig. 16.28, 2) is only available for **DIO**, **AOUT** and **AIN** channels and allows users to individually set the frequency of data acquisition based on the channel-type.

3. The **Port** (Fig. 16.28, 3) displays the *NC500* port number that is assigned to the channel. For **Microscope**, the **Port** column also displays all the ID numbers of the ROI(s). And for **Ephys** channels, the **Port** column also displays the ID numbers of the individual electrodes within the array.

4. The **Label / User Name** (Fig. 16.28, 4) can be edited (by double-clicking the default name) to assign a more intuitive/experiment-relevant label to the channel. For **Microscope** and **Ephys** channels, the **Label** column allows users to rename the individual ROI(s) and electrodes.

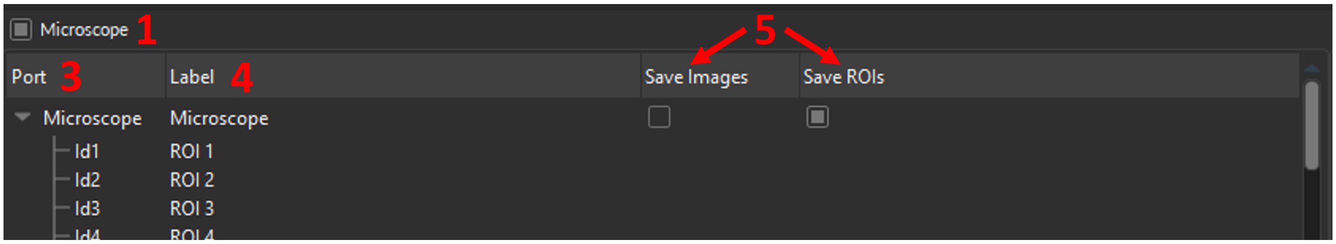
5. The **Save Data** check-box (Fig. 16.28, 5), when enabled, saves the signals, ROI(s), video, or images in the *.doric* file. Even when disabled, data from this channel is still acquired, and does NOT affect the visualization in **Acquisition View**.

6. The **View / Edit Settings** button (Fig. 16.28, 6) opens a pop-up window where users can view and/or edit the value of the parameters set when creating the channel. Channels created using **Live Processing** presets will have a *Live Process* button, while their un-editable dependencies have an *Info* button (which specifies the Master channel that they belong to).

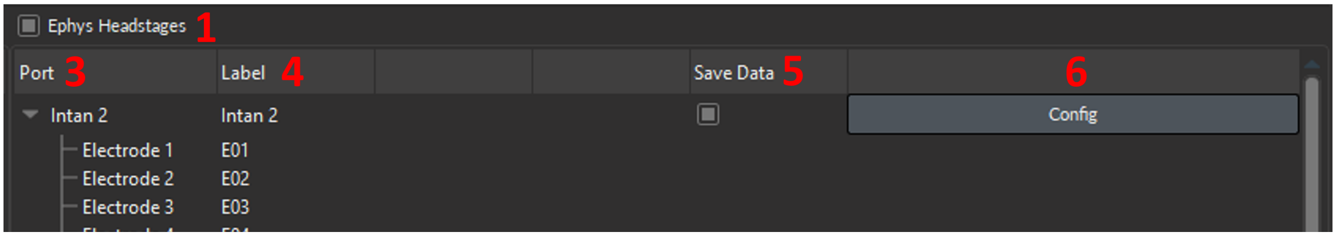
7. The **Serial Number** (Fig. 16.28f, 7) displays the unique identification number assigned to the device, allowing users to select the proper camera when multiple identical models are used.

8. The **Type** (Fig. 16.28f, 8) displays the model types of the camera.

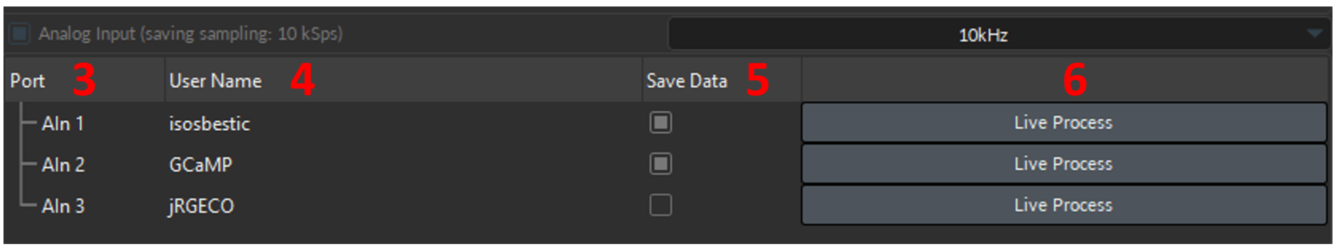
9. The **Format** (Fig. 16.28f, 9) displays the video file format that will be used to save the acquired footage. Currently only **.avi* is available.



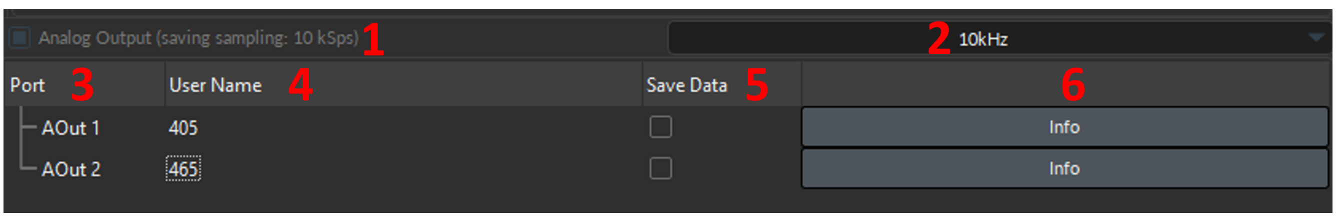
(a) *Microscope*



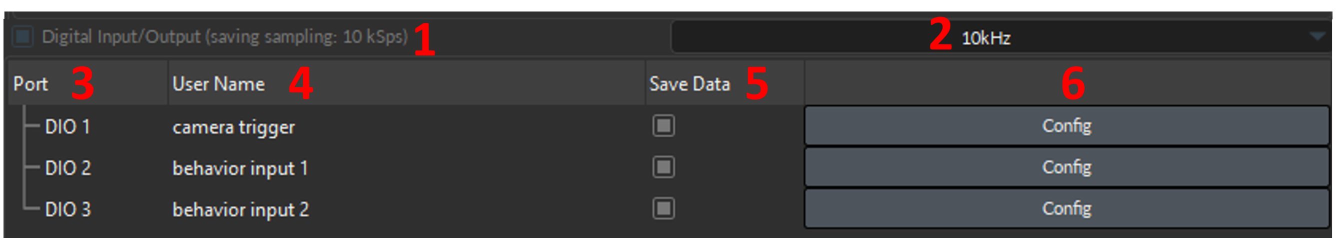
(b) *Ephys*



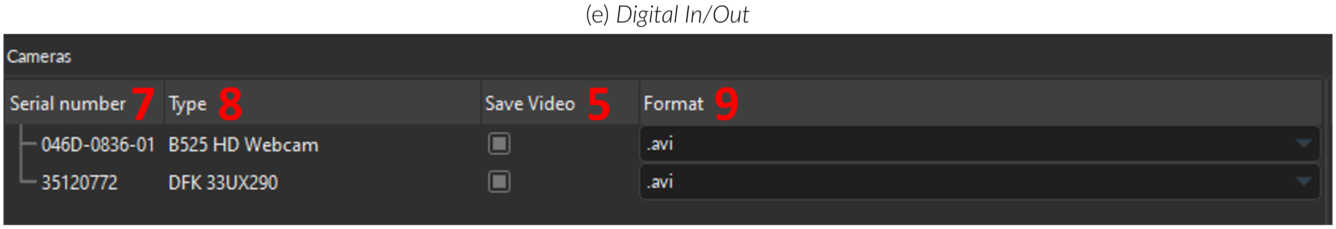
(c) *Analog In*



(d) *Analog Out*



(e) *Digital In/Out*



(f) *Camera*

Figure 16.28: *Configuration, channels organization*

16.6 Channel(s) Configuration

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. 16.23). This will open the **Channel(s) Configuration** window (Fig. 16.29).

Figure 16.29: *Channel Configuration window*

To generate a new **Channel** using the **Channel(s) Configuration** window (Fig. 16.29):

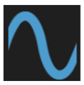
1. Select one of the available **Channel Type** icons from the left most column of the **Channel(s) Configuration** window (Fig. 16.29). Table 16.1 describes the use case of each type.

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 16.1 for hyperlinks to the relevant sections.

3. Select the **Add** button (Fig. 16.29) to generate the defined channel or to update an already configured channel. Note that pressing the **Add** button 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.

Table 16.1: *Types of channels and their use cases*

Icon	Channel Type	Use Case	Section
	Digital I/O	Records and/or generates a binary (TTL) signals commonly used to synchronize data collection	16.6.1
	Analog Output	Generates Analog Output signals between 0-5V, including sine, or customized output sequences	16.6.2
	Analog Input	Records analog signals between -10V and 10V	16.6.3
	Ephys Tethered	Record extracellular electrophysiological signals from tethered electrode array(s)	16.6.4
	Camera(s)	Collect video footage for behaviour experiments	16.6.5
	Rotary Joint(s)	Records the device's rotation to control for motor noise or compute animal motion.	16.6.6
	Event(s)	Manually flag behavior events or stimuli time-locked to the neural recording using customized Keyboard keys	16.6.7
	Microscope	Records single-cell calcium imaging from miniature microscope in moving animals (supports both cortical and deep brain recordings)	16.6.8
	IMU(s)	Inertial Measurement Unit, measures changes in acceleration, head movements and other parameters linked to subject displacement	Coming soon

16.6.1 Digital I/O Channel

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 a displayed stimuli or a measured behavior).

The *Channel(s) Configuration* window for the **Digital I/O** Channel is divided into three sections (Fig. 16.34): (1) the **Channel Options** (Section 16.6.1.1), (2) the **Sequence Options** & (3) **Preview** (both treated in Section 16.6.1.2).

Figure 16.30: *Channel(s) configuration, Digital I/O*

16.6.1.1 Channel Options

The **Channel Options** defines the channel, mode, and source of the digital signal, through **Digital I/O Options** and **Trigger Options**.

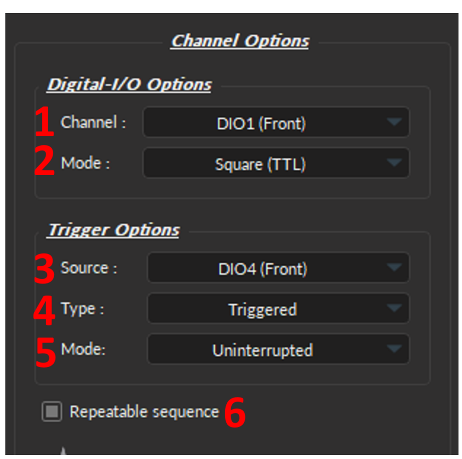
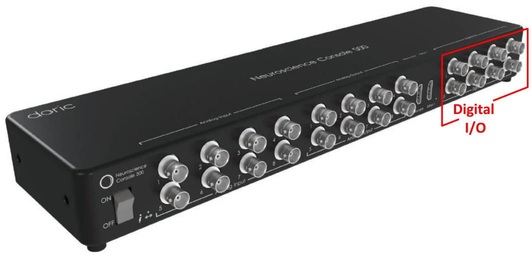


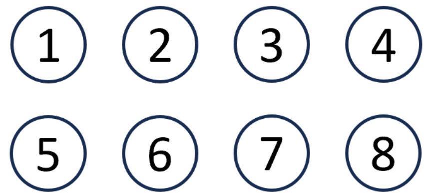
Figure 16.31: *Digital I/O, Channel Options*

Digital I/O Options:

1. The **Channel** (Fig 16.31, 1) identifies the port on the *NC500* assigned to the newly created Digital I/O. Each numbered channel on the physical console corresponds to the same number of digital channels within the software (Fig. 16.32).



(a) *Digital I/O port location*

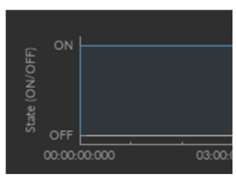


(b) *Port number schematic*

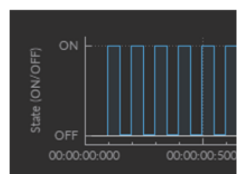
Figure 16.32: *NC500 channel ports*

2. The **Mode** (Fig 16.31, 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. 16.33a);
   - The **Square (TTL)** Mode (Fig. 16.33b);
   - The **Input** mode receives a signal that is 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 16.5, no. 2b). No **Sequence Options** or **Sequence Previews** are available for this mode.



(a) *Continuous*



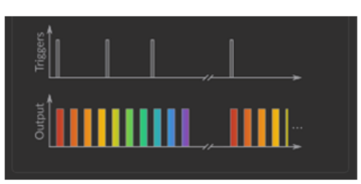
(b) *Square*

Figure 16.33: *DIO Output Modes*

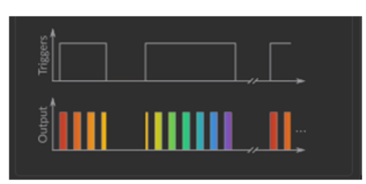
Trigger Options:

3. The **Source** trigger option (Fig 16.31, 3) 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.

4. The **Type** (Fig 16.31, 4) defines the type of trigger that is used to start/stop a sequence. The **Triggered** type can start and stop a sequence at a rising edge while the **Gated** type can start the sequence at a rising edge and stop it at a falling edge (Fig. 16.34). 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 16.35.



(a) *Trigger*



(b) *Gate*

Figure 16.34: *Trigger Options Modes*

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

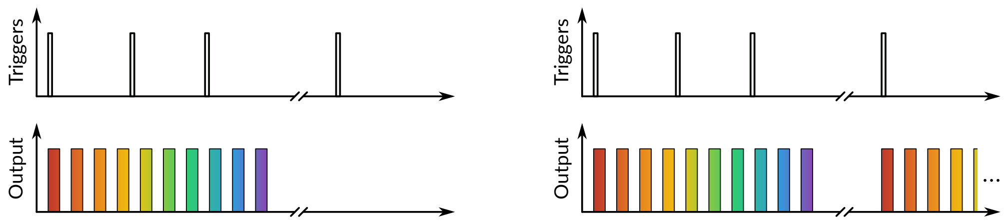
Figure 16.35: *Trigger options possibilities*

- In **Triggered** type (Fig. 16.34a), 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** button is pressed.
- In **Gated** type (Fig. 16.34b), the sequence will start once the voltage reaches 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***

5. The **Mode** (Fig 16.31, 5) field defines how the trigger activates a sequence. Each mode is not compatible with each combination of trigger type and repeatability. Figure 16.35 shows the different available combinations for the different Trigger Modes. Four Modes are available and are the following:

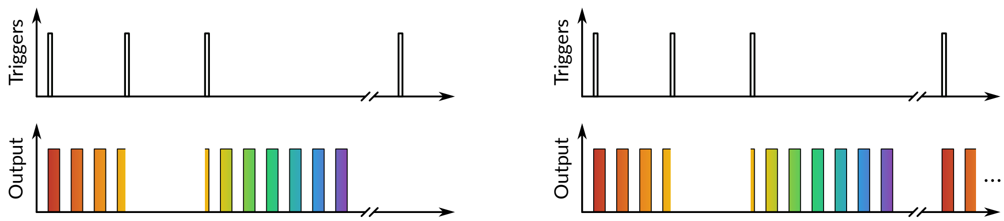
   - **Uninterrupted**: This mode activates the channel sequence when an input greater than 3.3 V is detected by the BNC input. Following input pulses will be ignored while the sequence is running (Fig. 16.36). When the **Repeatable sequence** checkbox is checked, the sequence will restart with the arrival of the first input pulse after the sequence has finished (Fig. 16.36b). 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. 16.37). Following input pulses (when *Triggered*, Fig. 16.37a) or falling edge (when *Gated*, Fig. 16.37c) 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. 16.37b and 16.37d).
   - **Continue**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 16.38). The following input pulse (when *Triggered*, Fig. 16.38a) or a falling edge (when *Gated*, Fig. 16.38c) 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 (Fig. 16.38b and 16.38d).
   - **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. 16.39a) or falling edge (when *Gated*, Fig. 16.39b) 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.

6. The **Repeatable sequence** check-box (Fig 16.31, 6), when selected, allows a sequence to be repeated. Not all modes and trigger types can be repeated. Please refer to the Fig. 16.35 to know the repeatable sequence combinations.

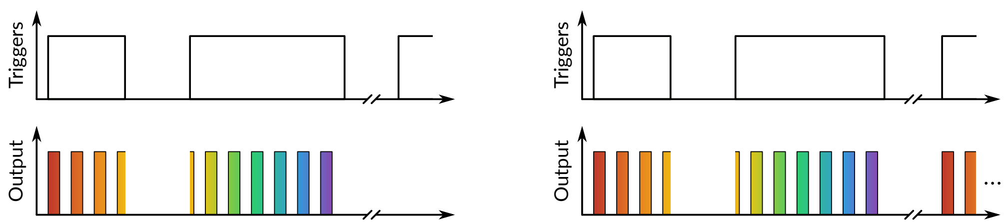


(a) *Triggered Non-Repeatable Sequence* (b) *Triggered Repeatable Sequence*

Figure 16.36: *Uninterrupted Sequence Mode*

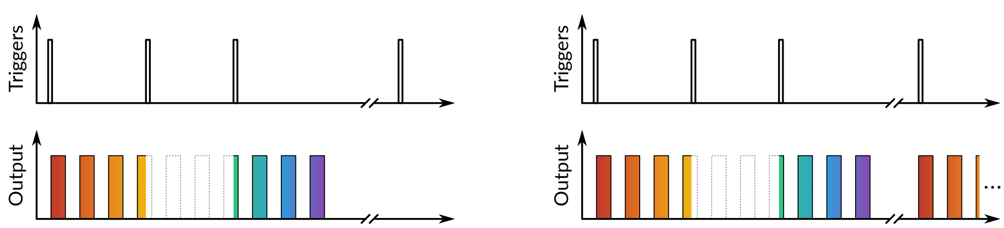


(a) *Triggered Non-Repeatable Sequence* (b) *Triggered Repeatable Sequence*

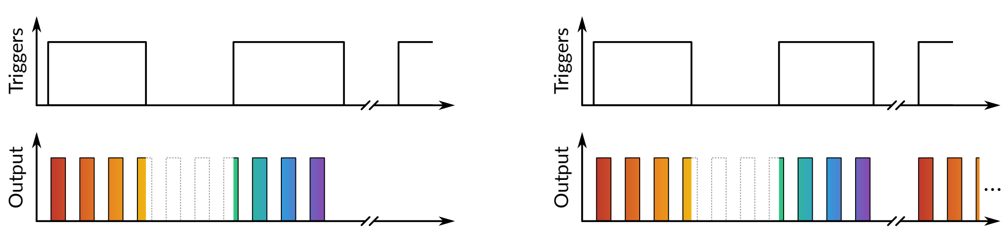


(c) *Gated Non-Repeatable Sequence* (d) *Gated Repeatable Sequence*

Figure 16.37: *Pause Sequence Mode*

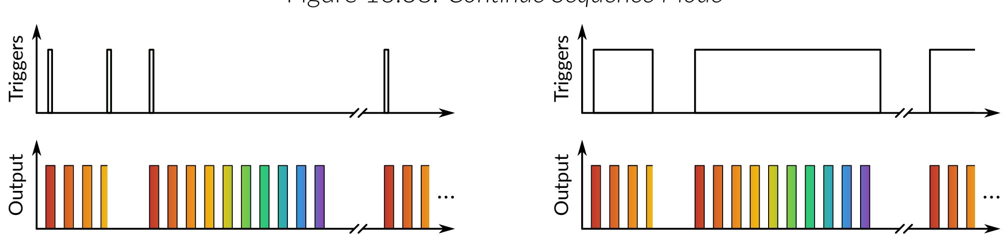


(a) *Triggered Non-Repeatable Sequence* (b) *Triggered Repeatable Sequence*



(c) *Gated Non-Repeatable Sequence* (d) *Gated Repeatable Sequence*

Figure 16.38: *Continue Sequence Mode*



(a) *Triggered Repeatable Sequence* (b) *Triggered Gated Sequence*

Figure 16.39: *Restart Sequence Mode*

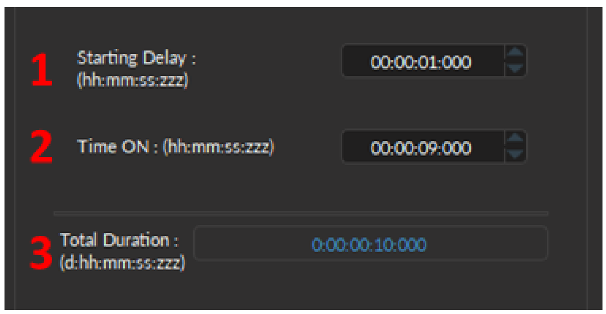
16.6.1.2 Sequence Options & Preview

The **Sequence options** section (Fig. 16.40a) contains the TTL pulse sequence parameters, while the **Sequence Preview** section (Fig. 16.40b) 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**.

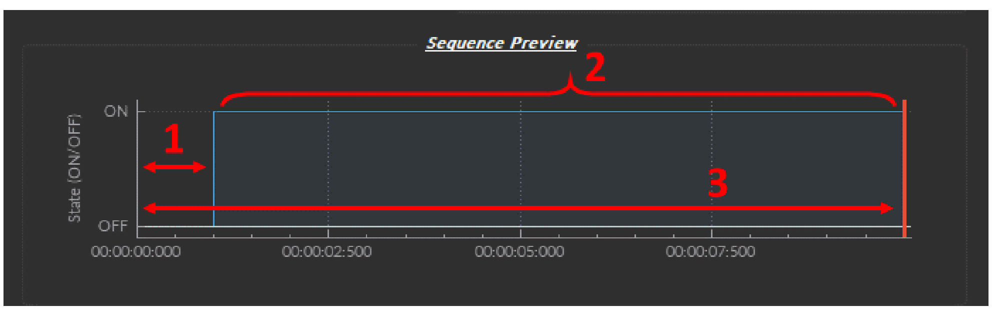
The parameters contained in the **Sequence Options** depend on the **Channel Mode** (selected in **Channel Options**, Fig. 16.31), as following:

- The **CW (Continuous Wave)** channel mode (Fig. 16.34) allows the creation of a continuous TTL pulse sequence. The following elements appear in the **Sequence Options** box.

    1. The **Starting Delay** (Fig. 16.40, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Time ON** (Fig. 16.40, 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. 16.40, 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*



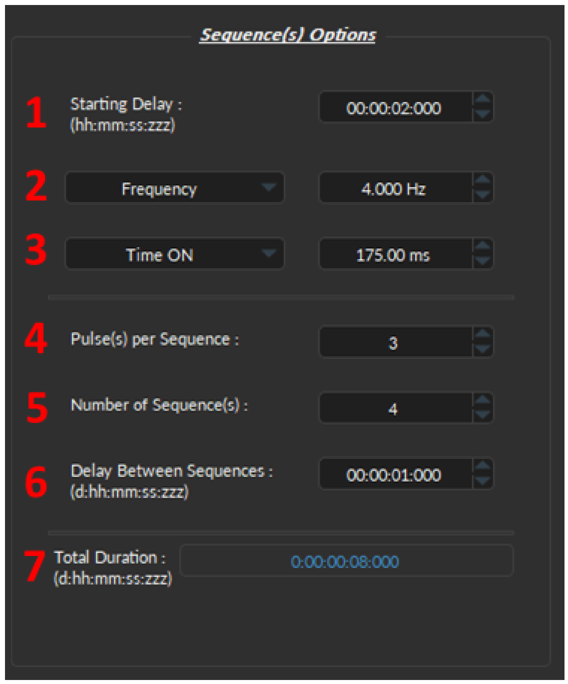
(b) *Sequence Preview*

Figure 16.40: *Channel(s) configuration window, Digital I/O - CW Mode*

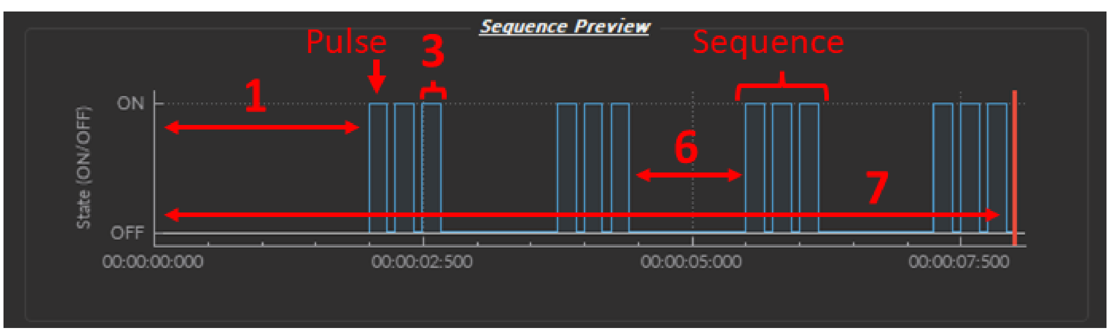
- The **Square** channel mode (Fig. 16.41) allows the creation of a square TTL pulse sequence. This includes the following elements:

    1. The **Starting Delay** (Fig. 16.40, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.
    2. The **Frequency** (Fig. 16.41a, 2) sets the frequency (in Hz), which is the number of pulses per second. The frequency can also be changed to the **Period** (Fig. 16.41a, 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. 16.41, 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. 16.41, 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. 16.41, 5) sets the number of times that the sequence will be repeated.
6. The **Delay between sequences** (Fig. 16.41, 6) sets the amount of time separating any two sequence (excluding the **Starting Delay**).
7. The **Total Duration** (Fig 16.41, 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 16.41: *Channel(s) configuration window, Digital I/O - Square Mode*

16.6.2 Analog Output Channel

The **Analog Output** channel type creates analog pulse sequences, between 0-5V. 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**, and **Custom** (Fig. 16.45).

Figure 16.42: *Channel(s) Configuration, Analog Output*

The **Channel Options** defines the channel, source, and mode of the digital signal, through **Analog Output Options** and **Trigger Options**.

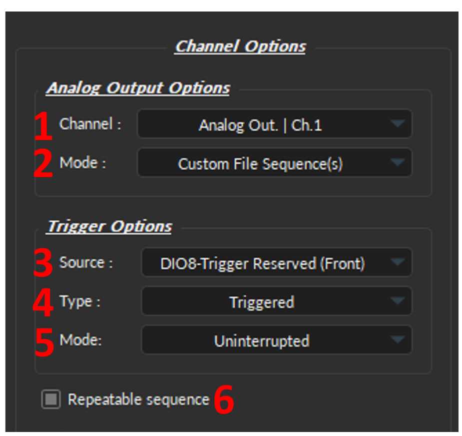
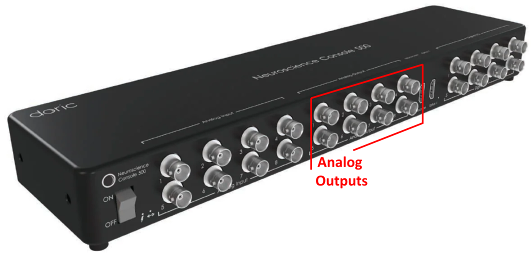


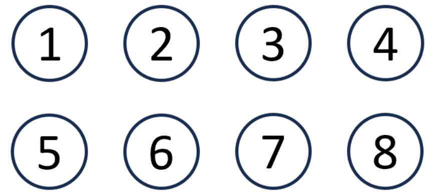
Figure 16.43: *Analog Output, Channel Options*

Analog Output Options:

1. The **Channel** (Fig 16.43, 1) identifies the port on the *NC500* assigned to the newly created Analog Output. Each numbered channel on the physical console corresponds to the same number of analog channels within the software (Fig. 16.44).



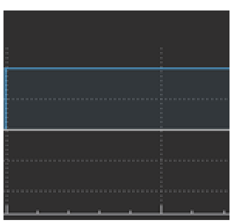
(a) *Analog Output ports*



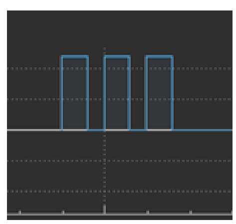
(b) *Port number schematic*

Figure 16.44: *NC500 channel ports*

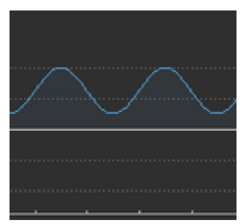
2. The **Mode** (Fig 16.43, 2) identifies the shape of the output sequence (Fig. 16.45). The **Sequence Option** are different depending on the mode. Thus each mode is treated in the following sections:
   - The **Continuous wave (CW)** mode (Fig. 16.45a) - Section 16.6.2.1.
   - The **Square (TTL)** mode (Fig. 16.45b) - Section 16.6.2.2.
   - The **Sine** mode (Fig. 16.45c) - Section 16.6.2.3.
   - The **Custom File Sequence(s)** mode (Fig. 16.45d) - Section 16.6.2.4.



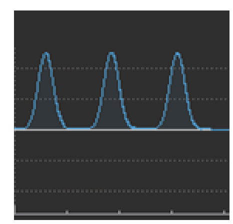
(a) *Continuous*



(b) *Square*



(c) *Sine*

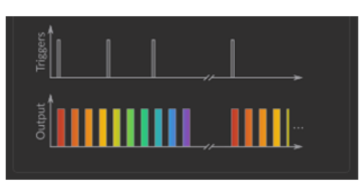


(d) *Custom Sequence(s)*

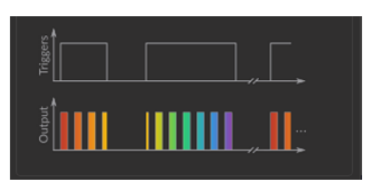
Figure 16.45: *Analog Output Modes*

Trigger Options:

3. The **Source** trigger option (Fig 16.43, 3) allows the choice of a **Manual Trigger** (activated by using the **Record**/**Live** buttons) or an **Input** trigger, coming from a **Digital I/O** channel set in input mode (Fig. 16.32).
4. The **Type** (Fig 16.43, 4) defines the type of trigger that is used to start/stop a sequence. The **Triggered** type can start and stop a sequence at a rising edge while the **Gated** type can start the sequence at a rising edge and stop it at a falling edge (Fig. 16.46). A more refined interaction of the trigger with the defined sequence can be set up using the **Mode** field. Not all Trigger Type is available for each combination of Trigger Mode and Repeatability. The different combinations are shown in Figure 16.47.



(a) *Triggered*



(b) *Gated*

Figure 16.46: *Trigger Options Modes*

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

Figure 16.47: *Trigger options possibilities*

- In **Triggered** type (Fig. 16.46a), 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** type (Fig. 16.46b), the sequence will start once the voltage reaches 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***

5. The **Mode** (Fig 16.43, 5) field defines how the trigger activates a sequence. Each mode is not compatible with each combination of trigger type and repeatability. Figure 16.47 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. 16.36). 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. 16.36b). 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. 16.37). Following input pulses (when *Triggered*, Fig. 16.37a) or falling edge (when *Gated*, Fig. 16.37c) 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. 16.37b and 16.37d).
   - **Continue**: This mode activates the channel sequence when a rising edge greater than 3.3 V is detected on the BNC input (Fig. 16.38). The following input pulse (when *Triggered*, Fig. 16.38a) or a falling edge (when *Gated*, Fig. 16.38c) 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 (Fig. 16.38b and 16.38d).
   - **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. 16.39a) or falling edge (when *Gated*, Fig. 16.39b) 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.

6. The **Repeatable sequence** check-box (Fig 16.43, 6), when selected, allows a sequence to be repeated. Not all modes and trigger types can be repeated. Please refer to the Fig. 16.47 to know the repeatable sequence combinations.

16.6.2.1 Continuous Wave (CW) Mode

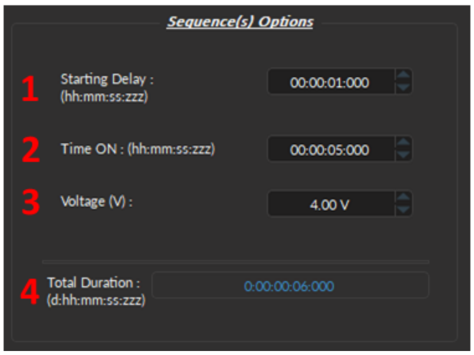
The **CW (Continuous wave)** channel mode (Fig. 16.48) allows the creation of a continuous analog signal. The following elements appear in the **Sequence Options** box (Fig. 16.48a).

1. The **Starting Delay** (Fig. 16.48, 1) defines the time between the activation of the sequence and the beginning of the signal.

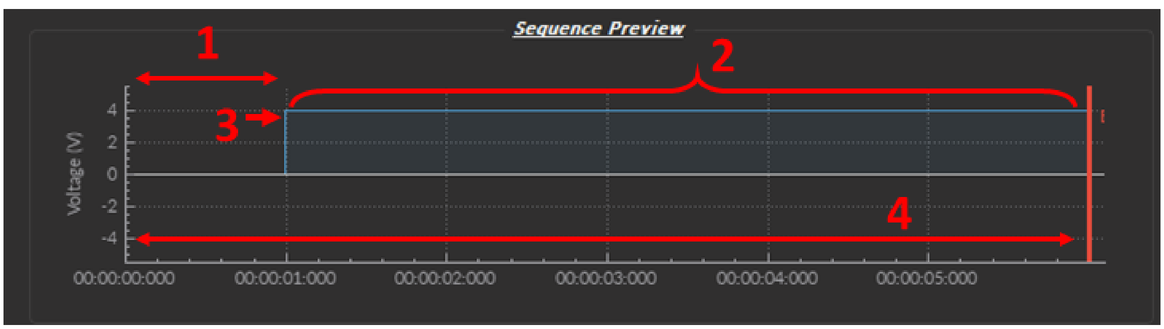
2. The **Time ON** (Fig. 16.48, 2) defines the length of time the continuous signal is active. Should the time chosen be 0, the signal will continue until the pulse sequence is stopped manually.

3. The **Voltage** (Fig. 16.48, 3) defines the voltage of the continuous signal, in Volts. The signal cannot go beyond +5 V.

4. The **Total Duration** (Fig. 16.48, 4) shows the total expected duration of the 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 16.48: *Channel(s) Configuration, Analog Output CW*

16.6.2.2 Square Mode

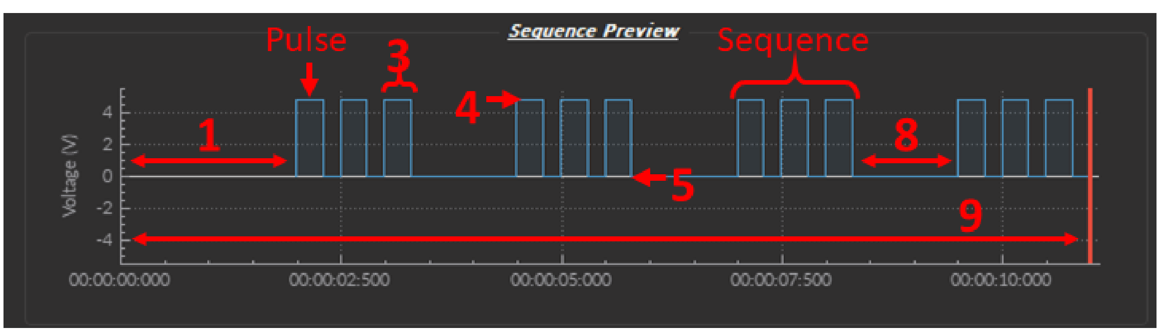
The **Square** channel mode (Fig. 16.49) creates a sequence of pulses with the minimum of the pulses at **V-** and the maximum of each pulse at **V+**.

1. The **Starting Delay** (Fig. 16.49, 1) defines the time between the activation of the pulse sequence and the beginning of the signal.

2. The **Frequency** (Fig. 16.49, 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. 16.49, 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. 16.49, 4) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +5 V.
5. The **Minimum Voltage (V-)** (Fig. 16.49, 5) defines the minimum voltage of each pulse, in volts. The signal cannot go below 0 V.
6. The **Pulse(s) per sequence** (Fig. 16.49, 6) sets 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. 16.49, 7) sets the number of times that the sequence will be repeated.
8. The **Delay between sequences** (Fig. 16.49, 8) sets the delay between each sequence.
9. The **Total Duration** (Fig. 16.49, 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*



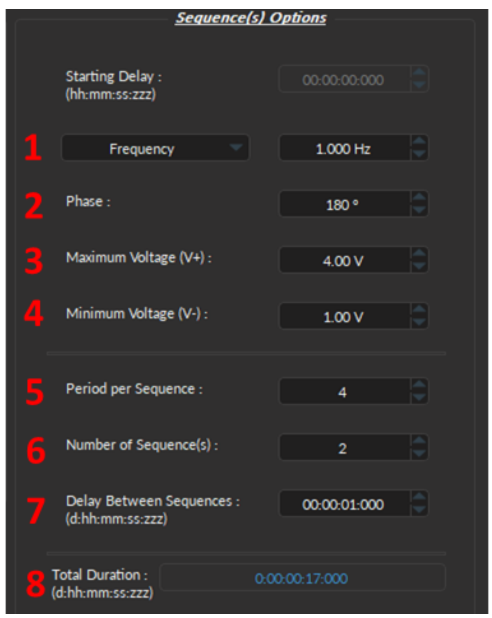
(b) *Sequence Preview*

Figure 16.49: *Channel(s) Configuration, Analog Output Square*

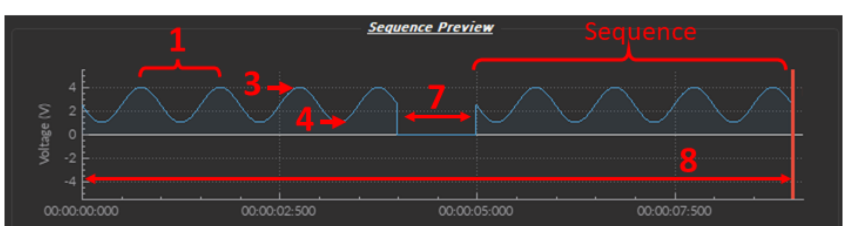
16.6.2.3 Sine Mode

The **Sine** mode (Fig. 16.50) creates a sinusoidal pulse sequence with peaks at **V+** and **V-** by specifying the following:

1. The **Frequency** (Fig. 16.50, 1) 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 sine wave every 100 ms (period), whereas a signal at 0.5 Hz (frequency) will output one sine wave every 2 seconds (period).
2. The **Phase** option (Fig. 16.50, 2) replaced **Time ON** (Fig. 16.49, 3). This allows the choice of the sine wave phase, in degrees.
3. The **Maximum Voltage (V+)** (Fig. 16.50, 3) defines the maximum voltage of each pulse, in volts. The signal cannot go beyond +5.0 V.
4. The **Minimum Voltage (V-)** (Fig. 16.50, 4) defines the minimum voltage of each pulse, in volts. The signal cannot go below 0 V.
5. The **Period per Sequence** (Fig. 16.50, 5) is similar to the **Pulse per Sequence** parameter in Square mode (Section 16.6.2.2, Square), but there the period is a single sine wave from peak to peak (Fig. 16.50b, 1).
6. The **Number of Sequence(s)** (Fig. 16.50, 6) sets the number of times that the sequence will be repeated.
7. The **Delay Between Sequences** (Fig. 16.50, 7) sets the delay between each sequence.
8. The **Total Duration** (Fig. 16.50, 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*



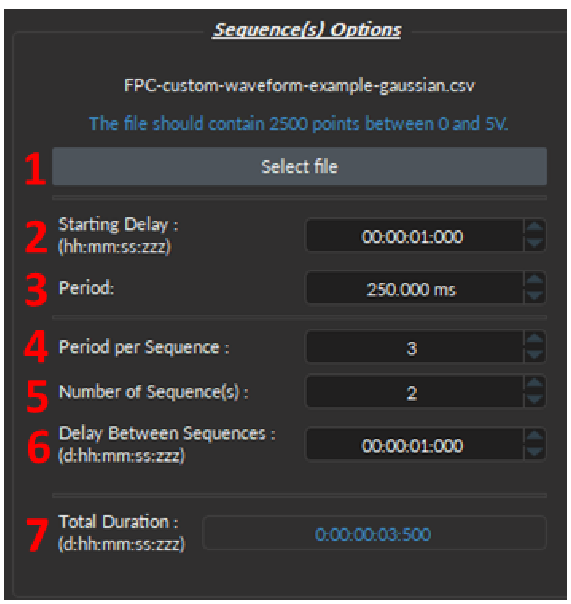
(b) *Sequence Preview*

Figure 16.50: *Channel(s) Configuration, Analog Output Sine*

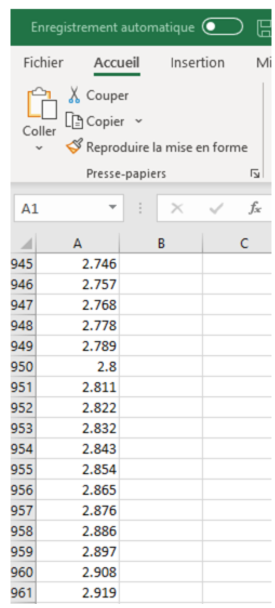
16.6.2.4 Custom File Sequence(s) Mode

The **Custom File Sequence(s)** mode (Fig. 16.51) provides a way to import a customized pulse sequence with a non-standard shape to fit experimental needs.

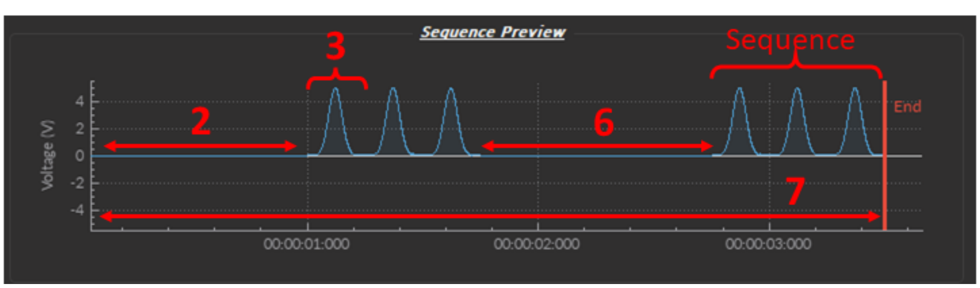
1. The **Select File** button (Fig. 16.51a, 1) is used to input a custom .csv file containing the data for the sequence. This must be a .csv format and requires 2500 values in column vector format (i.e. with *line break* between values), as in Fig. 16.51b. The values can be any value between **0V** and **+5V**.
2. The **Starting Delay** (Fig. 16.51, 2) defines the time between the activation of the sequence and the beginning of the signal.
3. The **Period** option (Fig. 16.51, 3) replaces the **Time ON** option (Fig. 16.49, 3). This option will stretch or shrink the 2500 value sequence to fit the specified amount of time.
4. The **Period per Sequence** (Fig. 16.51, 4) is similar to the **Pulse per Sequence** field found in **Square** modes (Fig. 16.49, 6), where the pulse is replaced by the period sequence (Fig. 16.51c, Sequence).
5. The **Number of Sequence(s)** (Fig. 16.51a, 5) sets the number of times that the sequence will be repeated.
6. The **Delay Between Sequences** (Fig. 16.51, 6) sets the delay between each sequence.
7. The **Total Duration** (Fig. 16.51, 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 16.51: *Channel(s) Configuration, Analog Out - Custom*

16.6.3 Analog Input Channel

The **Analog Input** channel type acquires analog data between -10V and 10V. This port can be used to record data from any analog devices/sensors such as Piezo sensors, photodetectors, or behavior/stimuli-related analog devices. Note that several preset options are available for **Analog Input**-type data, including **Filters**, **Lock-In**, and **Ephys** preset configuration, which can be found in the **Live Processing** section (see Table 16.3).

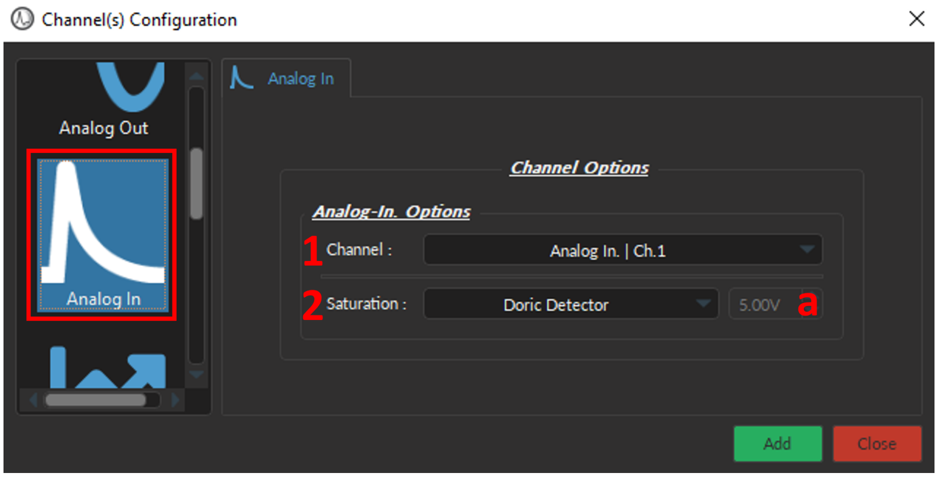


Figure 16.52: *Channel(s) Configuration, Analog Input*

The *Channel(s) Configuration* window for the **Analog Input** includes the following parameters:

1. The **Channel** (Fig. 16.52, 1) identifies which of the ports receives the analog input (Fig. 16.53a).

(a) *Analog Input ports*

(b) *Port number schematic*

Figure 16.53: *NC500 channel ports*

2. The **Saturation** (Fig. 16.52, 2) specifies the maximal voltage value (Fig. 16.52, a) that saturates the sensor/detector. Correctly setting this value is important since different devices support different maximal voltages and the **Saturation** value is used to warn users when the collected signal is near this maximum value, as in Fig. 16.18. See Table 16.2 for common detector types and their corresponding saturation levels. Use the **AIn** option to set the maximum saturation value (10V) handled by the *NC500*, or select the **Custom** option to set a specific saturation value between 0V and 10V.

Table 16.2: *Saturation of Preset Detectors*

Detector	Saturation
Doric detector	5V
Newport Detector	7.5V
Hamamatsu C10709	5V
AIn Channel	10V
Custom	0-10V

16.6.4 Ephys Tethered Channel

The **Ephys Tethered Channel** records extracellular electrophysiological signals from tethered multi-electrode array(s) (16 to 64 channels).

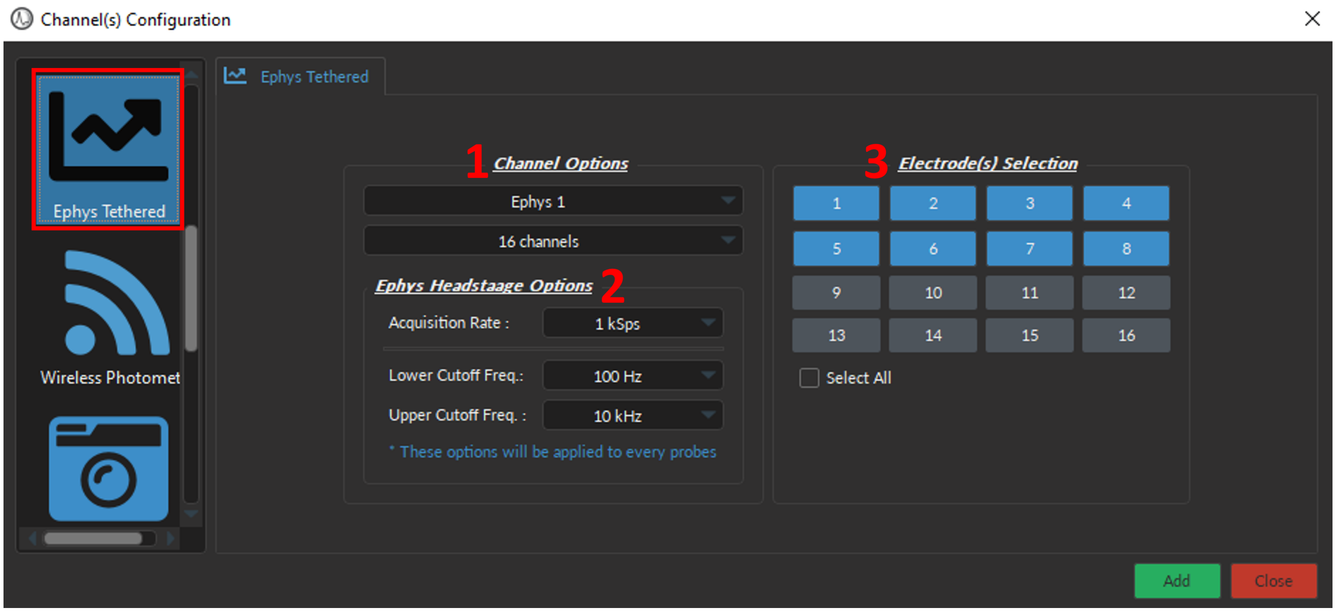


Figure 16.54: *Channel(s) configuration, Ephys Tethered*

1. The **Channel Options** (Fig. 16.54, 1) includes the following parameters:
   - **Ephys Port** - specifies which Ephys port (Ephys 1: front port, Ephys 2: back port) on the *NC500* is used for the current channel configuration.
   - **Channel Number** - specifies the number of channels contained on the multi-channel electrodes array connected to the *NC500*. Three options are currently available: 16, 32, and 64 channels.
2. The **Ephys Headstage Options** (Fig. 16.54, 2) includes the following parameters:
   - The **Acquisition Rate** - defines the sample rate of the electrophysiological data, which can range between 1 - 20 kSps.
   - The **Lower Cutoff Frequency** - defines the bandpass values, such that frequencies below the cutoff will be filtered from the data. This can be used to remove multi-unit activity, if unwanted.
   - The **Upper Cutoff Frequency** - defines the bandpass values, such that frequencies above the cutoff will be filtered from the data. This is useful to remove high-frequency noise.
3. The **Electrode(s) Selection** (Fig. 16.54, 3) selects which of the total **Channel Number** are enabled (in blue). Allows users to disable (greyed out) broken electrode sites or electrodes that are not in the region of interest. No data will be collected from the disabled electrode(s). Use the **Select All** checkbox to enable all electrodes simultaneously.

16.6.5 Camera Channel

It is natural to pair Doric neural recordings with behavior recording. Many behaviors, especially freely moving behaviors, require camera inputs for their measurement.

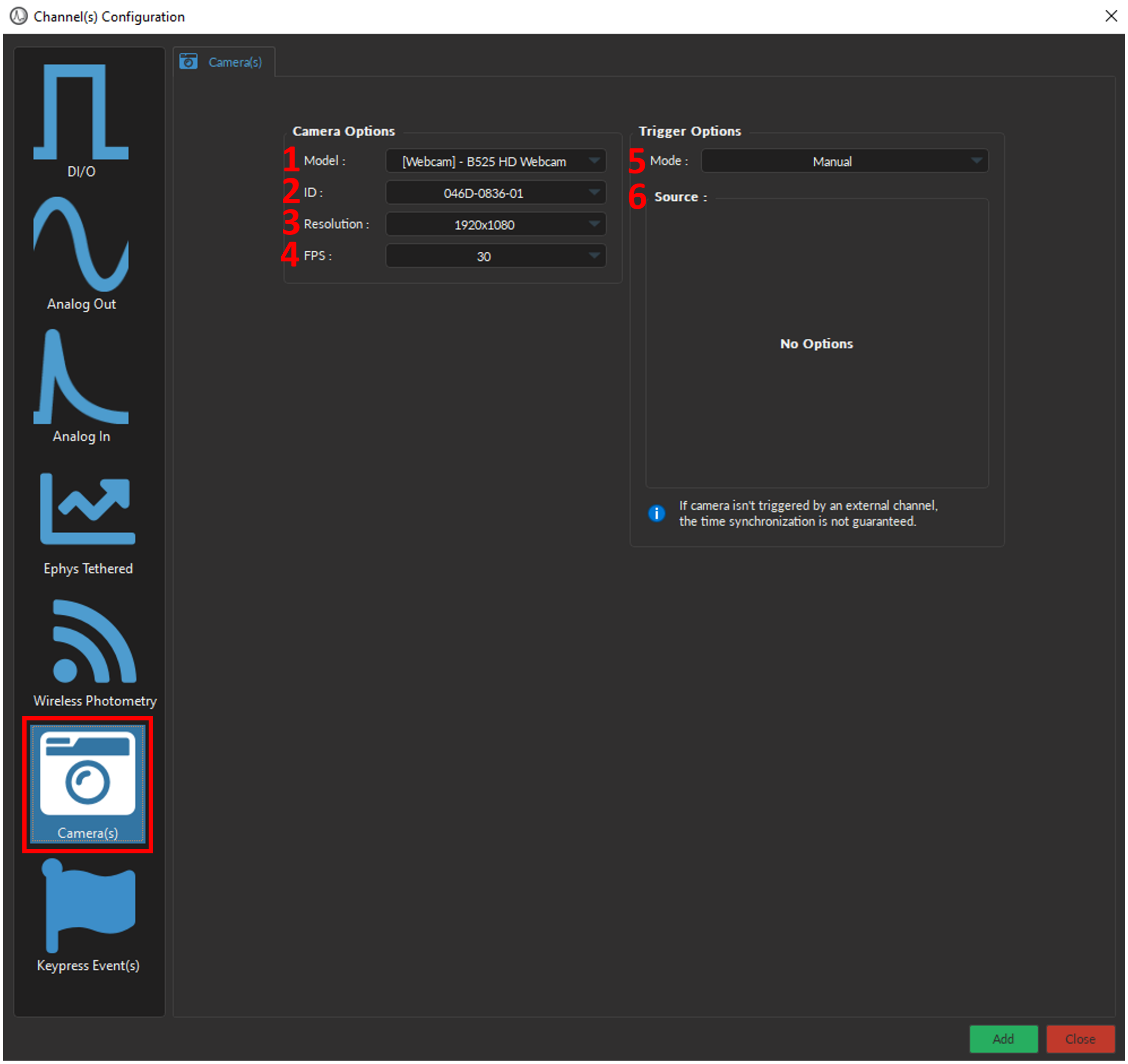


Figure 16.55: *Channel(s) configuration window, Camera*

WARNING:
A camera cannot be used for BOTH **NC500** and **Camera** modules.
When creating a Camera Channel, if *No available camera detected...*, disconnect the camera in the **Device Selection** window to close the extra module.

Camera Options:

1. The **Model** (Fig. 16.55, 1) allows you to select the camera of choice based on the type of camera. Note that a *Doric Behavior Camera* provides DIO synchronization option (such as **External** and **External Preconfigured** modes) that are not available with a typical *USB 3.0* web camera.

2. **ID** drop-down list (Fig. 16.55, 2) is used to select a camera based on its unique ID. The ID is beneficial when multiple cameras of the same model are required for the experiment.
3. The **Resolution** (Fig. 16.55, 3) is used to set the size of the image. The larger 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. 16.55, 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. 16.55, 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 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.*** A delay of around 1 second 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. 16.56). This attribute can be used to retroactively align the video and fiber photometry data during analysis.

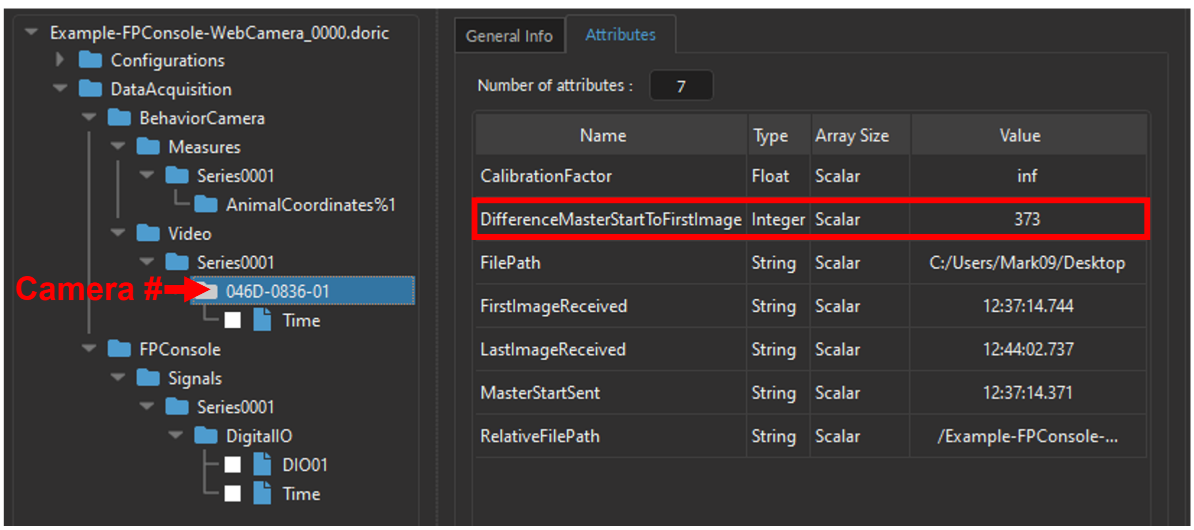


Figure 16.56: *Doric File Editor, Web Camera Attributes - Video Alignment Variable*

- **External** - This mode will drive the camera using external TTL signals 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. 16.55, 6 & Fig. 16.57, 6) 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. 16.57). For a detailed description of each Digital I/O parameter see Section 16.6.1. Briefly, key parameters include:

a) The **Channel** (Fig. 16.57, a) corresponds to the physical Digital I/O channel number on the NC500 that is connected to the trigger cable of the *Doric Behavior Camera*.

b) The **Mode** (Fig. 16.57, b) is by default set to *Square (TTL)*, which provides the external trigger signal to the camera. This parameter cannot be changed.

c) The **Frequency** (Fig. 16.57, 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. 16.57, d) is by default 50%. The frame will be taken at the start of each square pulse.

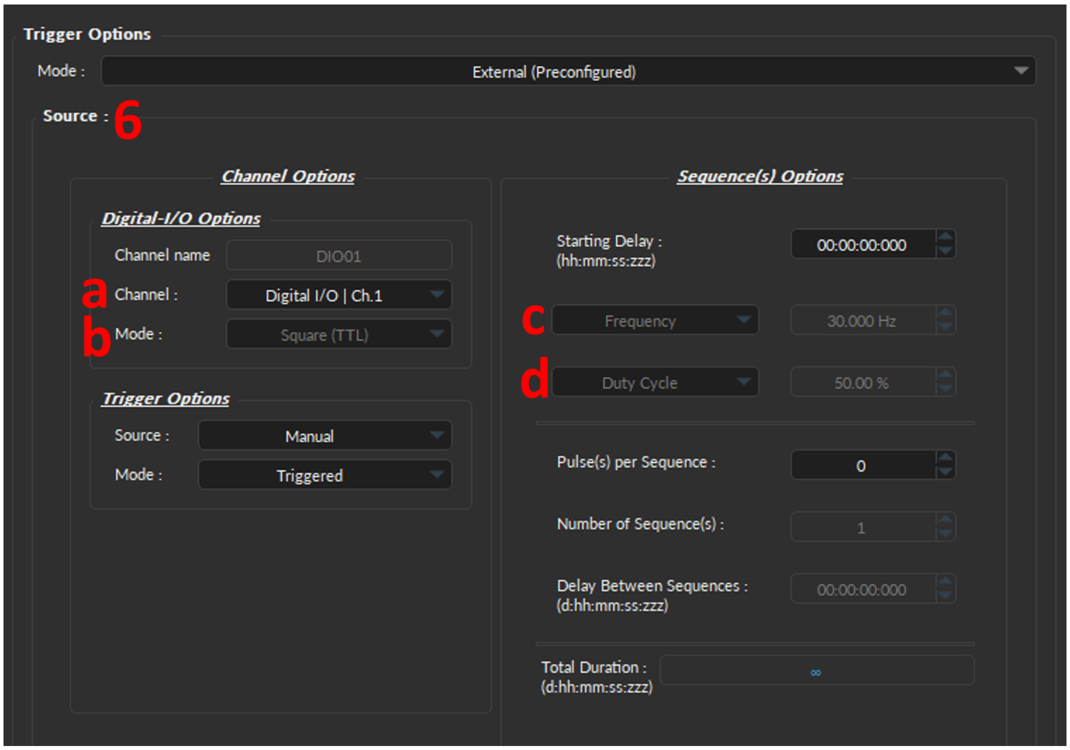


Figure 16.57: *Channel(s) configuration window, Camera - External (Preconfigured)*

16.6.6 Rotary Joint(s) Channel

Adding a **Rotary Joint** channel allows an easy way to control and record when the device's motor is turned on. This can be especially important when even small noise can impact experiments. Once the channel is added, a new **Assisted Rotary Joint** tab is automatically added in the **Control & Settings** of the interface. See Section 16.12 for more details.

The following rotary joint options must be specified when adding the channel:

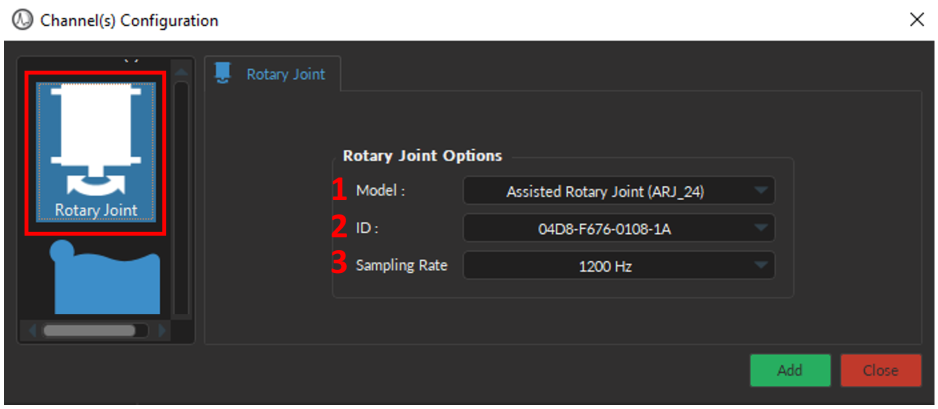


Figure 16.58: *Rotary Joint channel*

1. The **Model** (Fig. 16.58, 1) displays the type of rotary joint available. Assisted rotary joints (specifically *ARJ24*), when connected to the computer, will be automatically detected. Compatible devices include:
   - Assisted 1x1 Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 1x2 Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 2x2 Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 1x1 Pigtailed Fiber-optic & Electric Rotary Joints - 24 contacts
   - Assisted 2x2 Pigtailed Fiber-optic & Electric Rotary Joints - 24 contacts
2. The **ID** (Fig. 16.58, 2) displays the serial number of the rotary joint in question, which identifies the proper device when more than one rotary joint of the same model is connected to the computer.
3. The **Sampling Rate** (Fig. 16.58, 3) specifies the number of data points per second that are saved in the *.doric* file. The **Sampling Rate** can range between 10 Hz - 1200 Hz.

16.6.7 Events Channel

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.

<u>WARNING:</u>
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 **Event**, select the + sign at the bottom of the window (Fig. 16.59, left). To remove a KeyPress Event, use the - button (Fig. 16.59, right).

- <u>Note:</u> Selecting the + button (without clicking the *Add* button or the *Close* button of the *Channel Configuration* window) will **automatically** add the Event channel at the **bottom** of the Acquisition View window, below any pre-existing channels (Fig. 16.59).

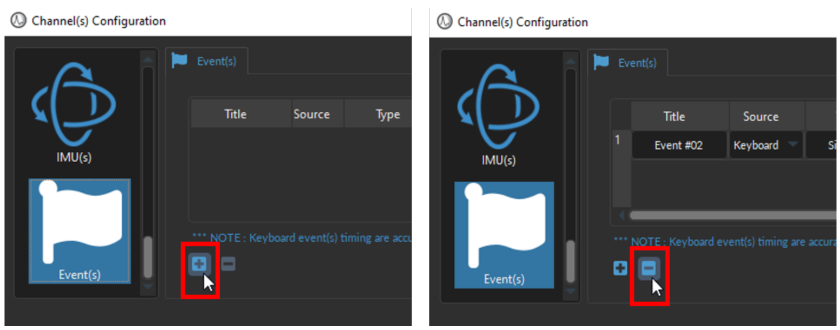


Figure 16.59: *Adding and Removing Events*

To edit a pre-existing **Event** Channel, select the left button (Fig. 16.60) in the **Acquisition View**.

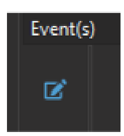
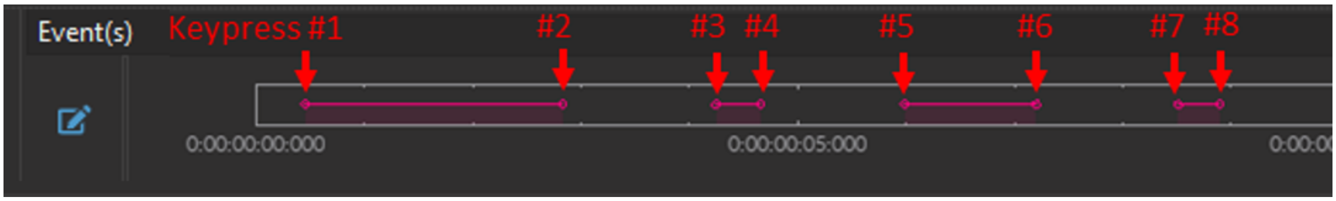


Figure 16.60: *Edit Keypress Event(s) Channel*

The following are the configurable parameters of a **Keypress Event,** per Fig. 16.62:

1. The **Title** (Fig. 16.62, 1) allows you to give a name for the Keypress event.
2. The **Source** (Fig. 16.62, 2) is by default *Keyboard*. However, when a DIO channel was added to the configuration and set in *Input Mode*, it is possible to select this DIO as being the source of the created Event.
3. Three **Types** of Event(s) (Fig. 16.62, 3) can be specified with the drop-down list:
   - **Single** - Records single event at the touch of a key (Fig. 16.61a).
   - **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. 16.61b).
   - **Timed** - Records an event for a predetermined duration of time (Fig. 16.61c). Every keypress is a new event, with the start of the event occurring when the key was depressed.

(a) *Single*



(b) *Toggled*

(c) *Timed*

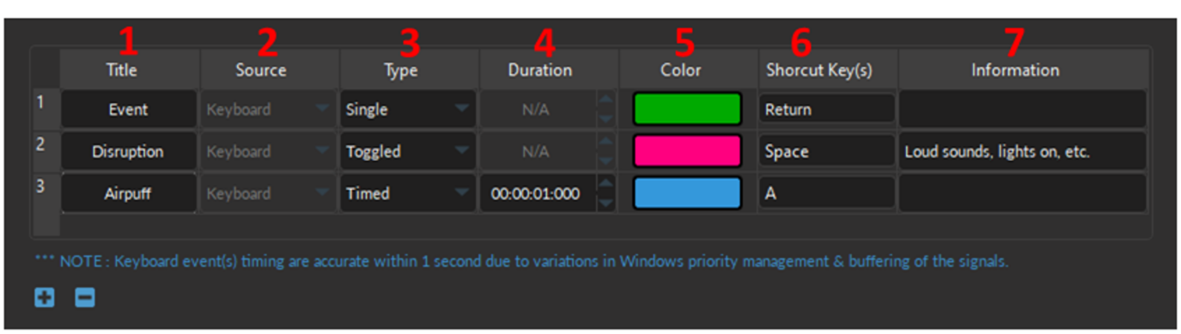
Figure 16.61: *Three types of Event(s)*

4. The **Duration** (Fig. 16.62, 4) is only used for the **Timed** Event 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 (Fig. 16.62, 5) to open the **Select Color** window. Basic colors are provided, in addition to custom colors that can be created and stored.

6. The **Shortcut Key(s)** (Fig. 16.62, 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 is properly set, it will appear in the *Shortcut Key(s)* cell (as in Fig. 16.62, column 6).

7. The **Information** column (Fig. 16.62, 7) provides space to make notes or write a short description of the Event.



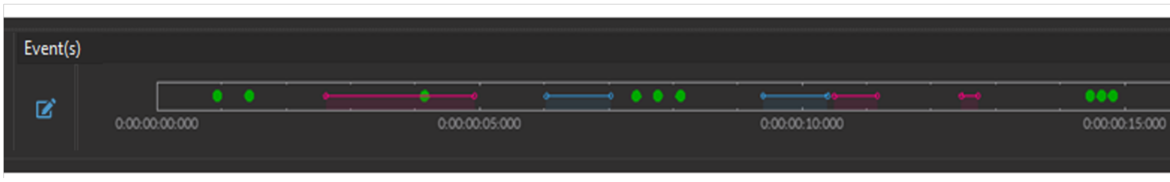


Figure 16.62: *Channel(s) configuration window, Event(s) channel*

16.6.8 Microscope Channel

The **Microscope** channel interfaces with *Doric* Gen 3.0 miniature-microscopes (such as the *eFocus*) and enables calcium imaging with cellular resolution over a larger brain area in freely-moving behaving animals. Adding this type of channel will automatically add **Microscope Control** (Section 16.8) and **Microscope Imaging Options** (Section 16.9) tabs to the **Control & Settings**.

<u>NOTE:</u> This channel option is only available in the **Channel Configuration** window once a miniature microscope is connected to the *Microscope* port of the *NC500*. If the miniature microscope was connected <u>after</u> the *NC500* was powered on, power cycle (turn OFF, then back ON) the *NC500* and try adding the channel once more.

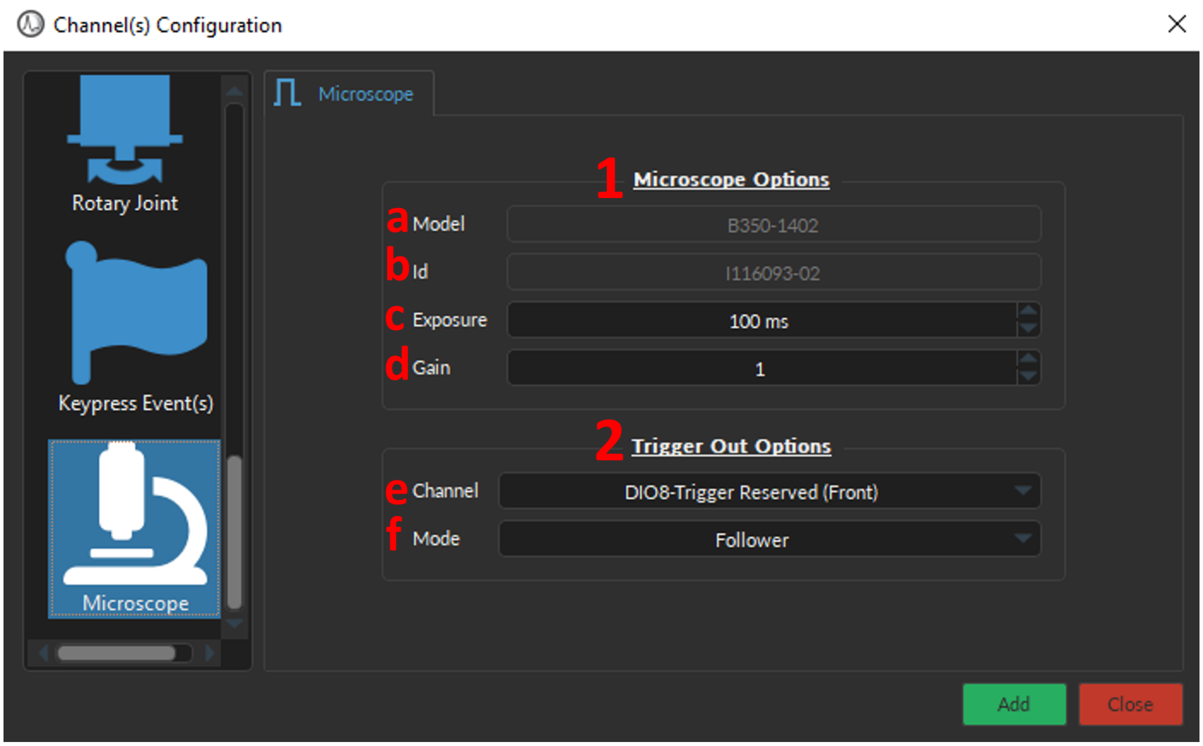


Figure 16.63: *Channel(s) configuration window, Microscope*

To create a **Microscope** channel, specify the following:

1. The **Microscope Options** (Fig. 16.63, 1) includes:

   a) The **Model** (Fig. 16.63, a) automatically detects the type of miniature microscope connected to the *NC500* port.

   b) The **ID** (Fig. 16.63, b) displays the serial number of the miniature microscope connected to the *NC500* port.

   c) The **Exposure** (Fig. 16.63, c) specifies the length of time (in ms) that the microscope sensor collects light from the sample. There are trade-offs between exposure time, image brightness, and phototoxicity.

   d) The **Gain** (Fig. 16.63, d) sets the relative amplification measure applied to the sensor. Note that increasing the gain will simultaneously increase both the signal and noise. Four options of gain (1-4) are available.

2. The **Trigger Out Options** (Fig. 16.63, 2) outputs a TTL train from the **Digital Output** port on the *NC500* to synchronize other experiment devices. Two parameters must be specified:

   e) The **Channel** (Fig. 16.63, e) sets the DIO port number that outputs the TTL pulse when the microscope is recording. By default, **DIO8 (front)** is reserved for this purpose.

   f) The **Mode** (Fig. 16.63, f) specifies the shape or pattern of TTL output:

   - **Follower** - outputs a signal that is continuously ON during the entirety of the recording.
   - **Each Frame** - outputs a TTL pulse at every time point when an image is captured.

16.7 Live Processing

The **Live Processing** Section offers preset configurations that process the data in real-time and save both the raw data and the processed output. These **Live Processing** calculations include filters, decimation, and demodulation.

The **Live Processing** Section is split into two sections (Fig. 16.64), each of which being treated in the following sections:

1. The **Live Processing Tab** - Section 16.7.1
2. The **Live Processing List** - Section 16.7.2

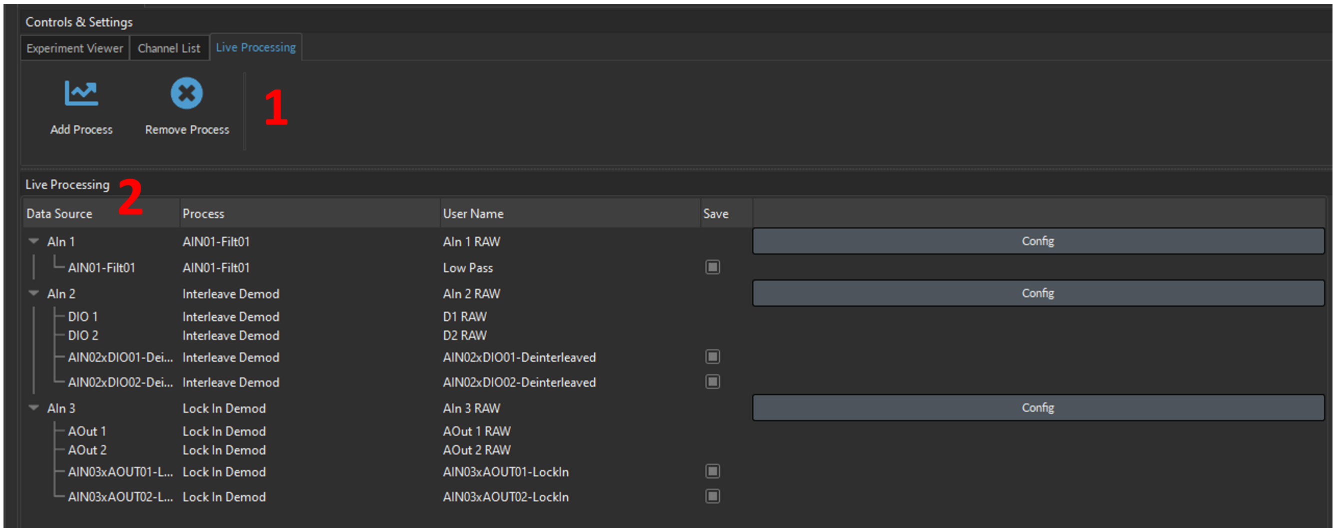


Figure 16.64: *Live Processing*

16.7.1 Live Processing Tab

The **Live Processing** tab contains buttons to add or remove pre-configured *Processes*, including the following:

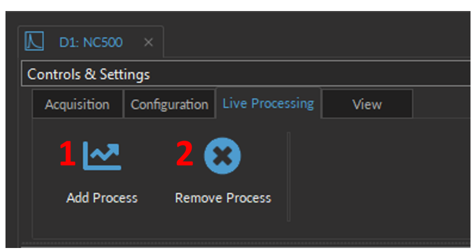


Figure 16.65: *Live Processing Tab*

1. The **Add Process** button (Fig. 16.65, 1) creates preset configurations where a live computation (such as filter, decimation, and/or demodulation) is applied in real-time as the data is collected. Some preset options create both input and output channels required for the process (e.g. **Lock-In**, **Interleaved**, and **Ephys**). Each preset configuration is treated in separate sections, as in Table 16.3.

2. The **Remove Process** button (Fig. 16.65, 2) deletes the selected preset. Only one preset option (and all its dependencies) can be deleted at a time. This option is permanent and cannot be recovered unless the settings were already saved using the **Save Configuration** button (Fig. 16.23, 4).

Table 16.3: *Types of presets and their use cases*

Icon	Live Process	Description	Application	Section
	Filters	Real-time data filtering to visualize signal without noise or undesired frequencies.	Photometry or Ephys	16.7.1.1
	Interleaved	Alternates both LED excitations and inputs recording.	Bundle Fiber Photometry	16.7.1.2
	LockIn	Simultaneously drive LEDs and demodulate signals.	Basic Fiber Photometry	16.7.1.3
	Ephys	Set individual filters for each electrode within a multi-unit electrode array.	Electrophysiology	16.7.1.4

16.7.1.1 Filters

The **Filters** pre-set (Fig. 16.66) applies a filtering operation over raw data in real-time. This pre-configured process is used to remove noise and/or unimportant frequencies from the **Analog Input** data, including for electrophysiology and fiber photometry applications.

However, we recommend testing out presets specially designed for those neuroscience techniques before customizing your own filters, as they cover the most common uses:

- *Ephys*: Sections 16.7.1.4.
- *Photometry*: Sections 16.7.1.2 & Sections 16.7.1.3 (**Interleaved** and **LockIn** presets, respectively).

The **Filter** parameters are separated into two sections (Fig. 16.66) and include the following:

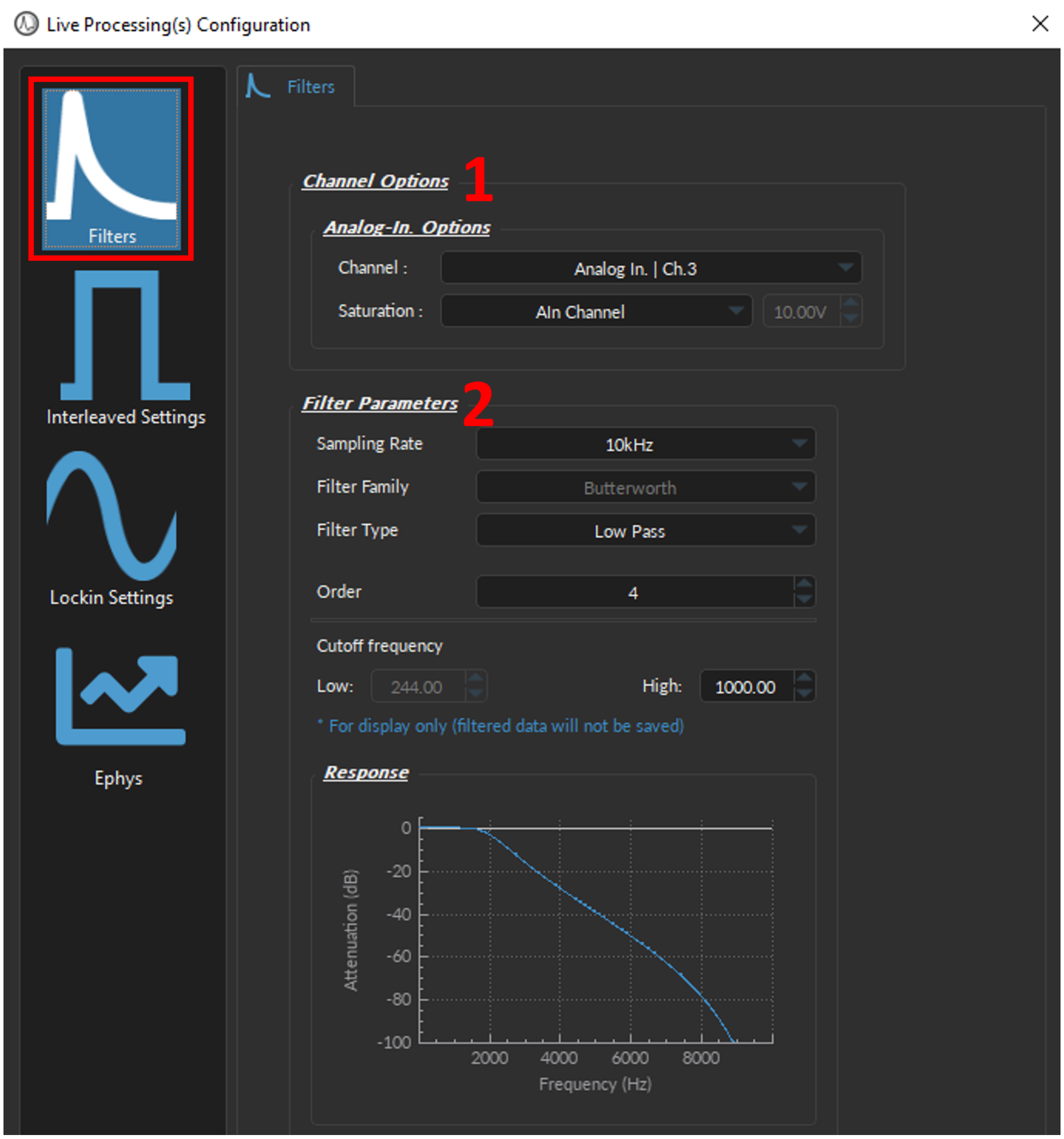


Figure 16.66: *Live Processing, Filters*

1. The **Channel Options** (Fig. 16.66, 1) contains the following parameters:

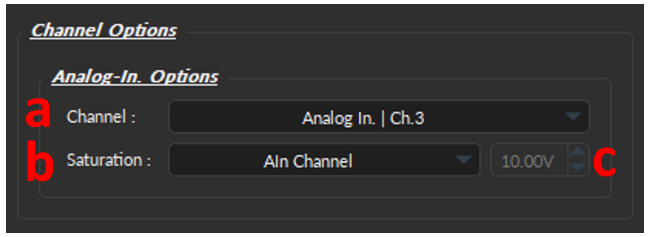


Figure 16.67: *Filters, Channel Options*

a) The **Channel** (Fig. 16.66, a) identifies which of the *NC500* ports receives the analog input data from the sensor/device (Fig. 16.68).

(a) *Analog Input ports* (b) *Port number schematic*

Figure 16.68: *NC500 channel ports*

b) The **Saturation** (Fig. 16.67, b) specifies the detectors used to collect the **Analog Input** data and automatically set its corresponding **Maximal Voltage** value (Fig. 16.67, c) that saturates it. Choosing the right detector is important as different detectors are saturated at different voltage levels (Table 16.2). If none of the common detectors are collecting data, use the **AIn** option to set the *NC500* **Maximum Voltage** (10V), or the **Custom** option to set it between 0V and 10V.

c) The **Maximum Voltage** (Fig. 16.67, c) displays the values at which preset detectors are saturated (Table 16.2). This value is used to warn users when the collected signal is saturating the detector, as in Fig. 16.18.

2. The **Filter Parameters** (Fig. 16.66, 2) contains the following parameters:

d) The **Sampling Rate** (Fig. 16.69, d) specifies the number of data points per second that are saved in the *.doric* file. The **Sampling Rate** can range between 10 Hz - 50 kHz. Reducing the **sampling rate** here reduces the file size and is equivalent to decimating the data as it does not affect the true acquisition sampling rate. Only the filtered (and not the raw **Analog In**) channel is decimated.

e) The **Filter Family** (Fig. 16.69, e) always corresponds to the *Butterworth* filters, which are ideal signal processing filters since they are designed to have a flat frequency response in the bandpass.

f) The **Filter Type** drop-down (Fig. 16.69, f) offers four filter choices, including **High-Pass**, **Low-Pass**, **Band-Pass**, and **Band-Stop** filters.

g) The **Order** (Fig. 16.69, g) specifies the number of cascaded stages within the *Butterworth* filter, such that the higher the order number, the closer the filter is to the ideal *brick wall* response.

h) The **Cut-off Frequency** (Fig. 16.69, h) defines 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 cutoff value is, by definition, always 3 dB below (Low Cutoff) or above (High Cutoff) the specified value.

i) The **Response Graph** (Fig. 16.69, i) displays the Frequency (Hz) vs Attenuation (dB) trace of the filter according to both the filter type and the cutoff values.

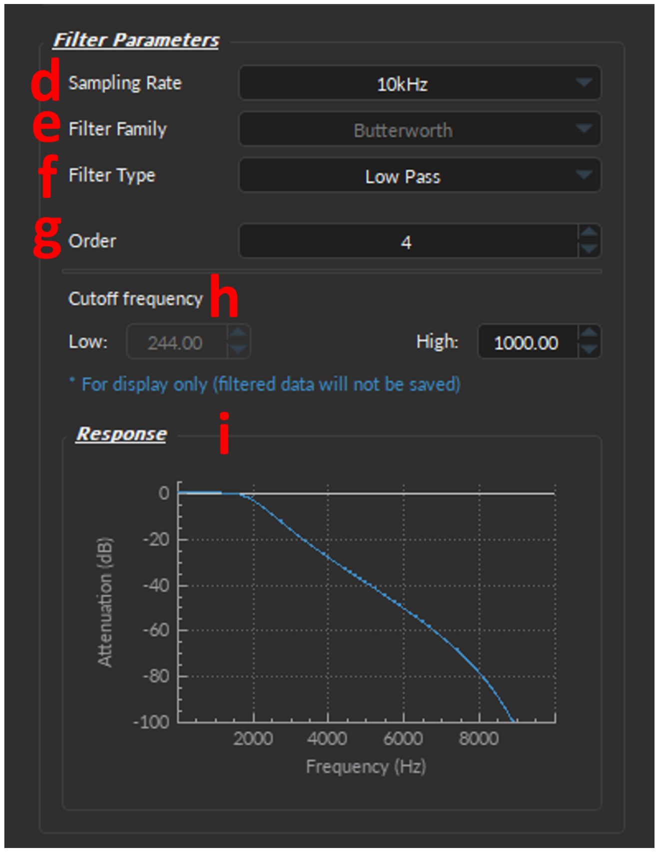


Figure 16.69: *Filters, Filter Parameters*

16.7.1.2 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. 16.70).

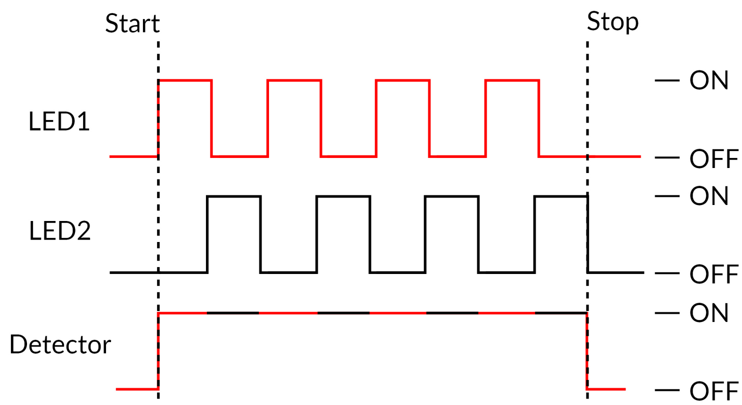


Figure 16.70: *Interleaved Acquisition Timing Diagram*

The interleave preset is using 50% duty cycle for each LED, without delay between them (Fig. 16.70). Thus, depending on the Rise/Fall time of the detector in use, there will be more or less crosstalk between the interleaved channels (Fig. 16.71).

WARNING:
Crosstalk occurs between **two interleaved** Digital I/O channels. If possible, use **LockIn 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 turned ON or OFF (Fig. 16.71). Figure 16.71 shows how the Digital Output channel of LED 1 has a small increase in voltage when 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. 16.71).

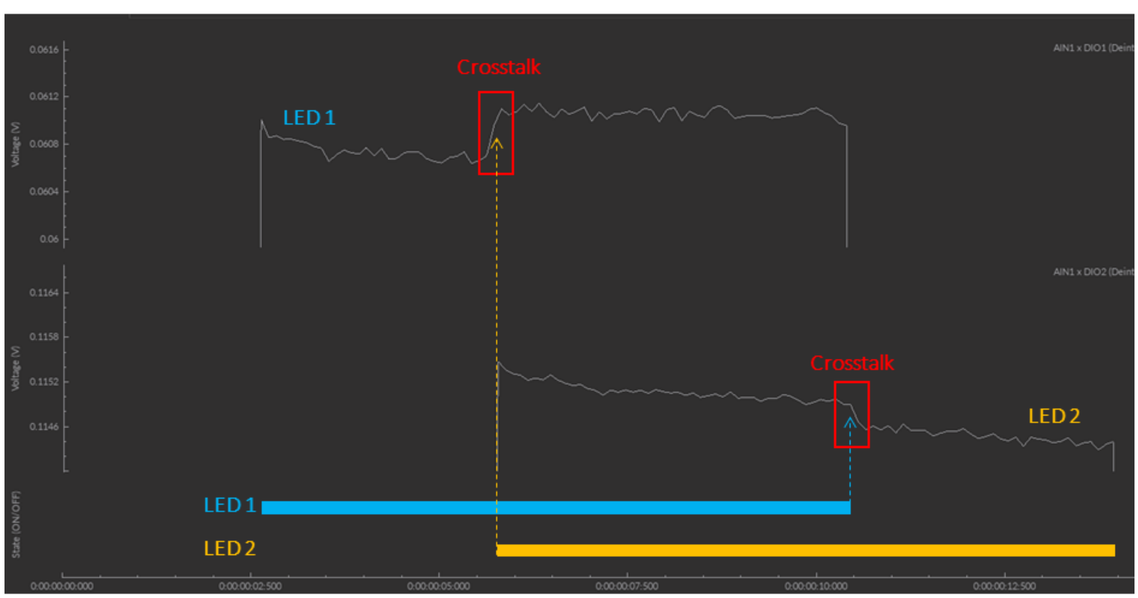


Figure 16.71: *Interleaved Cross-talk*

Strategies to Mitigate Cross-talk:

1. If the sampling rate of the triggered device(s) is high enough (>120Hz), use the **LockIn mode** (Section 16.7.1.3) instead of the **Interleaved mode**;
2. Switching to a detector with a smaller **Rise/Fall Time** (Fig. 16.73, d) will reduce the cross-talk. For instance, the *Doric* and *Newport Detectors* have a **Rise/Fall Time** of 15 ms, while Hamamatsu C10709 has one of 1 ms (Table 16.4).

Regardless of the Detector in use, **care should be taken not to misinterpret cross-talk as a real signal during data analysis.**

When creating an **Interleaved** Preset configuration, the following parameters must be specified:

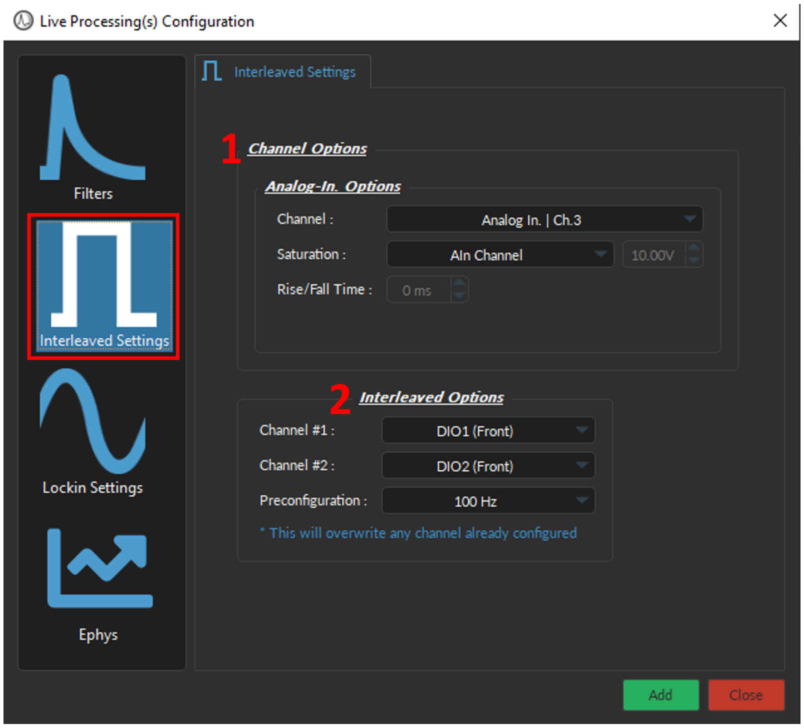


Figure 16.72: *Live Processing, Interleaved*

1. The **Channel Options** (Fig. 16.72, 1) contains the following parameters:

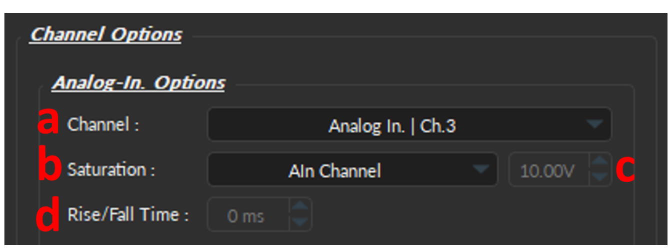


Figure 16.73: *Interleaved, Channel Options*

a) The **Channel** (Fig. 16.73, a) identifies which of the *NC500* ports receives the analog input data from the sensor/device (Fig. 16.74, a).

b) The **Saturation** (Fig. 16.73, b) specifies the detectors used to collect the **Analog Input** data and automatically set its corresponding **Maximal Voltage** value (Fig. 16.73, c) that saturates it. Choosing the right detector is important as different detectors are saturated at different voltage levels (Table 16.4). If none of the common detectors are collecting data, use the **AIn** option to set the *NC500* **Maximum Voltage** (10V), or the **Custom** option to set it between 0V and 10V.

c) The **Maximum Voltage** (Fig. 16.73, c) displays the values at which preset detectors are saturated (Table 16.4). This value is used to warn users when the collected signal is saturating the sensor, as in Fig. 16.18.

d) The **Rise/Fall Time** (Fig. 16.73, d) displays the time resolution of the chosen detector (*i.e.* how quickly it can respond to an instantaneous change in the input signal). See Table 16.4 for comparison of the **Rise/Fall Time** between common detectors.

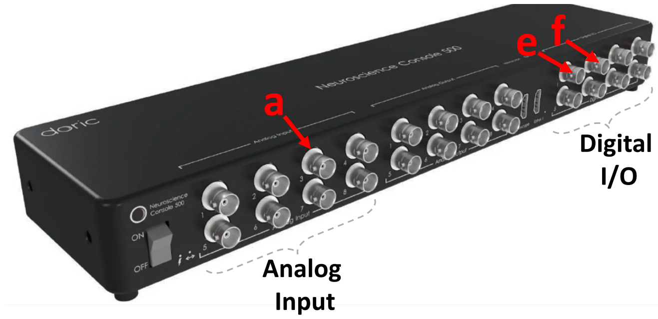


Figure 16.74: *Interleaved, NC500 port example*

Table 16.4: *Detector Comparison*

Detector	**Saturation**	**Rise/Fall Time**
Doric detector	5 V	15 ms
Newport Detector	7.5 V	15 ms
Hamamatsu C10709	5 V	1 ms
AIn Channel	10 V	NA
Custom	0-10 V	0-100 ms

2. The **Interleaved Options** (Fig. 16.72, 2) contains the following parameters:

Figure 16.75: *Interleaved Options*

e) The **Channel #1** (Fig. 16.75, e) identifies the **Digital Output** port on the *NC500* (Fig. 16.74, e) that will be used to drive the LED1 excitation required for the *Interleaved* preset configuration (Fig. 16.70, LED1).

f) The **Channel #2** (Fig. 16.75, f) identifies the **Digital Output** port on the *NC500* (Fig. 16.74, f) that will be used to drive the LED2 excitation required for the *Interleaved* preset configuration (Fig. 16.70, LED2).

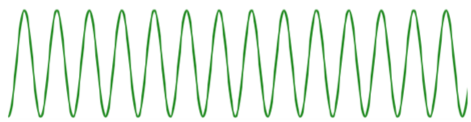
g) The **Preconfiguration** parameter (Fig. 16.75, g) identifies what interleaved frequency will be used to drive the two light sources one after the other. The same interleaved frequency is also required to de-interleave the raw signal from the **Analog Input** channel (Fig. 16.70, Detector). Four interleave frequencies are available: 10, 20, 50, or 100 Hz.

16.7.1.3 LockIn

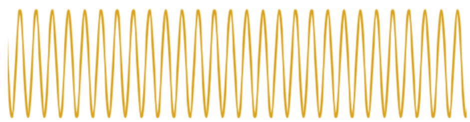
The **Lock-In** mode can detect fluorescence signals embedded in strong noise (e.g. Isosbestic and a fluorophore) or separate multiple signals from a single input during fiber photometry.

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.

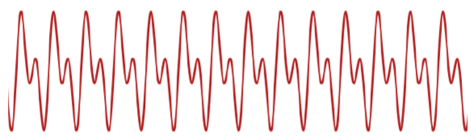
Each *LED light source* emits a sinusoidal illumination at a given frequency (Fig. 16.76a & 16.76b). The detector collects the fluorescent data at a frequency corresponding to the summation of the LED frequencies (Fig. 16.76c).



(a) *LED 1 frequency*



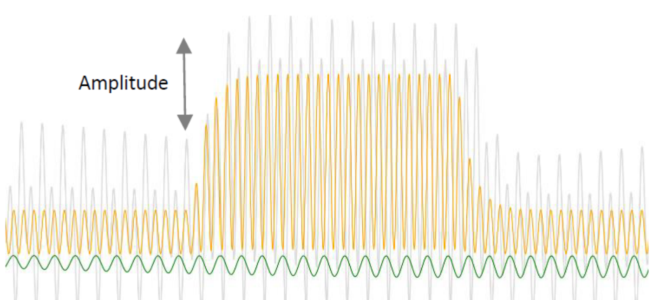
(b) *LED 2 frequency*



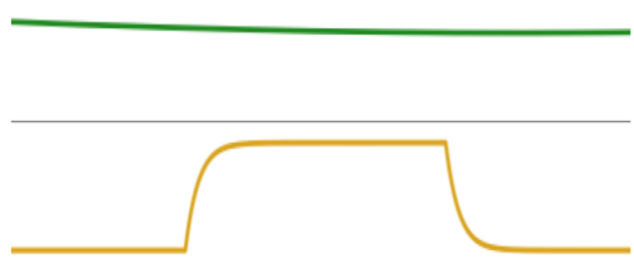
(c) *Raw signal*

Figure 16.76: *Lock-In Acquisition Timing Diagram*

The amplitude changes of the raw signal are due to the collected fluorescence and are dependent on the frequency (Fig. 16.77a). 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. 16.77). The result is separated from the ambient noise that occurred at different frequencies (Fig. 16.77b). The same principle can be applied to demodulate two fluorescent signals.



(a) *Amplitude changes (signal) are frequency dependent*



(b) *Separated signals*

Figure 16.77: *Demodulation separates noise from signal or two signals from each other*

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

To create a **LockIn** preset configuration, specify the following parameters (Fig. 16.78):

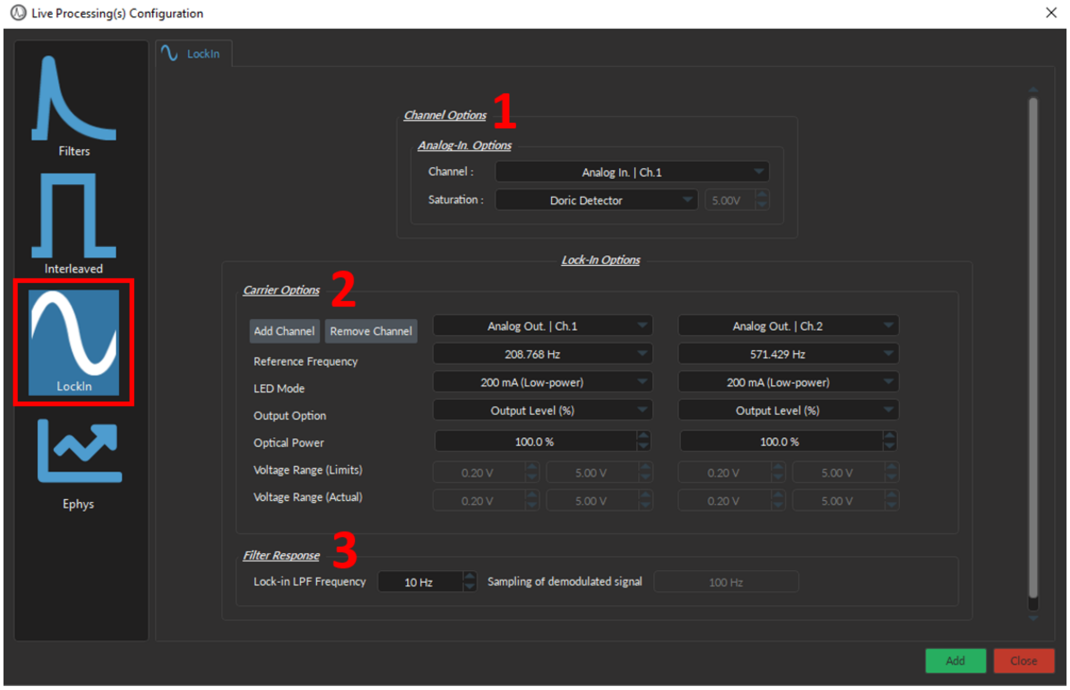


Figure 16.78: *Live Processing, LockIn*

1. The **Channel Options** (Fig. 16.78, 1) specifies the Analog Input-related information required for the **LockIn** present configuration, and includes the following parameters:

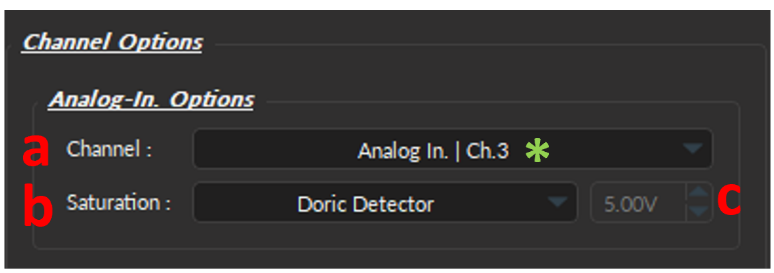


Figure 16.79: *LockIn, Channel Options*

   a) The **Channel** (Fig. 16.79, a) identifies which of the *NC500* ports receives the **analog input** data from the sensor/device (Fig. 16.80).

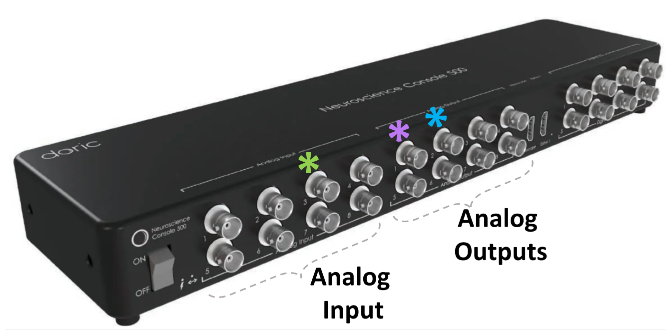


Figure 16.80: *LockIn, NC500 ports*

b) The **Saturation** (Fig. 16.79, b) specifies the detectors used to collect the **Analog Input** data and automatically set its corresponding **Maximal Voltage** value (Fig. 16.79, c) that saturates it. Choosing the right detector is important as different detectors are saturated at different voltage levels (Table 16.2). If none of the common detectors are collecting data, use the **AIn** option to set the *NC500* **Maximum Voltage** (10V), or the **Custom** option to set it between 0V and 10V.

c) The **Maximum Voltage** (Fig. 16.79, c) displays the values at which preset detectors are saturated (Table 16.2). This value is used to warn users when the collected signal is saturating the detector, as in Fig. 16.18.

2. The **Carrier Options** (Fig. 16.78, 2) specifies the **Analog Output** dependencies required for the **Lock-In** preset configuration, and includes the following parameters:

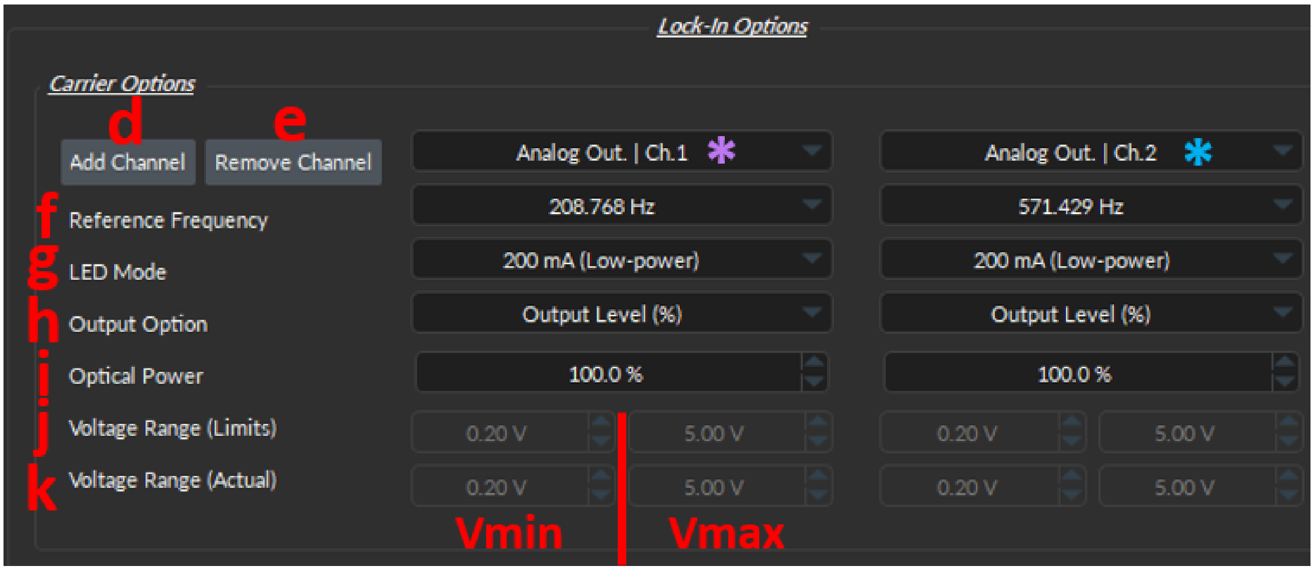


Figure 16.81: *Lock-In Settings, Carrier Options*

d) The **Add Channel** (Fig. 16.81, d) creates a new **Analog Output** channel column that will be used to drive the LED excitation required for the *LockIn* preset configuration. At least one channel must be added in the **Lock-In Options**.

e) The **Remove Channel** (Fig. 16.81, e) deletes the most recently added **Analog Output** channel (right-most column) from the **Lock-In Options**.

f) The **Reference Frequency** (Fig. 16.81, f) specifies which of eight different preset frequencies will be used as the oscillating **Analog Output** signal to drive the LED excitation. These **Reference Frequencies** have been carefully chosen to prevent cross-talk between multiple LED excitations related to the same **Analog Input** channel, and common noise sources (e.g. ambient light).

g) **LED mode** (Fig. 16.81, g) is the largest current that the LED can handle. This value should be set either to the ilFMC-G3 mode (required for G3 Photometry mini-cubes), the **200mA (Low Power)** mode (recommended for G1 & G2 mini-cubes) or, based on the intrinsic maximum current of the LED in use (500 mA or 1000 mA, depending on 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 the light source's normal maximal current. For example, a *LED 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 G1 or G2 Doric FMC or RFMC systems**
- **ilFMC-G3** - special mode created for all G3 mini cubes, whose maximum current is 500 mA at 5 V (100 mA/V). **Required for all G3 ilFMC systems**
- **500 mA** - the **LED maximum current** for the following LEDs: 365 nm, 385 nm, 405 nm & 420 nm. Corresponds to a **Vmax** = 1.25 V.

- **1000 mA** - the **LED maximum current** for most *Doric* LEDs, except the four mentioned above. Corresponds to a **Vmax** = 2.5 V.
- **Custom** - this setting allows users to manually adjust the **Vmax** and **Vmin** of the LED, regardless of **LED's 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. 16.82).

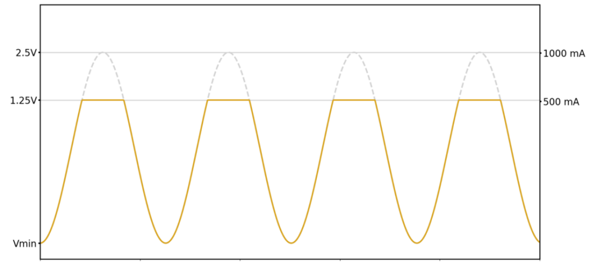


Figure 16.82: *Cropped LED excitation signal*

h) The **Output Option** (Fig. 16.81, h) selects the unit used to set the **LED Power**. The following options are available:

- **Output Level (%)** - uses percentage of **LED 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, 40 mA/V in low-power mode, and 100 mA/V for **ilFMC-G3** mode.
- **Power (uW/mW)** - directly uses the target power (in microwatts). **Only available when the PRONTO-Si power meter is connected to the computer**. To ensure an accurate target value, individually **calibrate** the fiber for each excitation wavelength of light every time this target is changed using the button in Fig. 16.83, ii. This opens a **Power Configuration** window in Fig. 16.85, where both the **target Power** and the correct **Wavelength** of the LED must be specified before calibration.

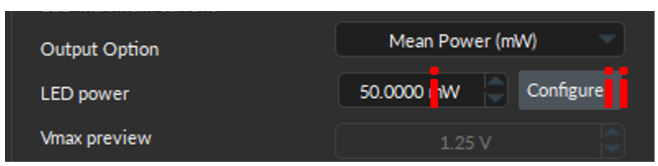


Figure 16.83: *Output Options, Mean Power*

i) **Optical power** (Fig. 16.81, i) converts either the specified percentage (**Output Level (%)**) or directly the target value in microwatts (**Mean Power (uW/mW)**, Fig. 16.83, i) into the output voltage that will be sent to the *LED Driver*, displayed in **Voltage Range (Actual)** (Fig. 16.81, j). **If you are using GCaMP and its isosbestic, we recommend that the isosbestic be about half the power of the GCaMP demodulated trace to reduce the risk of photobleaching.**

- **Configure** button (Fig. 16.83, ii) - opens the **Calibration** window (Fig. 16.85) where the target **Power** (in microwatts) and **Wavelength** of the excitation LED is specified, and the **Start Calibrating Output** button is located. The graph in Fig. 16.85 shows a successful calibration, where there is an initial rise in power, followed by an oscillation until the signal converges (flattens) to the target value.

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**.

j) **Voltage Range (Limits)** (Fig. 16.81, j)- automatically displays the maximum voltage based on the **LED Mode** (maximum current) and the **Optical Power** selected above (Fig. 16.81, i). **Vmax** (right column value) can be changed if the **Custom** LED maximum current mode is selected. The **Vmin** should never be below 0.2 V, nor the Vmax above 5 V.

k) **Voltage Range (Actual)** (Fig. 16.81, k) - the default value is set to 0.2 V but can be changed if the **Custom** LED maximum current mode is selected. The **Vmin** should never be below 0.1 V.

3. The **Filter Response** (Fig. 16.78, 3) contains the following parameters:

   l) The **Lock-in LPF Frequency** (Fig. 16.84, l) defines the **Cutoff Frequency** of the low-pass filter that extracts the signal and is set to 10 Hz by default. This value was selected because, in photometry experiments, the most significant noise source to the filter is around the carrier frequency above 200 Hz.

   m) The **Sampling of demodulated signal** (Fig. 16.84, m) is by default set to 100 Hz, and effectively serves as a 100x decimation. This value was selected because photometry signals are slow (>1 sec) and does not require such a high sampling resolution.

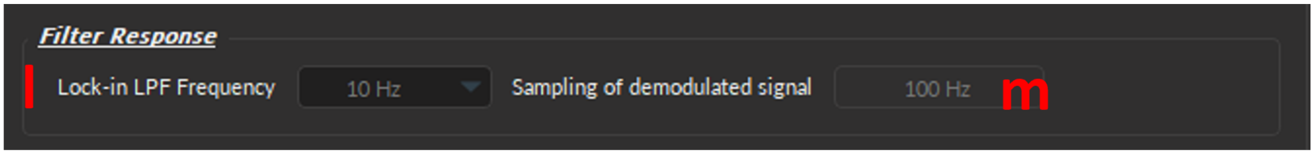


Figure 16.84: *Lock-In, Filter Response*

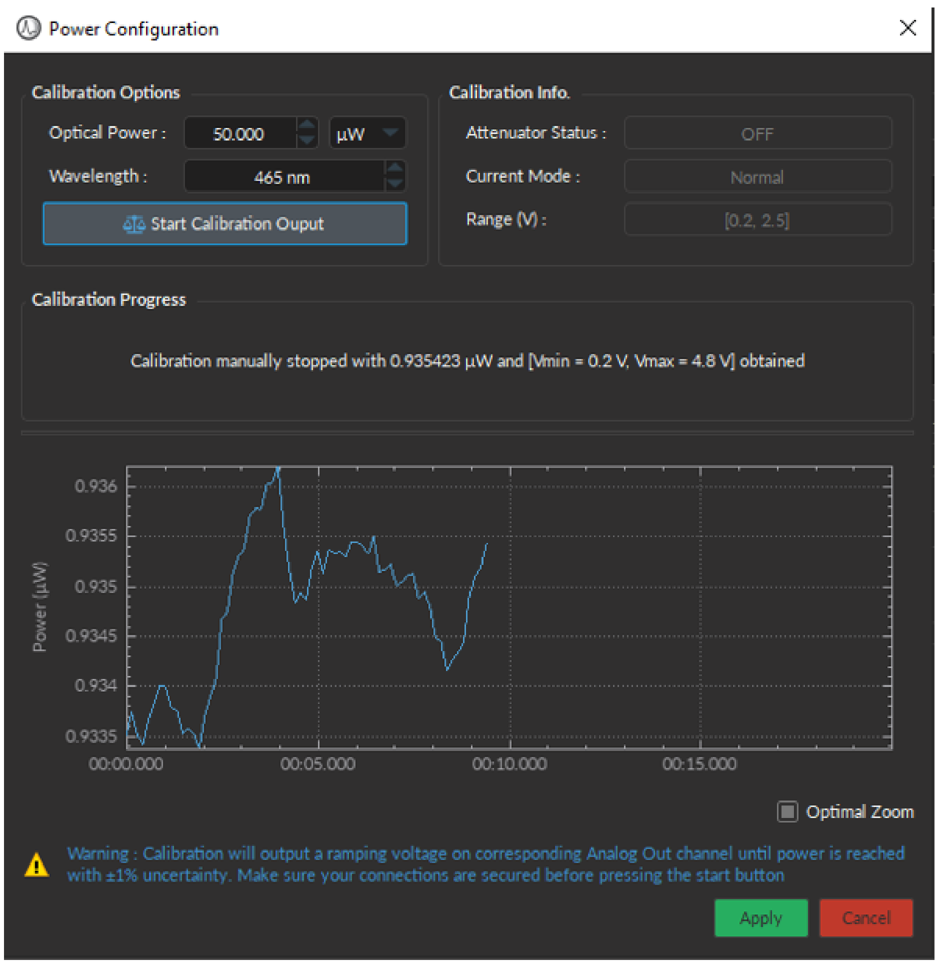


Figure 16.85: *Output Options, Power Calibration*

16.7.1.4 Ephys

The **Ephys** presets allows users to set individual filter parameters for each electrode within a multi-electrode array. The **Live Processing Ephys** configuration is split into five sections (Fig.16.86):

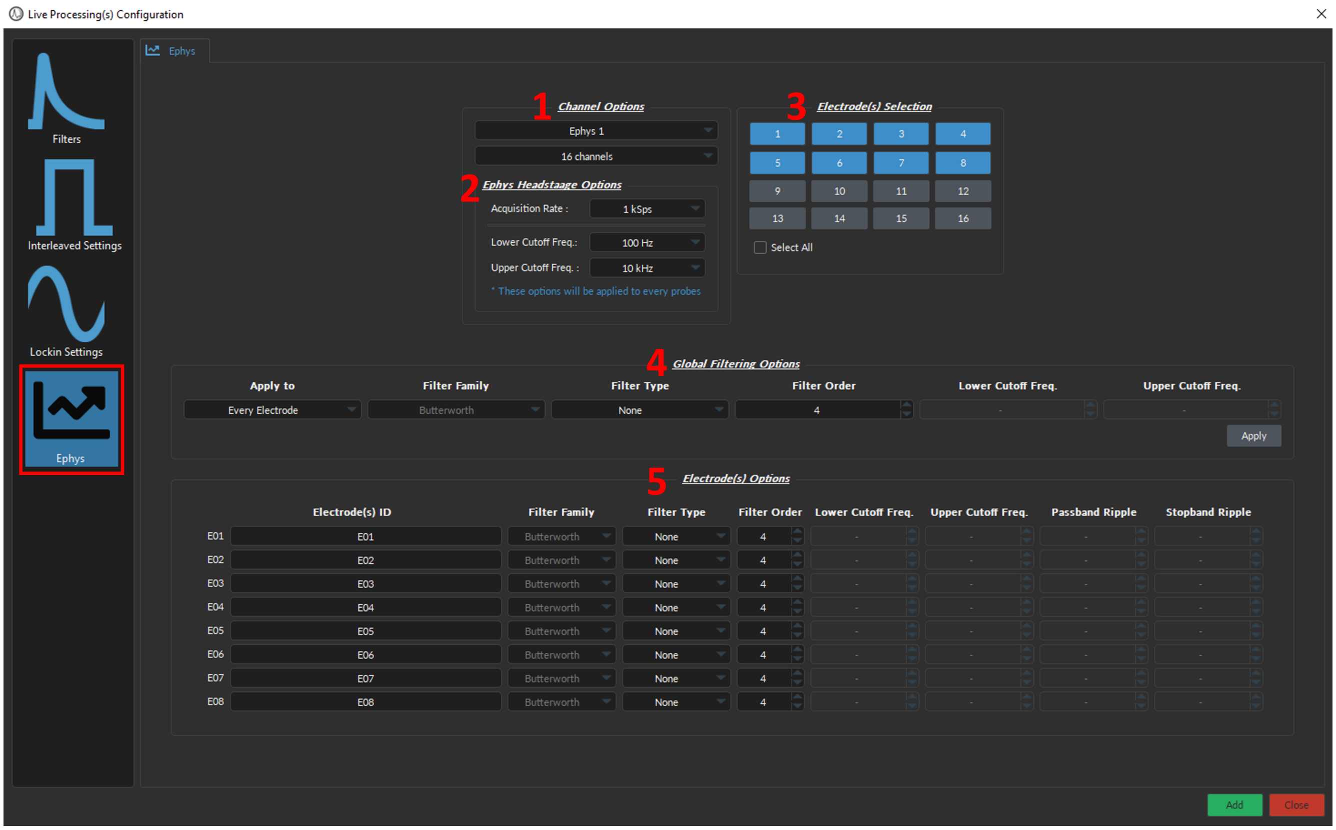


Figure 16.86: *Live Processing, Ephys*

1. The **Channel Options** (Fig. 16.86, 1) includes the following parameters:
   - **Ephys Port** - specifies which Ephys port (Ephys 1: front, Ephys 2: back) on the *NC500* is used for the current channel configuration.
   - **Channel Number** - specifies the number of channels contained on the multi-channel electrodes array connected to the *NC500*. Three options are available: 16, 32, and 64 channels.
2. The **Ephys Headstage Options** (Fig. 16.86, 2) includes the following parameters:
   - The **Acquisition Rate** - defines the sample rate of the electrophysiological data, which can range between 1 - 20 kSps.
   - The **Lower Cutoff Freq.** - defines the bandpass values, such that frequencies below the cutoff will be filtered from the data. This can be used to remove multi-unit activity, if unwanted.
   - The **Upper Cutoff Freq.** - defines the bandpass values, such that frequencies above the cutoff will be filtered from the data. Useful to remove high-frequency noise.
3. The **Electrode(s) Selection** (Fig. 16.86, 3) selects which of the total **Channel Number** are enabled (in blue). Allows users to disable (greyed out) broken electrode sites or electrodes that are not in the region of interest. No data will be collected from the disabled electrode(s). Use the **Select All** checkbox to enable all electrodes simultaneously.
4. The **Global Filtering Options** (Fig. 16.86, 4) include the following:

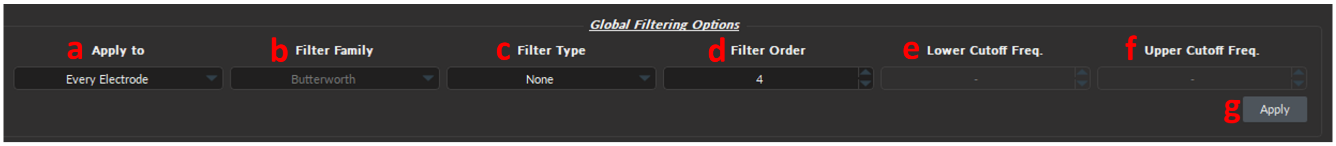


Figure 16.87: *Global Filtering Options*

a) The **Apply to** drop-down (Fig. 16.87, a) by default applies all the parameters in the **Global Filtering Options** to *Every Electrode* in the **Electrode(s) Options**. No other application options are currently available.

b) The **Filter Family** (Fig. 16.87, b) is the *Butterworth* filter which is a common signal processing filter that is designed to have a frequency response that is as flat as possible in the passband (i.e. had no ripples).

c) The **Filter Type** drop-down (Fig. 16.87, c) sets one of four available options, including:
   - *Band Pass* - allows signals within a selected range of frequencies (between the **Low Cutoff Freq.** & the **Upper Cutoff Freq.**) to pass unaltered while attenuating frequencies outside that range to very low levels.
   - *Band Stop* - attenuates frequencies within the specified range (between the **Low Cutoff Freq.** & the **Upper Cutoff Freq.**) while letting all other frequencies pass unaltered.
   - *Low Pass* - allows signals below a cutoff frequency (**Low Cutoff Freq.**) to pass unaltered, while attenuating frequencies above it.
   - *High Pass* - allows signals above a cutoff frequency (**Upper Cutoff Freq.**) to pass unaltered, while attenuating frequencies below it.

d) The **Filter Order** (Fig. 16.87, d) defines the ability of the filter to attenuate or suppress specific frequency ranges. Further, the filter order determines the sharpness of the transition band between the passband and stopbands. The **Filter Order** can be any value between 1-10.

e) The **Lower Cutoff Freq.** drop-down (Fig. 16.87, e) defines the bandpass values, such that frequencies below the cutoff will be filtered from the data. This can be used to remove multi-unit activity, if unwanted.

f) The **Upper Cutoff Freq.** drop-down (Fig. 16.87, f) defines the bandpass values, such that frequencies above the cutoff will be filtered from the data. Useful to remove high-frequency noise.

g) The **Apply** button (Fig. 16.87, g) updates the parameters of every electrode in the **Electrode(s) Options** to match the ones specified in the **Global Filtering Options**.

5. The **Electrode(s) Options** (Fig. 16.86, 5) individually sets the filtering options for each electrode within the array. The number of rows corresponds to the number of enabled electrodes in the **Electrode(s) Selection** (in blue). The filtering parameters functionalities (Fig. 16.88, b-f) are the same as the ones defined above in the **Global Filtering Options** (Fig. 16.87, b-f).

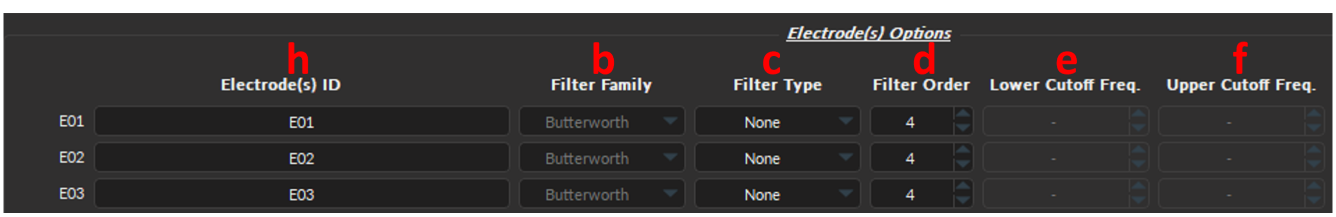


Figure 16.88: *Electrode Options*

h) The **Electrode(s) ID** text-box (Fig. 16.87, h) assigned a more intuitive/informative label to each enabled electrode.

16.7.2 Live Processing View

The **Live Processing** view contains a list of the added processes and all of their dependencies (Fig. 16.89). Users can delete, rename, change saving options within this list, or view/edit the configuration settings.

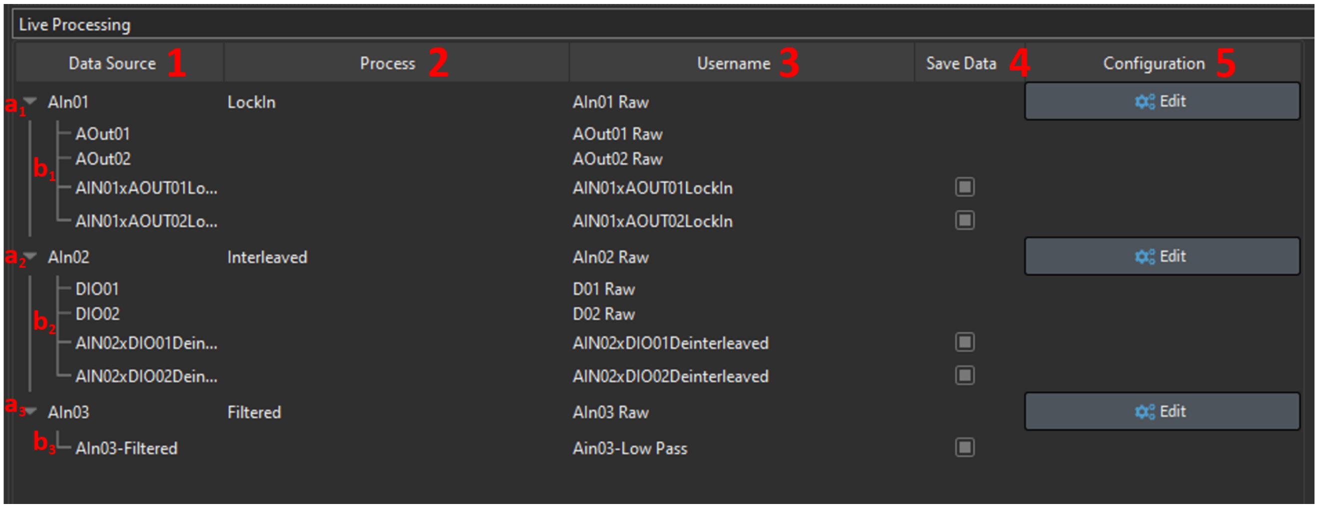


Figure 16.89: *Live Processing, List*

The **Live Processing** list is split into the following columns:

1. The **Data Source** column (Fig. 16.89, 1)
   a) The **Analog In** channels (Fig. 16.89, a) receives and saves the raw data from the sensor(s) without any live processing applied to the data.
   b) The **Dependencies** (Fig. 16.89, b) includes either DIO, AOUT and/or processed AIN channels required for the **Live Processing**.
2. The **Process** column (Fig. 16.89, 2) displays the type of *Live Process* calculation done of the raw data from **Analog In** channel (Fig. 16.89, a).
3. The **User Name** column (Fig. 16.89, 3), when double-clicked, specifies a new label for the channel. This label is the name that will be used to represent that channel in the **Graph List** in the **Acquisition View** (Section 16.4.2). Renaming a dependency also changes the **User Name** in the **Configuration** section.
4. The **Save Data** column (Fig. 16.89, 4) contains check-boxes which, if enabled, saves the channel data during the recording in the *.doric* data file. If disabled, all acquired data from that channel, even if viewed, will be lost.
5. The **Configuration** column (Fig. 16.89, 5) opens a **Channel(s) Editing** pop-up window where users can quickly check the settings used in the process configuration. In this window, users can even modify select parameters after creating the channel.

To **Delete** a **Live Process**, select the **Analog In** channel or one of its dependencies + the *Delete* keyboard key. This opens a pop-up window to confirm the deletion (Fig. 16.90). Note that deleting a **Live Process** also deletes all of its dependencies.

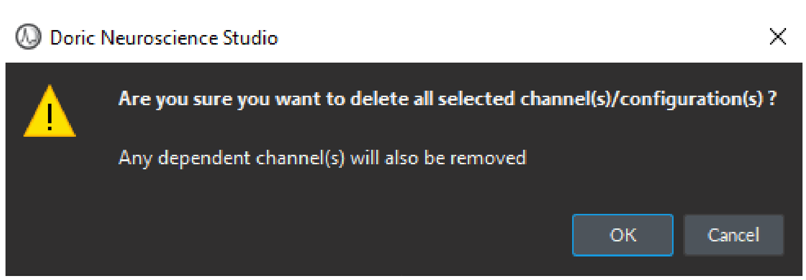


Figure 16.90: *Live Processing, Confirm deletion*

16.8 Microscope Control

The **Microscope Control** (Fig. 16.91) specifies microscope settings that change the quantity/quality of images the microscope sensor collects. These settings directly affect data recording in *.doric* file, and include:

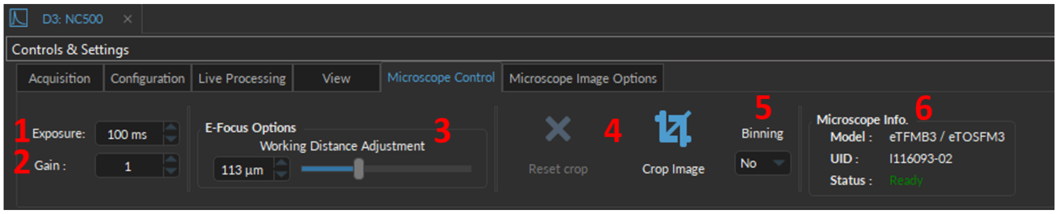


Figure 16.91: *Microscope Control Tab*

1. The **Exposure (ms)** textbox (Fig. 16.91, 1) specifies the length of time that the microscope sensor collects light from the sample. This value is inversely related to the **FPS** of the device, such that a 100 ms exposure corresponds to 10 FPS (accordingly, a 200 ms exposure corresponds to 5 FPS, and so on).

   NOTE: There are trade-offs between exposure time, image brightness, and phototoxicity.

2. The **Gain** text-box (Fig. 16.91, 2) corresponds to the relative amplification measure applied to the sensor. Note that increasing the gain will simultaneously increase both the signal and noise. Four options of gain (1-4) are available.

3. The **E-Focus Options** (Fig. 16.91, 3) allows users to adjust the **Working Distance** of the microscope focus using a slide bar from 0 to 350 um for Twist-on *efocus fluorescence microscope*.

4. The **Crop Image** button (Fig. 16.91, 4) allows to select a square portion of the image by dragging the mouse cursor on the selected image region. Releasing the cursor will automatically crop the displayed image. To reset the image to its original pixel size, Press the **Reset crop** button. Note: In order to crop an image, a frame must be loaded into the **Microscope View**, which can be done using either **Live** or **Record** buttons (Fig. 16.7, 1 & 2).

5. The **Binning** drop-down (Fig. 16.91, 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. 16.91, 6) displays the following device information:

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

16.9 Microscope Image Options

The **Microscope Image Options** adjusts the visualization parameters for the **Microscope View**. Importantly, none of the parameters set in the **Microscope Image Options** changes the images saved. See Section 16.8 to control the settings of the microscope itself. The following imaging options are available:

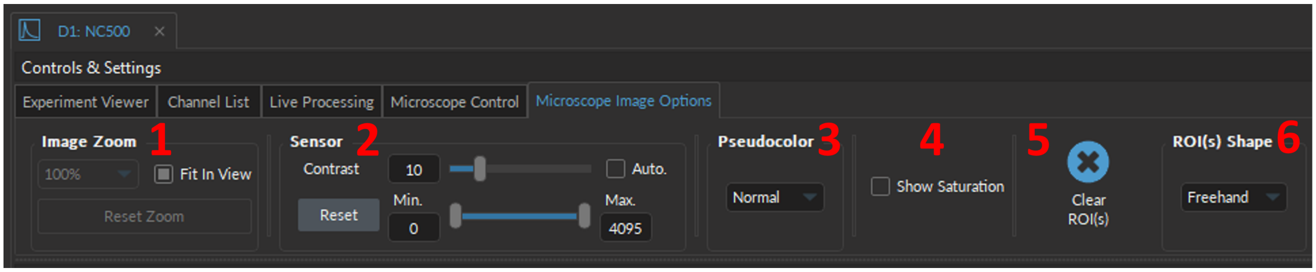


Figure 16.92: *Microscope Image Options Tab*

1. The **Image Zoom** (Fig. 16.92, 1) 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 **Microscope View**.
   - The **Rest Zoom** button returns the zoom factor to 100%.
2. The **Sensor** (Fig. 16.92, 2) is used to adjust the contrast on a given sensor.
   - The **Contrast** slider sets 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 a lower count will display a minimal value.
   - The **Maximum** slider sets the largest pixel value cutoff. Should the Max be below 4095, all pixels with a higher count will appear saturated.
   - The **Auto Contrast** check-box activates a contrast adjustment algorithm that optimizes 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.
3. The **Pseudocolor** drop-down (Fig. 16.92, 3) maps the pixels values to a pseudocolor range (of 13 possible options) to facilitate viewing.
4. The **Show Saturation** checkbox (Fig. 16.92, 4) is available when using the **Auto Contrast** setting. When enabled, saturation pixels will turn red. This function is only available if no pseudocolor is selected (Pseudocolor set to *Normal*).
5. The **Clear ROI(s)** button (Fig. 16.92, 5) will delete all drawn regions of interest within the **Microscope View**. Note that unless the ROI(s) were previously saved, these ROI(s) cannot be recuperated.
6. The **ROI(s) Shape** drop-down (Fig. 16.92, 6) 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). Five **ROI(s) Shapes** are available: *Freehand*, *Circle*, *Rectangle*, *Square*, and *Polygon* (Fig. 16.93, 1-5). Multiple shapes can be used within the **Microscope View** simultaneously.

Figure 16.93: *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 **Live** or **Record** buttons (Fig. 16.7, 1 & 2).

16.10 View Tab

The **View** tab contains the buttons to modify the visualization of the **Acquisition View**.

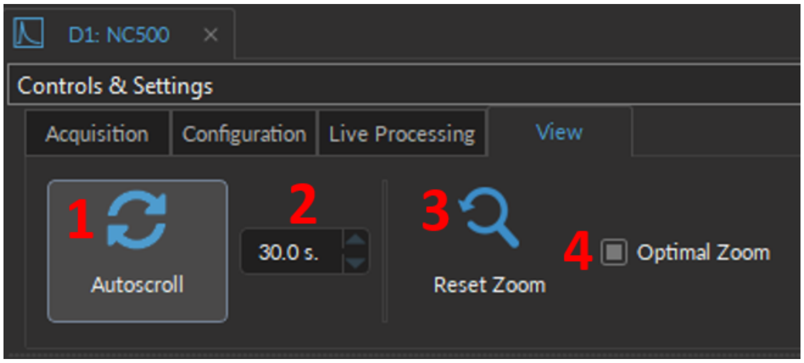


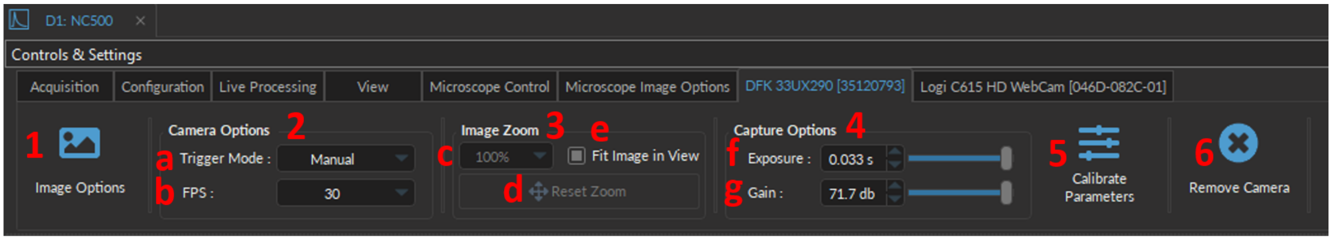
Figure 16.94: *Acquisition Tab*

The **Acquisition** Tab contains the following tools (Fig. 16.94):

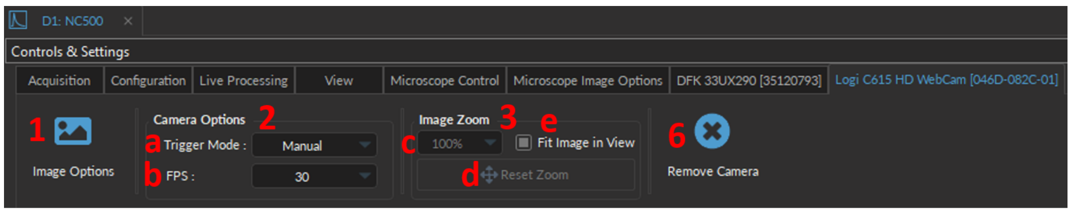
1. The **Autoscroll** button (Fig. 16.94, 1), when selected, automatically scrolls as new data appears, keeping the range of the Time axis to the value specified in the **Zoom range** text-box.
2. The **Zoom range** text-box (Fig. 16.94, 2) sets the maximum range of the time axis for the **Autoscroll** and **Reset Zoom** functions (Section 16.3, no. 1 & 3).
3. The **Reset Zoom** button (Fig. 16.94, 3) readjusts the graph Y-axis to the default value, or the **Optimal Zoom** value if the Optimal Zoom check-box is selected.
4. The **Optimal Zoom** check-box (Fig. 16.94, 4) automatically adjusts the graph Y-axis range based on the values of the data collected. Smaller values will lead to greater zoom, and vice versa.

16.11 Camera Controls

The **Camera Control** tab is only available after the creation of a **Camera** channel (Section 16.6.5), which is compatible with both the Doric Behavior Camera and most USB 3.0 cameras. If multiple **Camera Channels** were created, each camera would have its own tab, with a unique camera name. Each camera tab contains the following parameters:



(a) *Doric Behavior Camera*



(b) *Web Camera*

Figure 16.95: *Acquisition, Camera Tabs*

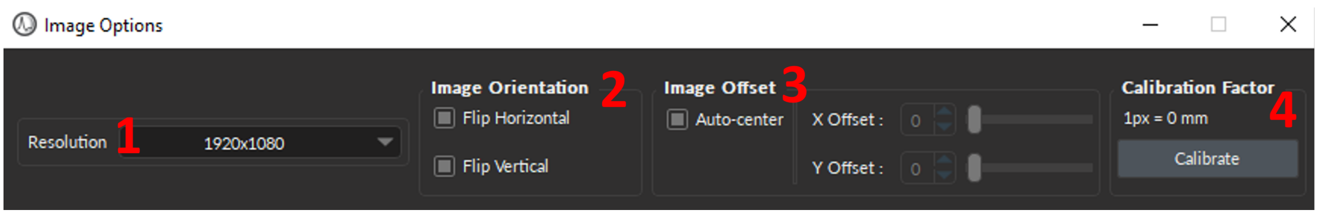
1. The **Image Options** (Fig. 16.95, 1) opens the **Image Options** window, which is detailed in Section 16.11.1
2. The **Camera Options** (Fig. 16.95, 2) includes:
   - The **Trigger Mode** (Fig. 16.95, a) sets how the camera will be controlled and synchronized with the rest of the recording system.
     - **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.
   - The **FPS**, the *frames per seconds* (Fig. 16.95, b), sets the frequency at which images are captured. 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.
3. The **Image Zoom** (Fig. 16.95, 3) 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.
   - The **Zoom %** drop-down list (Fig. 16.95, c) specifies the zoom factor for the image display, which ranges between 10%-500%.
   - The **Reset Zoom** button (Fig. 16.95, d) returns the zoom factor to 100%.
   - The **Fit Image in View** checkbox (Fig. 16.95, e) automatically adjusts the image to fit the entire Acquisition View.
4. The **Capture Options** (Fig. 16.95, 4) are only available for *Doric Behavior Cameras* and controls the brightness of the image in two different ways:
   - **Exposure** (in ms) (Fig. 16.95, f) - the duration when the camera sensors are exposed to light. The larger the exposure, the brighter the image.
   - **Gain** (in dB) (Fig. 16.95, g) - is an amplification factor applied to all pixel values. Increasing the gain will increase the brightness of the signal and noise evenly.

5. The **Calibrate Parameters** button (Fig. 16.95, 5) automatically calibrates the exposure, gain, and white balance based on the current level of ambient light detected and generally gives a clearer image. You can always optimize the exposure or gain manually after the initial calibration processes.

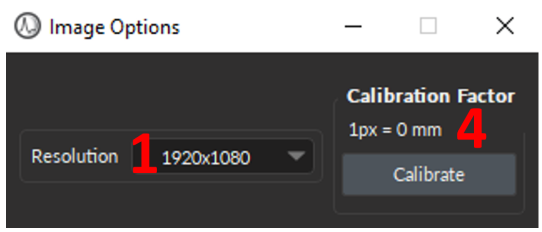
6. The **Remove Camera** button (Fig. 16.95, 6) disconnects the **Camera** from the software. If multiple cameras are integrated within this module, this button will only close the camera of that current tab.

16.11.1 Camera Image Options

The **Camera Image Options** depend on whether the camera in question is a *Doric Behavior Camera* or a different (but compatible) web camera. While common **Image Options** are identical between the two types of cameras, *Doric Behavior Camera* have extra options/parameters (for comparison, see Fig. 16.96a & 16.96b).



(a) *Doric Behavior Camera*



(b) *Web Camera*

Figure 16.96: *Camera Image Options*

1. The **Resolution** (Fig. 16.96, 1) specifies 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.

2. The **Image Orientation** (Fig. 16.96, 2) contains parameters that control the direction of the image displayed in the **Acquisition View**:
   - The **Flip Horizontal** checkbox displays a mirrored image where the left side becomes the right, and vice versa.
   - The **Flip Vertical** checkbox displays a mirrored image where the top becomes the bottom and vice versa.

3. The **Image Offset** (Fig. 16.96, 3) 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 of the **Image Zoom**.
   - The **Auto-center** checkbox centers the camera and is the default setting. Disabling the box unlocks the X & Y slider setting to manually set the offset.
   - The **X Offset** slider allows users to move the camera image horizontally by the selected number of pixels.
   - The **Y Offset** slider allows users to move the camera image's vertical axis by the selected number of pixels.

4. The **Calibration Factor** (Fig. 16.96, 4) contains:
   - 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.

- The **Calibrate** button opens the hidden **Calibration Settings** box which is 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.

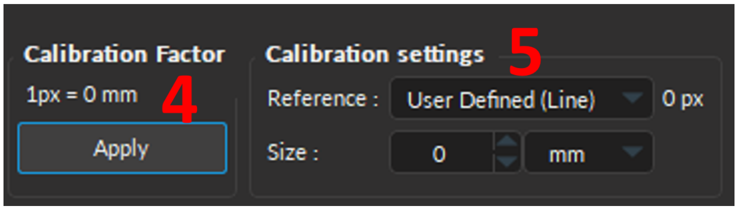


Figure 16.97: *Camera Image Options, Calibration Settings*

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.

   a) The **Reference** drop-down list 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. 16.98). This line can online be horizontal or vertical. For optimal results use an object/dimension that fills most of the image.

   b) The **Current Reference Dimensions** (in pixels) is displayed to the right of the drop-down list.

   c) The **Size & Units** text-boxes specify the real dimensions of the reference and its unit (mm, cm, or inches). Select the **Apply** button to recalculate the **Calibration Factor** using the new **Size & Units**.

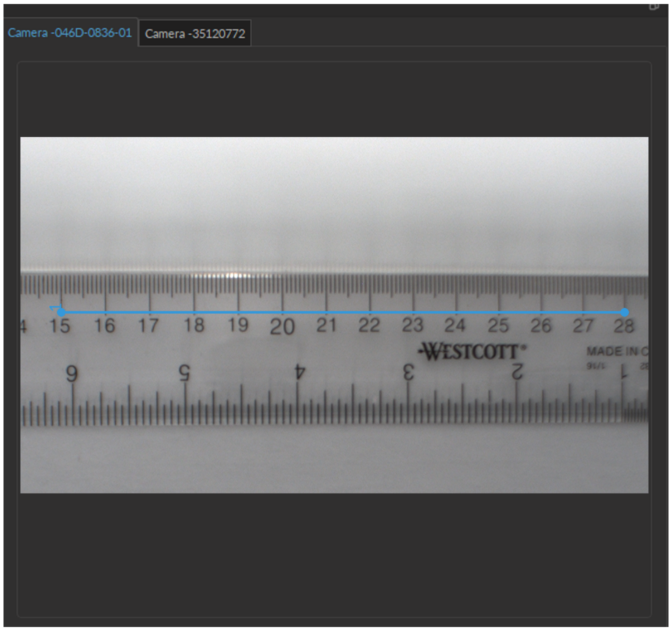


Figure 16.98: *Camera Module - Calibration User-defined line*

16.12 Assisted Rotary Joint Controls

The **Assisted Rotary Joint** tab contains the settings that control the rotary joint and records when the motor is rotating. If multiple **Rotary Joint Channels** were added to the interface, each device would have its own tab, with a unique device ID.

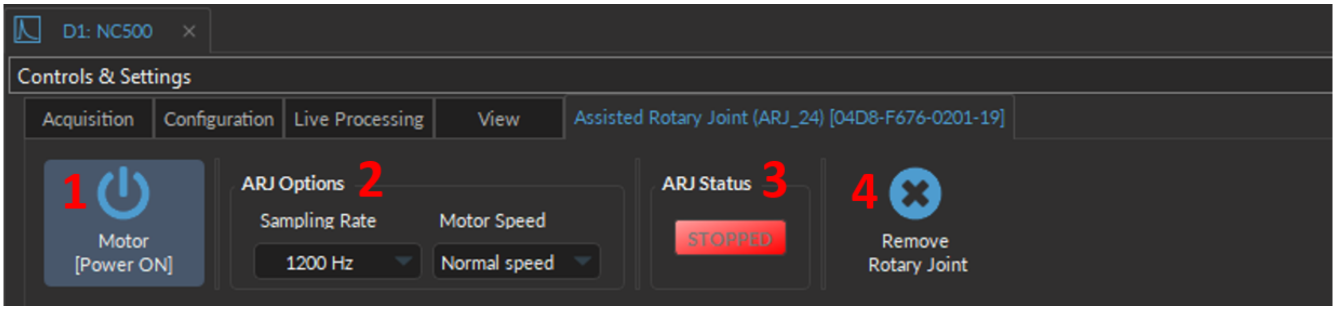
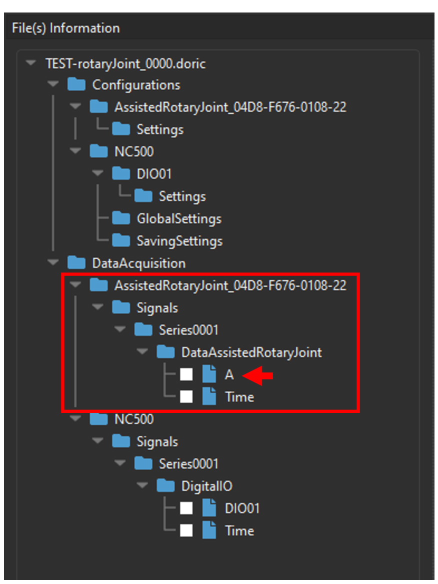


Figure 16.99: *Rotary Joint tab*

1. The **Motor Power ON** (Fig. 16.99, 1) remotely turns ON or OFF the device from the software.
2. The **ARJ Options** (Fig. 16.99, 2) define parameters that affect directly data collection/control of the device, including:
   - The **Sampling Rate** - specifies the number of data points per second that are saved in the *.doric* file. The **Sampling Rate** can range between 10 Hz - 1200 Hz.
   - The **Motor Speed** - defines how quickly the motor will turn in response to rotational motion. Two speed options are available: *Half-Speed* and *Normal Speed*.
3. The **ARJ Status** (Fig. 16.99, 3) displays whether the rotary joint is currently *Stopped* or *Active*. The **Status** will change when either the **Motor [Power ON/OFF]** button is selected or the physical button on the device is used.
4. The **Remove Rotary Joint** (Fig. 16.99, 4) disconnects the device from the software. If multiple rotary joints are being used, this button will only close that tab's device.

NOTE: There is no graphical visualization of the recorded data in the **Acquisition View**. However, during a recording, a single digital vector is collected, representing whether the motor is rotating (1) or not (0) (Fig. 16.100). This data is located within the *DataAcquisition* folder of the *.doric* File, and under the *AssistedRotaryJoint-ID* branch (Fig. 16.100a).



(a) *File Structure*

	Time	A
0	0.000833333333333	0
1	0.00583333333333	0
2	0.0108333333333	0
3	0.0158333333333	0
4	0.0208333333333	0
5	0.0258333333333	0
6	0.0308333333333	0
7	0.0358333333333	0
8	0.0408333333333	0
9	0.0458333333333	0
10	0.0508333333333	0
11	0.0558333333333	1
12	0.0608333333333	1
13	0.0658333333333	1
14	0.0708333333333	1
15	0.0758333333333	1

(b) *Example*

Figure 16.100: *Rotary Joint Data*

17 Support

17.1 Contact us

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

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