Miniaturized Fluorescence Microscopy: Advanced Systems Configuration and Synchronized Recording

Application Note

Version 1.0.0

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System Overview

The Miniature Fluorescence Microscopy Systems has been developed to perform freely-moving imaging of neuronal activity. Multiple hardwares are available to provide specific functionality and flexibility in the experiments. The controlling software, Doric Neuroscience Studio, also contains multiple features that helps achieve advanced configurations. It is therefore possible, providing the right tools, to perform fluorescence microscopy combined with optogenetic opsin activation/inhibition, external triggering of the microscopy system, or synchronization of a behavior tracking camera. The present section presents the different electronic devices available to perform Fluorescence Microscopy and their functions.

1.1 Fluorescence Microscope Driver

The Fluorescence Microscope Driver (FMD) is used for microscope control and video rate data collection by the computer via a high speed Ethernet communication. The microscope is controlled using the Doric Neuroscience Studio software. The output of the FMD is shown in Figure 1.1.

- **BNC Trigger Input**: Is used to trigger the acquisition of the microscope.
- **BNC Trigger Output**: Sends a 0-4.75 V TTL pulse. In Normal mode, the pulse is high when the microscope is in function. In Trig with each frame mode, a 4.75 V pulse is sent at each acquired frame of the microscope.
- **Microscope HDMI port**: Connects the microscope to the microscope driver.

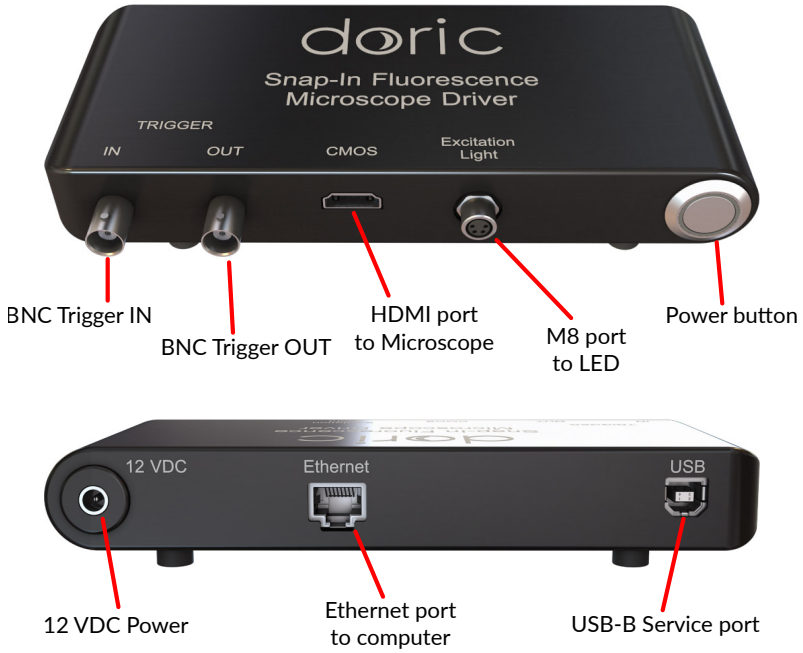

Figure 1.1: *Fluorescence Microscope Driver (FMD) and Ports*

- **M8 port to the LED**: Connects to a LED light source and is used for fluorescence microscopy without optogenetic excitation. For optogenetic experiments, the LED used for the fluorescence excitation is integrated in the Ce:Yag optical head and is therefore controlled using the Ce:Yag + LED Driver.
- **12 VDC power**: Connects to a 12 VDC Power supply
- **Ethernet port**: Connects to the computer via a high speed Ethernet communication.
- **USB-B Service port**: Used for maintenance of the microscope driver such as for firmware update. This port should NOT be connected to the computer during imaging sessions.

1.2 Optogenetics TTL Pulse Generators

The Doric Optogenetics TTL Pulse Generator (OTPG) is used to generate modulated pulse trains as well as the triggering of devices (fig. 1.2). The OTPG connects to a computer using a USB-B to USB-A cable and each channel ports can be connected to a device using a BNC cable. For the OTPG_4, which contains 4 ports, all channels can either be used as an output to deliver a pulse train, or as an input to receive trigger signals. For the OTPG_8, which contains 8 ports, all ports can be used as outputs but only 4 ports (# 5 to 8) can be used as inputs.

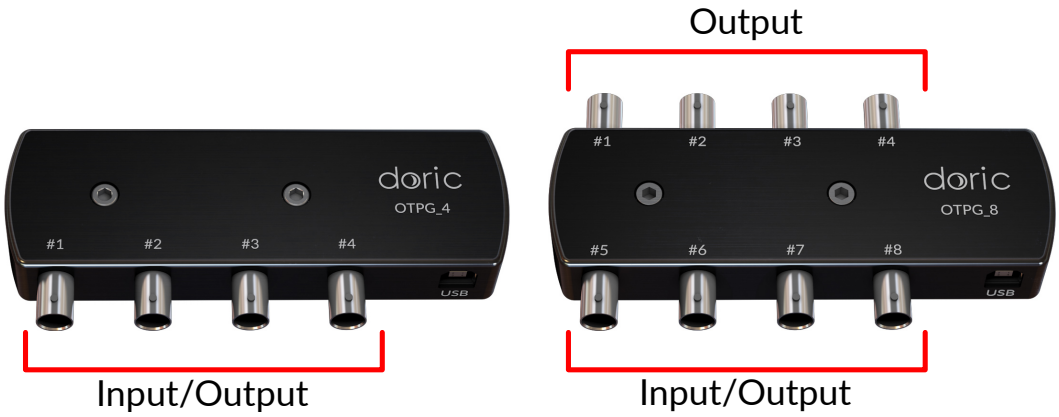

Figure 1.2: *Optogenetics TTL Pulse Generators. Left: OTPG_4. Right: OTPG_8.*

1.3 Ce:YAG + LED Driver

The Ce:YAG + LED Driver (fig. 1.3) is used to control the Ce:YAG + LED Fiber Optical Head. The driver controls the Ce:YAG light source and the LED light source independently, which are used for optogenetic stimulation and fluorescence imaging respectively. The driver contains the following elements:

- **LCD Screen**: Displays the driver information (operating mode, status, etc...).
- **Control Knobs**: Are used to control the driver in stand-alone mode as well as setting the light source current in all modes.
- **12 V port**: Connects the driver to its 12 VDC power supply.
- **HDB15 Connector**: Connects the driver to the light source using a HDB15 type cable. This cable uses a custom pinout and other HDB15 cables should not be used with this system.
- **BNC Input Ports**: Are used as input for external analog signals to control the light sources. For a light source to be driven by an external source, the corresponding light source channel needs to be set to TTL mode.
- **BNC Output Ports**: Are used to monitor the current of each light source.
- **USB port**: Is used to connect the driver to a computer using a USB-A/USB-B cable. This allows the driver to be controlled using the Doric Neuroscience Studio software.

Figure 1.3: *Ce:YAG + LED Driver and ports.*

- **Interlock Connector Plug**: Is used to connect the driver to a safety interlock system. It is recommended to connect the interlock plug to a laboratory interlock system.
- **Power On/Off Switch**: Turns the driver on and off.
- **Key Switch**: The Key must be inserted in the key switch to enable light emission. Note that, despite its similar form factor, the power key is not a standard micro SD card such as those used in some digital cameras. Do not attach the Key to a key fob or similar holder; this may prevent proper insertion of the Key Switch.

1.4 Behavior Tracking Camera

The Behavior Tracking Camera (fig. 1.4) is used with Miniature Fluorescence Microscopes Systems to track a subject's motion during an experiment. The camera can be synchronized with the microscope and other hardware to precisely correlate the subject's behavior to the microscope recorded images. The principal ports are the following:

- **USB3.0 Micro-B Port**: Connects the Camera to the computer via a *USB3.0 Micro-B to USB3.0-A* cable.
- **Trigger Port**: Is used to trigger the camera by synchronizing it to an external device such as the Microscope Driver or the OTPG via a *12-pin Hirose to BNC* cable.

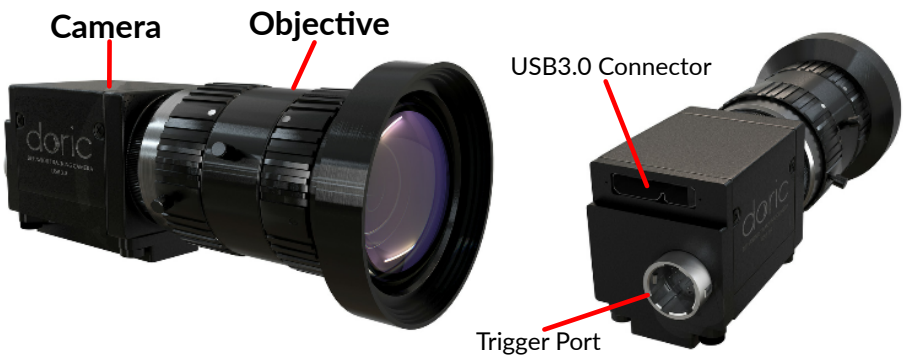

Figure 1.4: *Behavior Tracking Camera and its ports.*

1.5 Doric Neuroscience Studio

The Doric Neuroscience Studio software is a freeware developed to control all Doric Neuroscience hardwares and is available on our website. For more information on the functionality of Doric Neuroscience Studio, please refer to the user manual.

2

Setting up Advanced Configurations

With the different hardware available to perform Miniature Fluorescence Microscope, many configurations are possible to perform different types of experiments. This chapter explains some common configuration, such as microscopy combined with optogenetic stimulation, external triggering, and behavior camera synchronization, and the necessary device connections and computer settings necessary to achieve them.

2.1 Configuration GCaMP6 + NpHR3

The present section explains the setup necessary to perform fluorescence imaging of GFP-like fluorophore combined with NpHR3 opsin activation. In this configuration, the OTPG is the master device and drives the other devices.

2.1.1 Connections (Fig. 2.1)

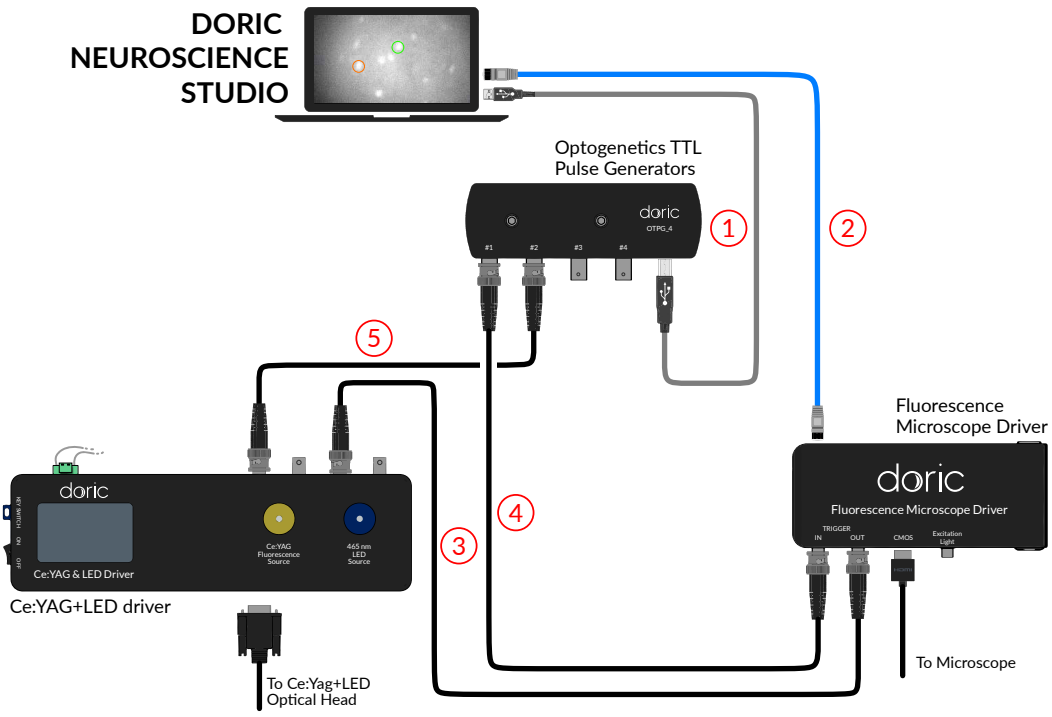

Figure 2.1: *Electronic connection necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with NpHR3 opsin activation.*

1 - Connect the OTPG to the computer

Connect the OTPG to the computer using the provided **USB-A - USB-B connection cable**. This allows the OTPG to be configured and controlled with the Doric Neuroscience Studio software.

2 - Connect the Microscope Driver to the computer

Connect the Microscope Driver to the computer using the provided **Ethernet cable**. This allows the Microscope Driver to be configured and controlled with the Doric Neuroscience Studio software.

3 - Connect the Microscope Driver Trigger OUT to the LED Light Source

Connect the **TRIGGER OUT** BNC output of the Microscope Driver to the **LED IN** of the Ce:YAG + LED Driver with a BNC cable. This allows the blue LED of the Ce:YAG + LED Optical Head to be in function when the microscope is recording.

4 - Connect the Microscope Driver Trigger IN to the OTPG

Connect the **TRIGGER IN** of the Microscope Driver to one of the **BNC Port** of the OTPG. The OTPG can then generates Pulses that will trigger the microscope.

5 - Connect the Ce:Yag Light Source to the OTPG

Connect the **Ce:YAG IN** of the Ce:YAG + LED Driver to a **BNC Port** of the OTPG. The Ce:YAG light source, used for opsin activation, can then be controlled with the OTPG to produce pulse patterns.

2.1.2 Configuration Example

This section explains how to setup the Ce:YAG + LED Driver and the different Tabs in Doric Neuroscience Studio for an example of an experiment involving GFP-like fluorophore and NpHR3 halorhodopsin activation. The experiment parameters are the following (fig 2.2).

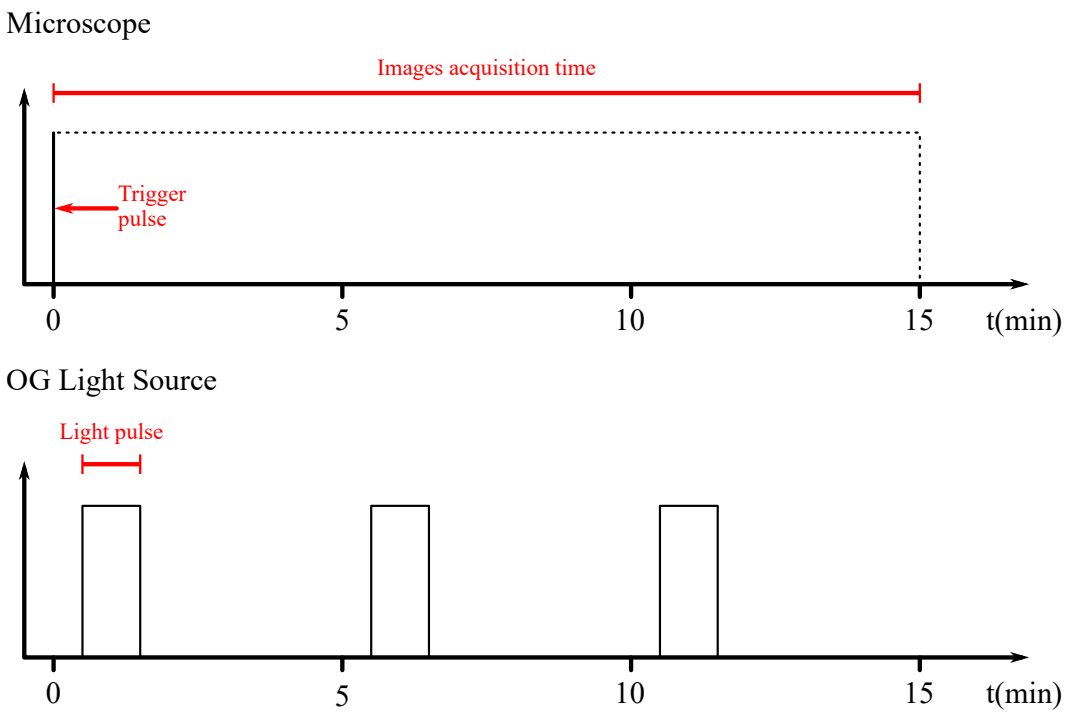

Figure 2.2: *Schematic of the output OTPG pulses. For the microscope, a short pulse triggers the microscope acquisition and for the OG light source, 3 pulses of 60 seconds, with an initial delay of 30 seconds, enable the light source.*

Microscope

- Microscope Driver Trigger IN connected to the OTPG channel 1.
- Continuous image recording for 15 minutes.

- Microscope exposure at 100 ms (10 images/second).
- LED light source with an intensity of 20 %.

OG stimulation

- Ce:YAG Trigger IN connected to the OTPG channel 2.
- OG stimulation every 5 minutes starting 30 seconds after recording for a total of 3 OG stimulations.
- A 60 seconds continuous OG light exposition for each OG stimulation.
- OG light source (i.e. Ce:YAG light source) at half the maximum intensity (>25 mW/mm^2 for eTOSFM).

Configure the Ce:YAG + LED Driver

For the light sources to be driven by the OTPG and the Microscope Driver, they need to be set in TTL mode.

1. Turn ON the Ce:YAG + LED Driver.
2. Push the *Ce:YAG Control Knob* a few time until the *TTL Mode* is displayed on the screen.
3. Turn the *Control Knob* to increase the current applied to the Ce:YAG light source. A maximum of 1200 mA can be applied on the light source. To have half the maximum intensity, set the current at 600 mA.
4. Push the *LED Control Knob* a few time until the *TTL Mode* is displayed on the screen.
5. Turn the *Control Knob* to increase the current applied to the LED light source to reach the desired output power. A maximum of 1000 mA can be applied on the light source. For a 20 % illumination, a current of 200 mA should be applied. It is recommended to not use the LED at maximum power as it could photobleach the fluorophores and/or saturate the imaging detector.
6. Ensure that the *Safety Key* is in the Driver and that the *Interlock* is in a closed loop.
7. The driver is ready to be triggered. The light sources will only generate light if the Microscope Driver or the OTPG is triggering the driver.

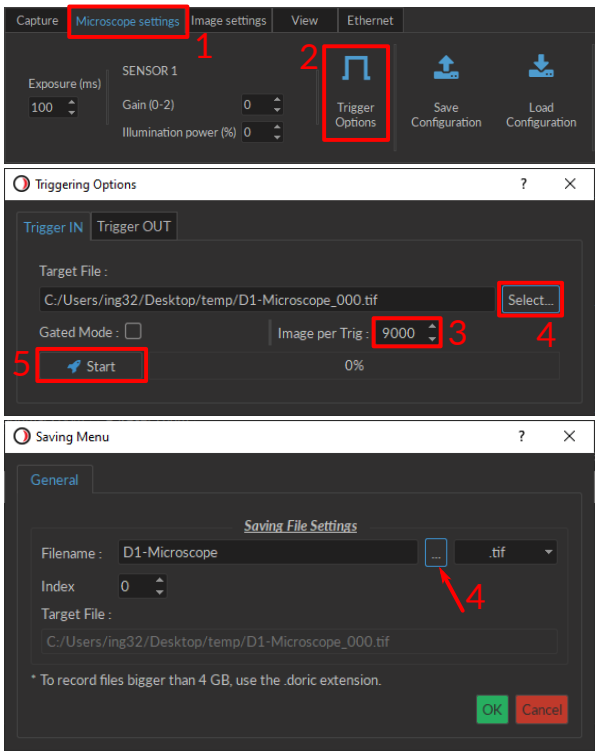

Figure 2.3: *Configuration of the Microscope in Doric Neuroscience Studio.*

Configure the Microscope Tab (Fig. 2.3)

For the microscope to be driven by the OTPG, it needs to be set in trigger mode.

1. On the Microscope Main Tab, select the *Microscope Settings* Tab.
2. Click on *Trigger Options*. An external window will open.
3. Set number of *Image per Trig* to 9,000 (10 images/second for 15 minutes).
4. Click *Select...* and set the saving Filename and Path. For files larger than 4GB, it is recommended to use the .doric extension file (HDF5 based file format) to save the microscope images. Click *OK* to close the saving menu.
5. Click *Start*. The microscope is now ready to start acquiring images. No images will be acquired until a trigger is sent from the OTPG.

Configure the OTPG Tab (Fig. 2.4)

The OTPG needs to be configured to send trigger pulse to the OG Light source and the Microscope Driver.

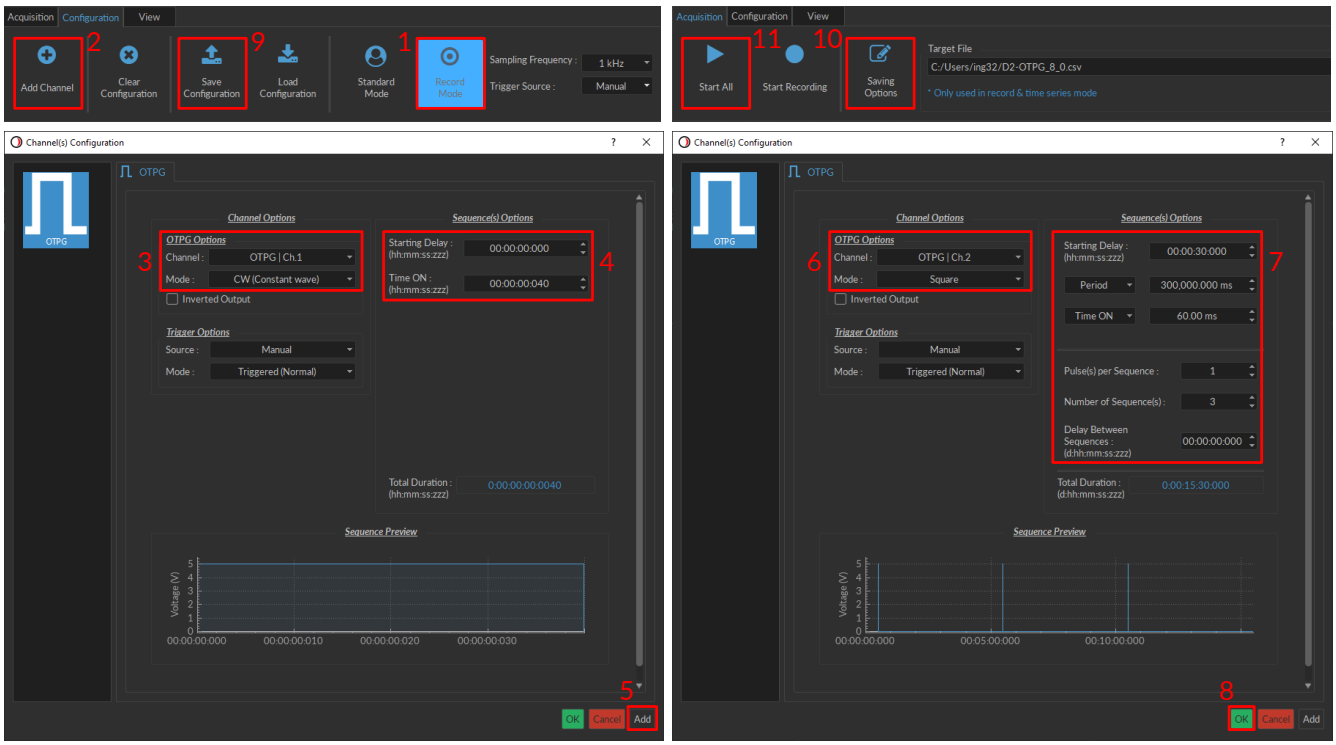

Figure 2.4: *OTPG Tab Configuration in Doric Neuroscience Studio.*

1. If you wish to record the Input/Output signals of the OTPG, set the OTPG in *Record Mode* in the *Configuration* Tab[1].

Microscope Trigger Configuration

2. In the *Configuration* Tab, click *Add Channel*.
3. Set the following parameters in *OTPG Options*:
   - Channel: Channel OTPG|Ch.1 (Channel used for the Microscope Trigger IN).
   - Mode: CW.
4. Set the following parameters in *Sequences Options*:

[1]Record Mode is only available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher.

- Starting Delay: 00:00:00:000.
- Time ON: 00:00:00:040.

5. Click *Add* to save the current channel and add a new channel.

OG Light Control

6. Set the following parameters in *OTPG Options*:
    - Channel: Channel OTPG|Ch2. (Channel used to trigger the Ce:YAG Light source).
    - Mode: Square.
7. Set the following parameters in *Sequences Options*:
    - Starting Delay: 00:00:30:000.
    - Frequency: change to Period: 300,000.000 ms.
    - Time ON: 60,000.00 ms.
    - Pulse per Sequence: 1.
    - Number of Sequences: 3.
    - Delay between sequences: 00:00:00:000
8. Click *OK* to save the selection.

Save and Start

9. To save the configuration for future use, click *Save Configuration* and save the file with the desired filename. The configuration can be loaded in Doric Neuroscience Studio using the *Load Configuration* button.
10. To save the OTPG signal, select the desired path and filename in the *Saving Option* menu in the *Acquisition* tab.
11. When ready to start the imaging session, click *Start Recording* to record the OPTG signal or *Start All* to start the OTPG without recording the signal. This will also sent the trigger to the microscope to start recording images and start the illumination pattern of the OG light source (as shown in Fig. 2.2).

2.2 Configuration RCamp2 + ChR2

The present section explains the setup necessary to perform fluorescence imaging of RFP-like fluorophore combined with ChR2 opsin activation. In this configuration, the OTPG is the master device and drives the other devices.

2.2.1 Connections (Fig. 2.5)

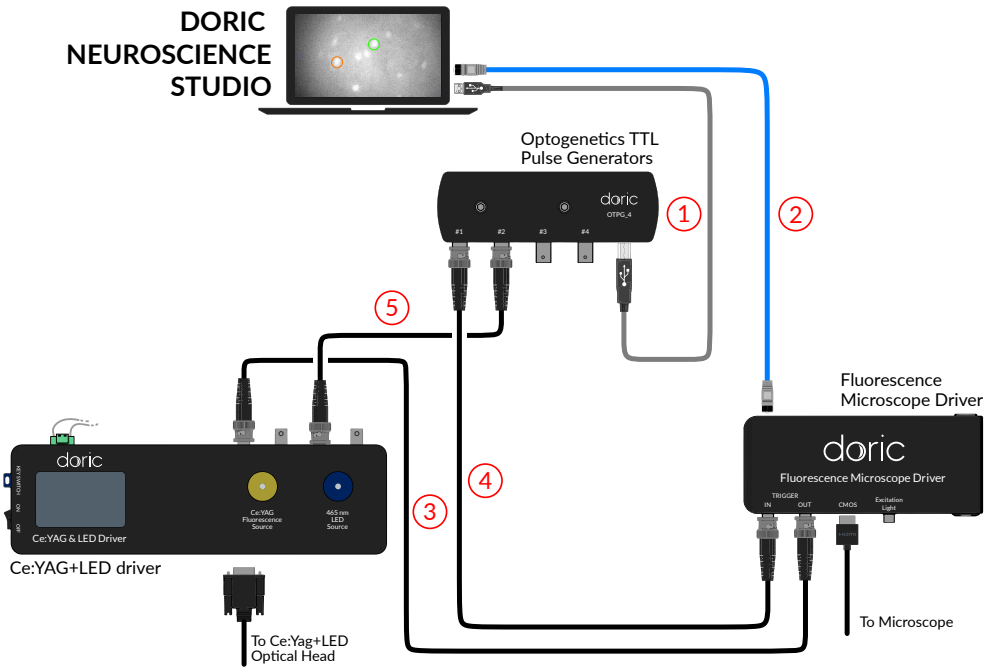

Figure 2.5: *Electronic connection necessary to perform Miniature Fluorescence Microscopy of RFP-like fluorophore combined with ChR2 opsin activation.*

1 - Connect the OTPG to the computer

Connect the OTPG to the computer using the provided **USB-A - USB-B connection cable**. This allows the OTPG to be configured and controlled with the Doric Neuroscience Studio software.

2 - Connect the Microscope Driver to the computer

Connect the Microscope Driver to the computer using the provided **Ethernet cable**. This allows the Microscope Driver to be configured and controlled via the Doric Neuroscience Studio software.

3 - Connect the Microscope Driver Triger OUT to the Ce:YAG Light Source

Connect the **TRIGGER OUT** BNC output of the Microscope Driver to the **Ce:YAG IN** BNC output of the Ce:YAG + LED Driver with a BNC cable. This allows the Ce:YAG light source of the Ce:YAG + LED Optical Head to be in function when the microscope is recording.

4 - Connect the Microscope Trigger IN to the OTPG

Connect the **TRIGGER IN** of the Microscope Driver to one of the **BNC Port** of the OTPG. The OTPG can then generates Pulses that will trigger the microscope.

5 - Connect the OG Light Source to the OTPG

Connect the **Ce:LED IN** of the Ce:YAG + LED Driver to a **BNC Port** of the OTPG. The LED, used for opsin activation, can then be controlled with the OTPG to produce pulse patterns.

2.2.2 Configuration Example

This section explains how to setup the Ce:YAG + LED Driver and the different Tabs in Doric Neuroscience Studio for an example of an experiment involving RFP-like fluorophore and ChR2 opsin activation. The parameters are the following (fig 2.6).

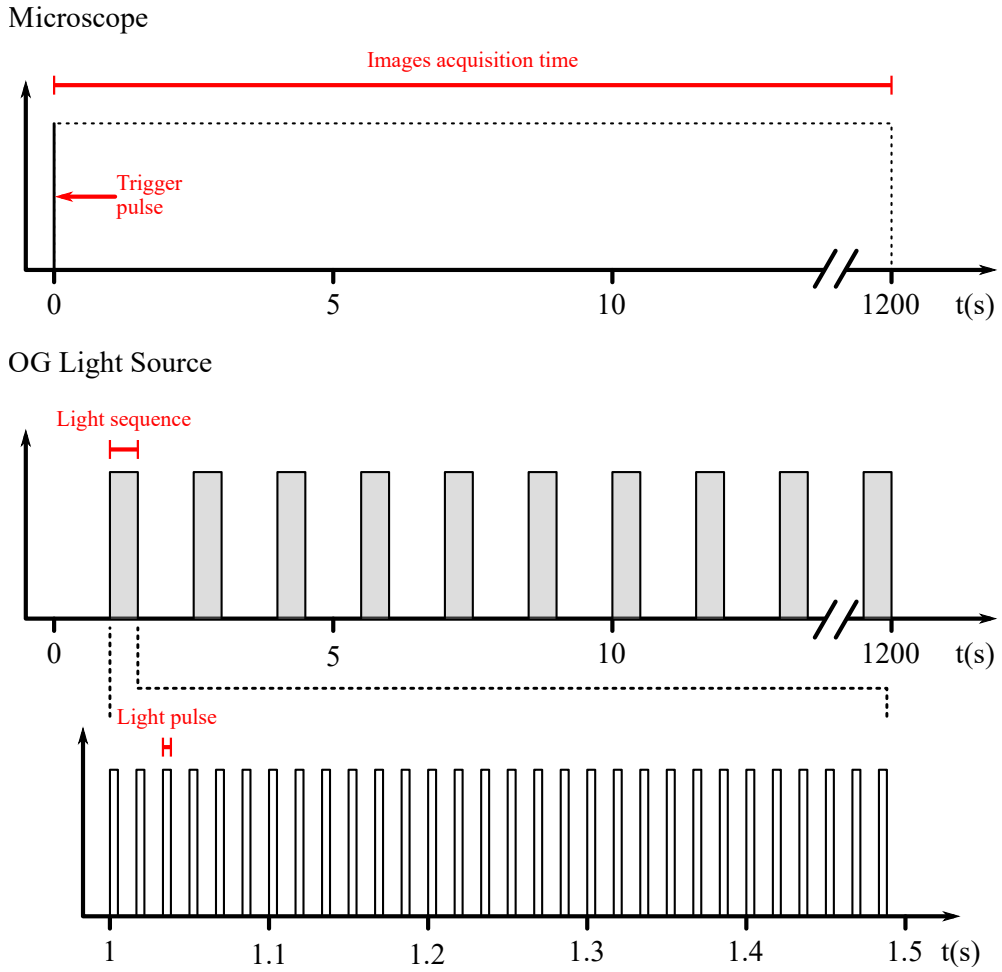

Figure 2.6: *Schematic of the output OTPG pulses. For the microscope, a short pulse triggers the microscope acquisition, and for the OG light source, 0.5 seconds sequences containing 5 ms pulses at 60 Hz every 1.5 seconds enable the light source.*

Microscope

- Microscope Trigger IN connected to the OTPG channel 1.
- Continuous image recording for 20 minutes.
- Microscope exposure at 100 ms (10 images/second).
- Ce:YAG light source with an intensity of 20 %.

OG stimulation

- LED Trigger IN connected to the OTPG channel 2.
- OG stimulation sequences of 0.5 second with 1 second between each sequence.
- Each sequence produces 5 ms pulses at a frequency of 60 Hz.
- OG light source (i.e. LED light source) at half the maximum intensity (>5 mW/mm^2).

Configure the Ce:YAG + LED Driver

For the light sources to be driven by the OTPG and the Microscope Driver, they need to be set in TTL mode.

1. Turn ON the Ce:YAG + LED Driver.
2. Push the *Ce:YAG Control Knob* a few time until the *TTL Mode* is displayed on the screen.
3. Turn the *Control Knob* to increase the current applied to the Ce:YAG light source. A maximum of 1200 mA can be applied on the light source. For a 30 % illumination, a current of 360 mA should be applied. It is recommended to not use the light source at maximum power as it could photobleach the fluorophores and/or saturate the imaging detector.
4. Push the *LED Control Knob* a few time until the *TTL Mode* is displayed on the screen.
5. Turn the *Control Knob* to increase the current applied to the LED light source to reach the desired output power. A maximum of 1000 mA can be applied on the light source. To have half the maximum intensity, set the current at 500 mA.
6. Ensure that the *Safety Key* is in the Driver and that the *Interlock* is in a closed loop.
7. The driver is ready to be triggered. The light sources will only generate light if the microscope or the OTPG is triggering the driver.

Configure the Microscope Tab (Fig. 2.7)

For the microscope to be driven by the OTPG, it needs to be set in trigger mode.

1. On the Microscope Main Tab, select the *Microscope Settings* Tab.

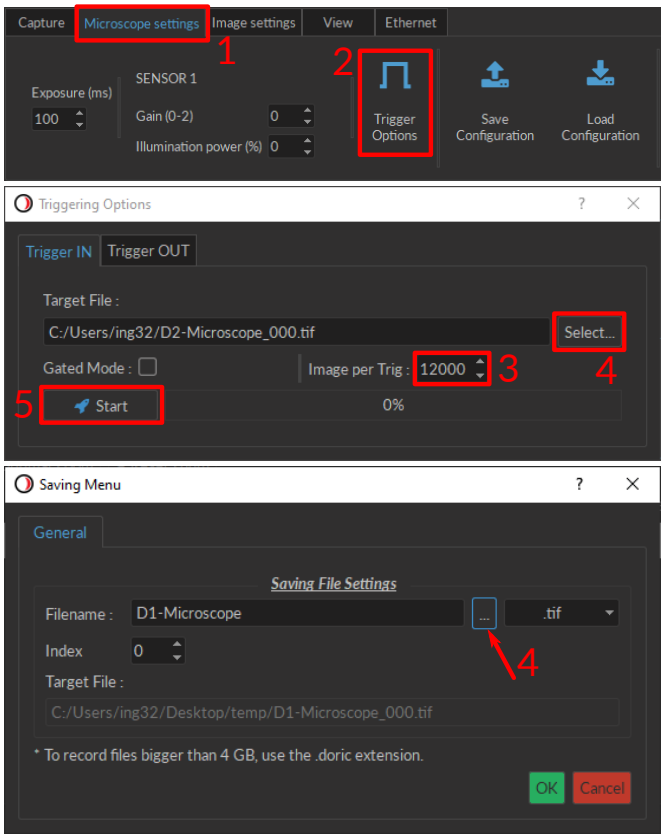

Figure 2.7: *Configuration of the Microscope on Doric Neuroscience Studio.*

2. Click on *Trigger Options*. An external window will open.
3. Set number of *Image per Trig* to 12,000 (10 images/second for 20 minutes).
4. Click *Select...* and set the saving Filename and Path. For files larger than 4GB, it is recommended to use the .doric extension file (HDF5 based file format) to save the microscope images. Click *OK* to close the saving menu.
5. Click *Start*. The microscope is now ready to start acquiring images. No images will be acquired until a trigger is sent from the OTPG.

Configure the OTPG Tab (Fig. 2.8)

The OTPG needs to be configured to send trigger pulse to the OG Light source and the Microscope Driver.

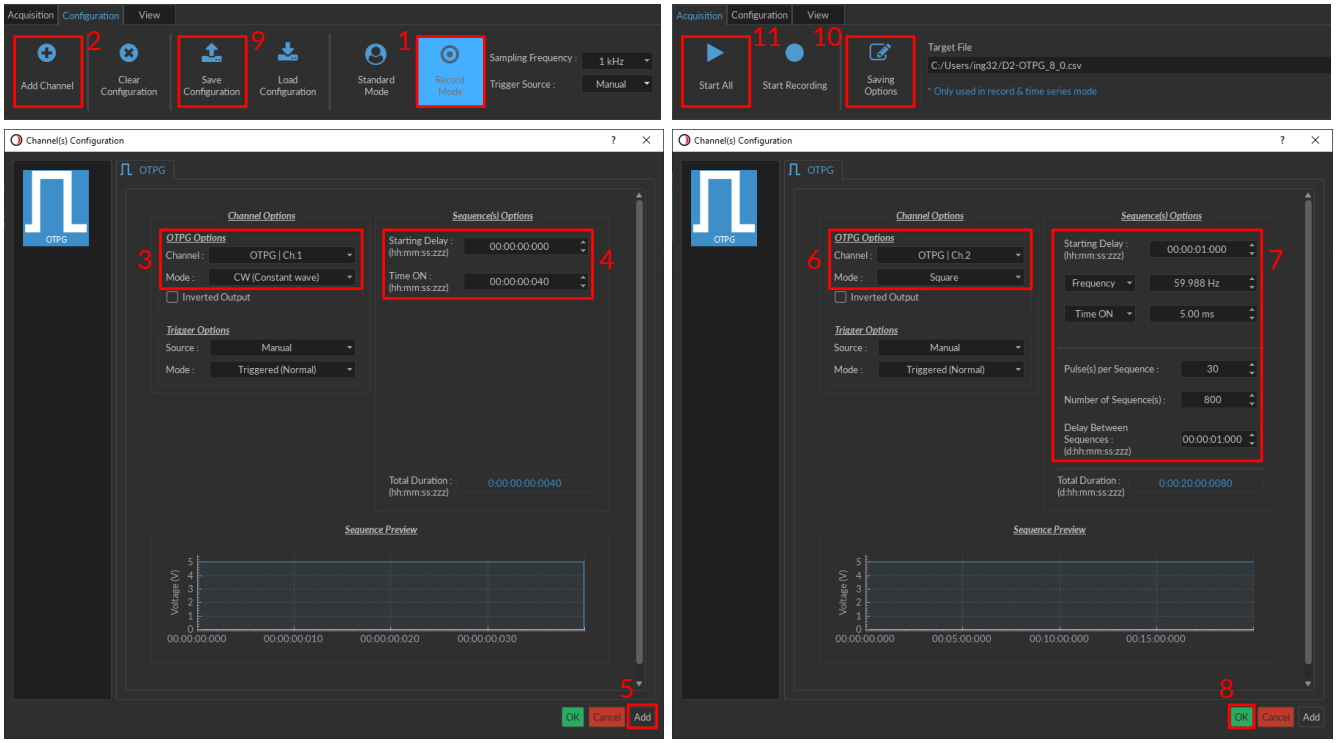

Figure 2.8: *OTPG Tab Configuration in Doric Neuroscience Studio.*

1. If you wish to record the Input/Output signals of the OTPG, set the OTPG in *Record Mode* in the *Configuration* Tab[2].

Microscope Trigger Configuration

2. In the *Configuration* Tab, click *Add Channel*.
3. Set the following parameters in *OTPG Options*:
   - Channel: Channel OTPG|Ch.1 (Channel used for the Microscope Trigger IN).
   - Mode: CW.
4. Set the following parameters in *Sequences Options*:
   - Starting Delay: 00:00:00:000.
   - Time ON: 00:00:00:040.
5. Click *Add* to save the current channel and add a new channel.

[2] Record Mode is only available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher.

OG Light Control

6. Set the following parameters in *OTPG Options*:
    - Channel: Channel OTPG|Ch2. (Channel used to trigger the Ce:YAG Light source).
    - Mode: Square.
7. Set the following parameters in *Sequences Options*:
    - Starting Delay: 00:00:01:000.
    - Frequency: 60.000 Hz.
    - Time ON: 5.00 ms.
    - Pulse per Sequence: 30.
    - Number of Sequences: 800.
    - Delay between sequences: 00:00:01:000
8. Click *OK* to save the selection.

Save and Start

9. To save the configuration for future use, click *Save Configuration* and save the file with the desired filename. The configuration can be loaded in Doric Neuroscience Studio using the *Load Configuration* button.
10. To save the OTPG signal, select the desired path and filename in the *Saving Option* menu in the *Acquisition* tab.
11. When ready to start the imaging session, click *Start Recording* to record the OPTG signal or *Start All* to start the OTPG without recording the signal. This will also sent the trigger to the microscope to start recording images and start the illumination pattern of the OG light source (as shown in Fig. 2.6).

2.3 Configuration with an External Trigger

It is possible to configure the microscope system to start an acquisition at the input of an external trigger. It can be particularly useful when we want an experiment to start at the event of an external element. This section explains how to setup the microscopy system in such way that an imaging session starts at the reception of an external trigger. In this configuration, the OTPG is the master device and drives the other devices while an external device sends a trigger to start the experiment.

2.3.1 Connections of the External Trigger to the System (Fig. 2.9)

Connect the external source trigerring the microscope system to one of the **BNC port of the OTPG** (Connection 6 in Fig. 2.9). ***For OTPG_8, only ports # 5 through 8 are input ports.*** The rest of the connections are identical to the ones displayed in section 2.1.1 for the GCaMP6 + NpHR3 configuration (shown as reference in Fig. 2.9) and in section 2.2.1 for the RCamp2 + ChR2 configuration.

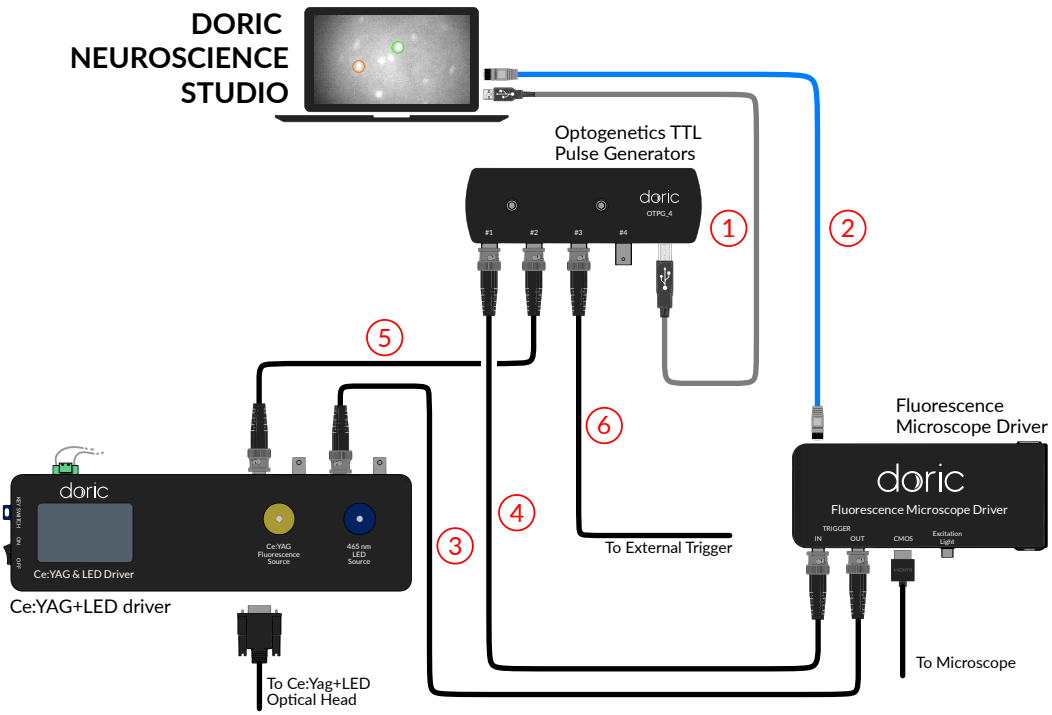

Figure 2.9: *Electronic connection necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with NpHR3 opsin activation and triggered by an External Trigger.*

2.3.2 Configuration Example

This section explains how to setup the Ce:YAG + LED Driver and the different Tabs in Doric Neuroscience Studio for an example of an experiment involving GFP-like fluorophore imaging, NpHR3 halorhodopsin activation and an external triggering source. The parameters are similar to section 2.1.2 with the addition to the external trigger and are the following (fig 2.10):

Microscope

- Microscope Trigger IN connected to the OTPG channel 1.
- Continuous image recording for 15 minutes.
- Microscope exposure at 100 ms (10 images/second).
- Light source with an intensity of 20 %.

OG stimulation

- Ce:YAG Trigger IN connected to the OTPG channel 2.
- OG stimulation every 5 minutes starting 30 seconds after recording for a total of 3 OG stimulations.
- A 60 seconds continuous OG light exposition every OG stimulation.
- OG light source (i.e. Ce:YAG light source) at half the maximum intensity (>25 mW/mm^2).

External Trigger

- The External Trigger connected to the OTPG channel 5 (The OTPG_8 is used for this demonstration. For an OTPG_4, connect the External Trigger to any available port)

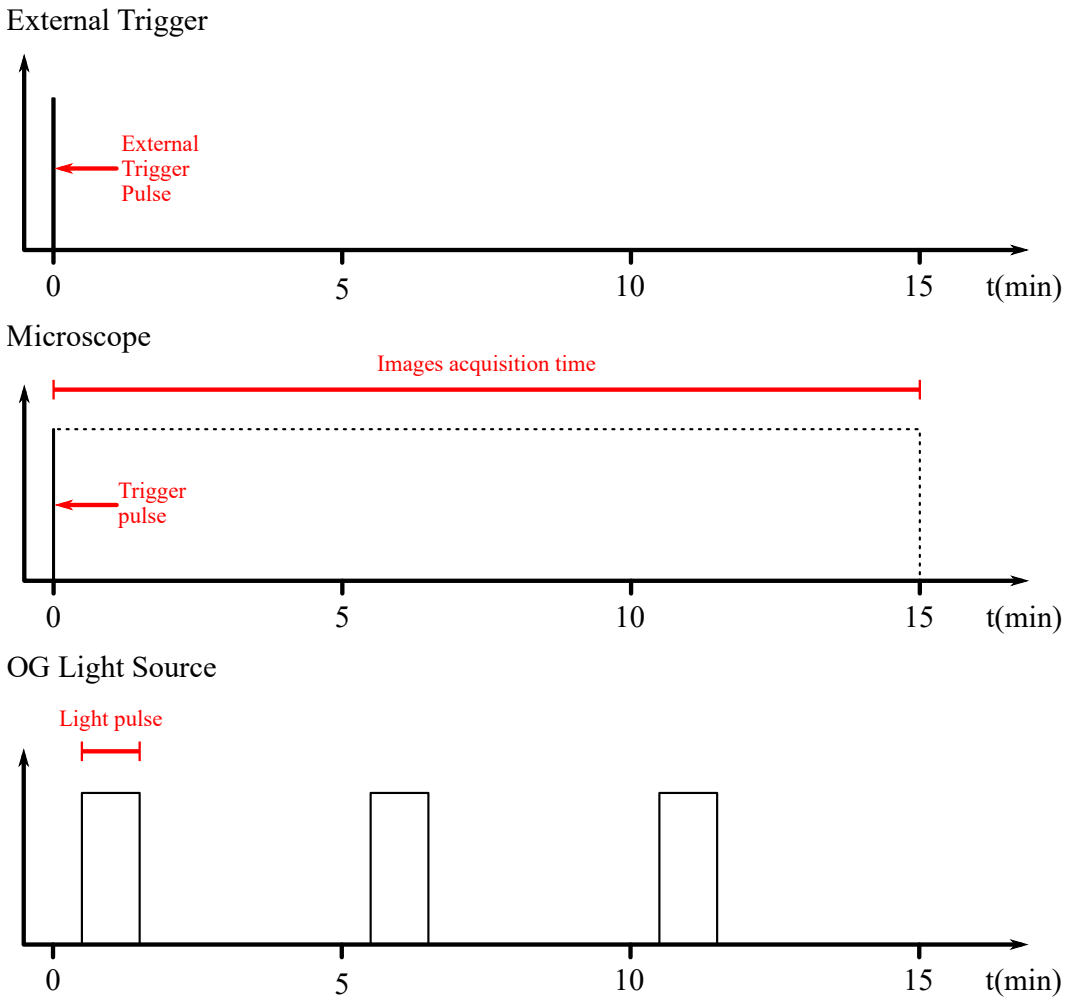

Figure 2.10: *Schematic of the OTPG input/output signals and the microscope acquisition time. For the microscope, a short pulse triggers the microscope acquisition, and for the OG light source, 3 pulses of 60 seconds, with an initial delay of 30 seconds, enable the light source. The beginning of the acquisition start at the reception of an External Trigger pulse.*

Configure the Ce:YAG + LED Driver

For the light sources to be driven by the OTPG and the Microscope Driver, they need to be set in TTL mode.

1. Turn ON the Ce:YAG + LED Driver.
2. Push the *Ce:YAG Control Knob* a few time until the *TTL Mode* is displayed on the screen.

3. Turn the *Control Knob* to increase the current applied to the Ce:YAG light source. A maximum of 1200 mA can be applied to the light source. To have half the maximum intensity, set the current at 600 mA.
4. Push the *LED Control Knob* a few time until the *TTL Mode* is displayed on the screen.
5. Turn the *Control Knob* to increase the current applied to the LED light source to reach the desired output power. A maximum of 1000 mA can be applied to the light source. For a 20 % illumination, a current of 200 mA should be applied. It is recommended to not use the LED at maximum power as it could photobleach the fluorophores and/or saturate the imaging detector.
6. Ensure that the *Safety Key* is in the Driver and that the *Interlock* is in a closed loop.
7. The driver is ready to be triggered by the OTPG. The light sources will only generate light if the microscope or the OTPG is triggering the driver.

Configure the Microscope Tab (Fig. 2.11)

For the microscope to be driven by the OTPG, it needs to be set in trigger mode.

1. On the Microscope Main Tab, select the *Microscope Settings* Tab.
2. Click on *Trigger Options*. An external window will open.
3. Set number of *Image per Trig* to 9,000 (10 images/second for 15 minutes).

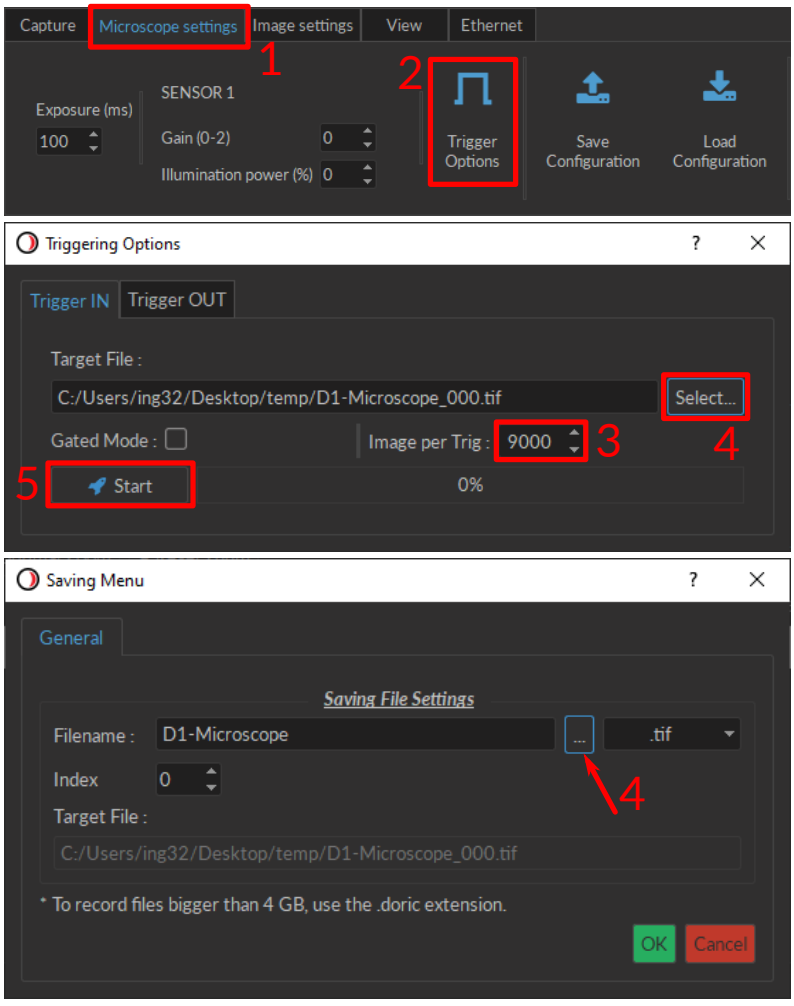

Figure 2.11: *Configuration of the Microscope in Doric Neuroscience Studio.*

4. Click *Select...* and set the saving Filename and Path. For files larger than 4GB, it is recommended to use the .doric extension file (HDF5 based file format) to save the microscope images. Click *OK* to close the saving menu.
5. Click *Start*. The microscope is now ready to start acquiring images. No images will be acquired until a trigger is sent from the OTPG.

Configure the OTPG Tab (Fig. 2.12)

The OTPG needs to be configured to receive the external trigger and send trigger pulses to the OG Light source and the Microscope.

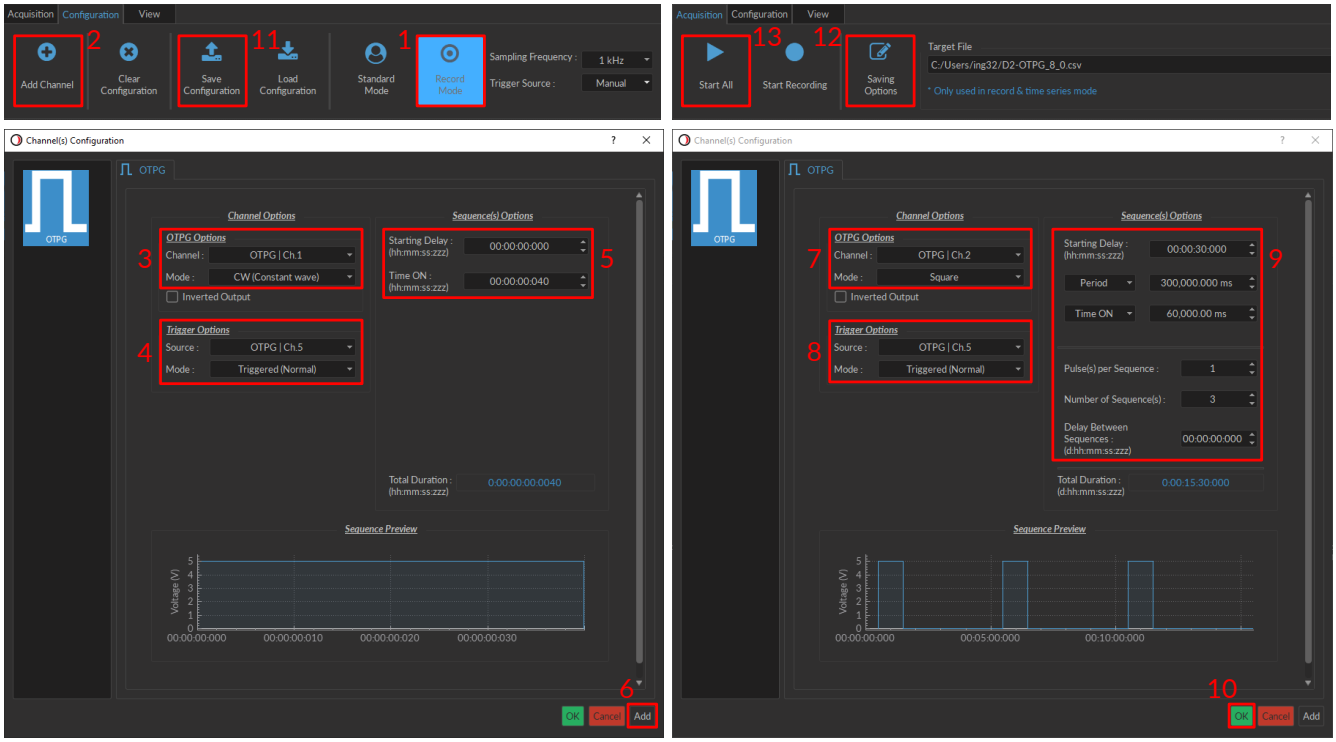

Figure 2.12: *OTPG Tab Configuration in Doric Neuroscience Studio.*

1. If you wish to record the Input/Output signals of the OTPG, set the OTPG in *Record Mode* in the *Configuration* Tab[3].

Microscope Trigger Configuration

2. On the OTPG main Tab, click *Add Channel*.
3. Set the following parameters in *OTPG Options*:
   - Channel: OTPG|Ch.1 (Channel used for the Microscope Trigger IN).
   - Mode: CW.
4. Set the following parameters in *Trigger Options*:
   - Source: OTPG|Ch.5 (Input channel of the External Trigger).
   - Mode: Triggered (Normal).
5. Set the following parameters in *Sequences Options*:
   - Starting Delay: 00:00:00:000.
   - Time ON: 00:00:00:040 ms.
6. Click *Add* to save the current channel and add a new channel.

[3] Record Mode is only available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher.

OG Light Control

7. Set the following parameters in *OTPG Options*:
    - Channel: OTPG|Ch2. (Channel used to trigger the Ce:YAG Light source).
    - Mode: Square.
8. Set the following parameters in *Trigger Options*:
    - Source: OTPG|Ch.5
    - Mode: Triggered (Normal).
9. Set the following parameters in *Sequences Options*:
    - Starting Delay: 00:00:30:000.
    - Frequency: change to Period: 300,000.000 ms.
    - Time ON: 60,000.00 ms.
    - Pulse per Sequence: 1.
    - Number of Sequences: 3.
    - Delay between sequences: 00:00:00:000
10. Click *OK* to save the selection.

Save and Start

11. To save the configuration for future use, click *Save Configuration* and save the file with the desired filename. The configuration can be loaded in Doric Neuroscience Studio using the *Load Configuration* button.
12. To save the OTPG signal, select the desired path and filename in the *Saving Option* menu in the *Acquisition* tab.
13. When ready to start the imaging session, click *Start Recording* to record the OPTG signal or *Start All* to start the OTPG without recording the signal. The channels are then waiting for an external trigger input to start their configured sequence. Once the OTPG receive the external trigger, it will also sent the trigger to the microscope to start recording images and start the illumination pattern of the OG light source (as shown in Fig. 2.10).

2.4 Configuration for a Behavior Camera and a Microscope Only

Many experiments require the synchronization of the Miniature Fluorescence Microscope with a Behavior Tracking Camera. It is recommended to synchronize the camera to the microscope using external triggers as it is more accurate than internal triggers. This section explains the simplest case of how to synchronize the Behavior Camera with the microscopy system where the Microscope Driver is the master device that drives the camera. Note that in this configuration, the microscope and the camera have the same framerate and doesn't involve optogenetic stimulation, as it would require a different configuration.

2.4.1 Connections (Fig 2.13)

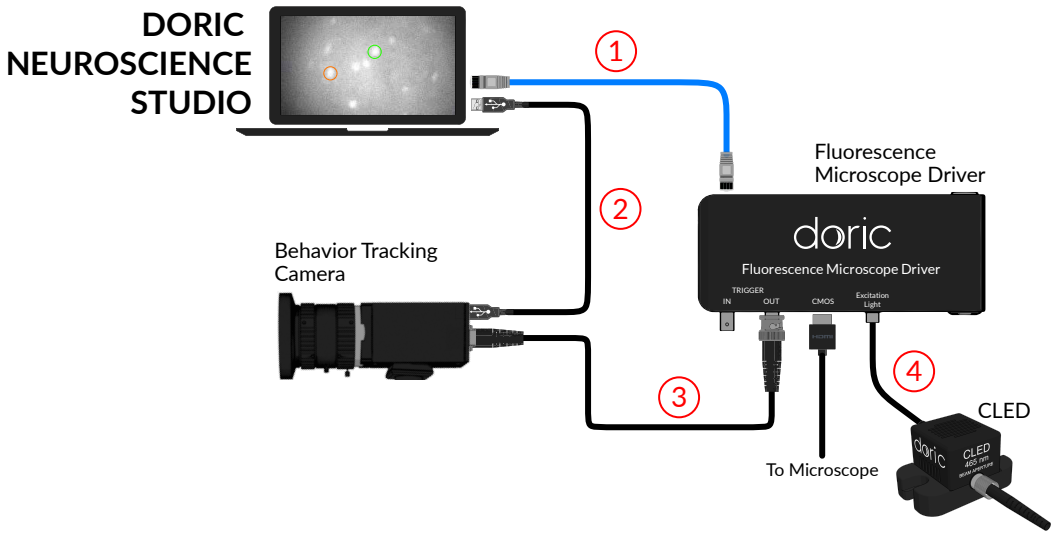

Figure 2.13: *Electronic connections necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with a behavior recording of the subject.*

1 - Connect the Microscope Driver to the computer

Connect the Microscope Driver to the computer using the provided **Ethernet cable**. This allows the Microscope Driver to be configured and controlled with the Doric Neuroscience Studio software.

2 - Connect the Camera to the computer

Connect the provided USB 3.0 cable of the camera to a **USB3 port of the computer**. To install the camera driver, please refer to the Behavior Tracking Camera user manual. The Behavior Camera can then be configured by the computer and images can be acquired.

3 - Connect the Camera to the Microscope Driver

Connect the triggering cable of the camera to the **Trigger OUT** of the Microscope Driver to synchronize the Behavior Camera with the microscope.

4 - Connect the LED to the Microscope Driver

Connect the **LED to the M8 output** of the Fluorescence Microscope Driver. As no optogenetic stimulation is necessary, the fluorescence illumination will be produced via a standalone LED connected to the Microscope Driver.

2.4.2 Configuration Example

This section explains how to setup the different Tabs in Doric Neuroscience Studio for an example of an experiment involving GFP-like fluorophore with a Behavior Camera monitoring. The parameters are the following (fig 2.14):

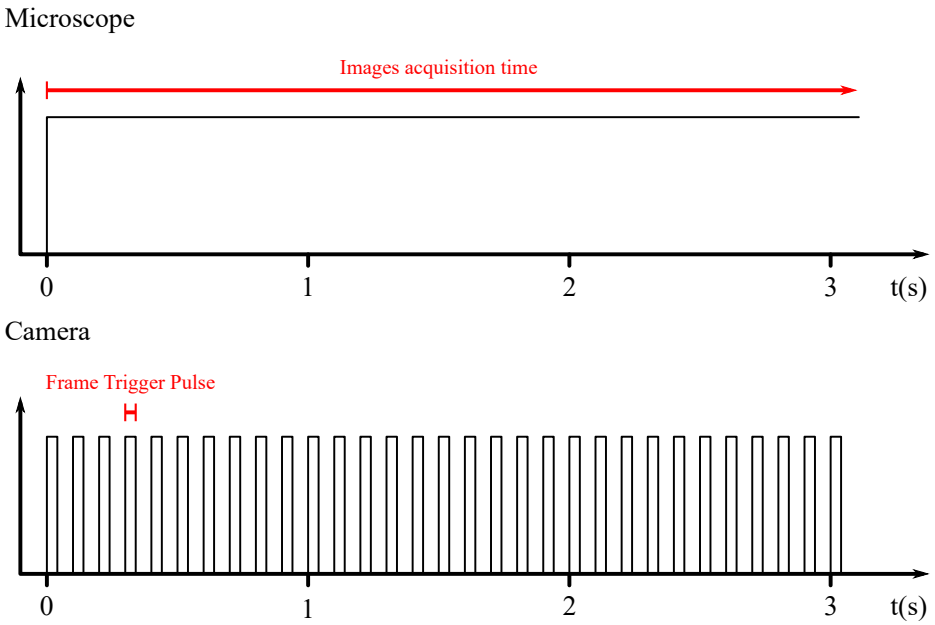

Figure 2.14: *Schematic of the microscope acquisition and camera input signal. The microscope is manually triggered, and the Camera frame acquisition is triggered by the microscope output signal.*

Microscope

- Continuous image recording.
- Microscope exposure at 100 ms (10 images/second).
- Light source with an intensity of 20 %.

Behavior Camera

- External Trigger connected to the Microscope Driver Trigger OUT.
- Frame rate of 10 FPS (For a proper synchronization of the camera with the microscope images and for the time stamps to be accurate, the frame rate of the camera has to match the one of the microscope).

Configure the Camera Tab (Fig. 2.15)

The camera will be triggered by the Microscope Driver and needs to be configured in External Trigger mode.

1. In the Capture Tab, click *Saving options*. An external window will open. Set the saving Filename and Path. Click *OK* to close the saving menu.
2. Select the *Settings* Tab.
3. In the *FPS* field, select 10. This sets the camera at 10 frames/seconds to match the acquisition rate of the microscope. The other camera settings in this tab can be set to optimize the image settings at the user's preference.
4. Select the *Synchronization* Tab.
5. In the *Trigger mode* field, select *External*.
6. Select the *Capture* Tab and click *Record* to enable the acquisition. The camera recording will start with the microscope recording. Once the Microscope finished recording, the Camera is still in record mode to continue recording images if another trigger is received from the microscope. Click *Stop* to exit record mode and save the video.

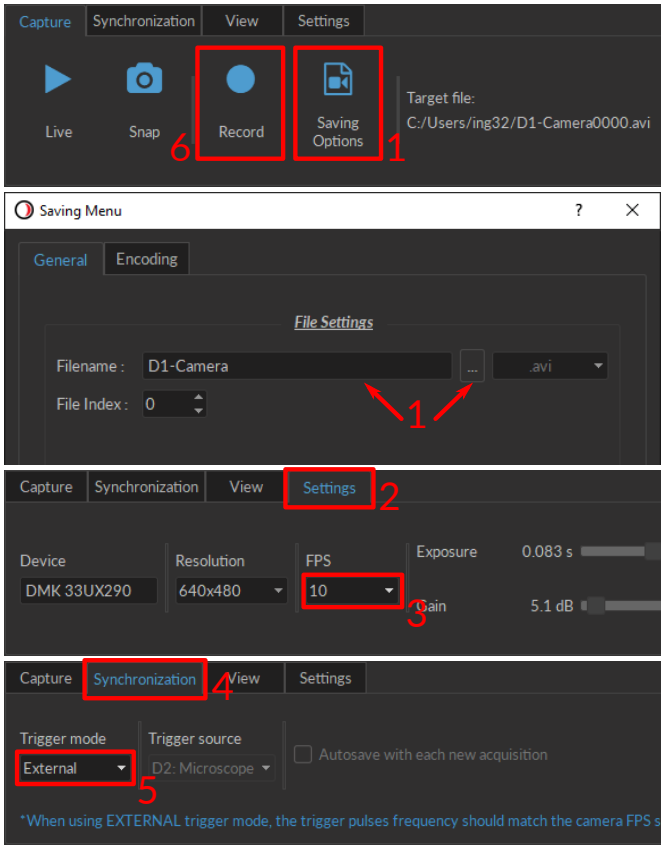

Figure 2.15: *Camera Configuration in Doric Neuroscience Studio.*

Configure the Microscope Tab (Fig. 2.16)

The microscope and the LED illumination power is configured via this Tab.

1. On the Microscope Main Tab, select the *Microscope Settings* Tab.
2. In the *Illumination Power* field, type 20. This will provide a 20 % LED illumination power to the microscope.
3. Click on *Trigger Options*. An external window will open.
4. Click on the *Trigger OUT* Tab and select the *Triggered w/ each frame* mode. In this mode, the microscope driver outputs a TTL pulse at the beginning of each frame.
5. Close the window and select the *Capture* Tab.
6. Click *Saving options* and set the saving Filename and Path. For files larger than 4GB, it is recommended to use the .doric extension file (HDF5 based file format) to save the microscope images. Click *OK* to close the saving menu.
7. When ready to start recording, click *Record* and the acquisition will start.
8. To stop recording, click *Stop*. Do not forget to also stop the acquisition of the camera in the Camera Tab to save the behavior recording.

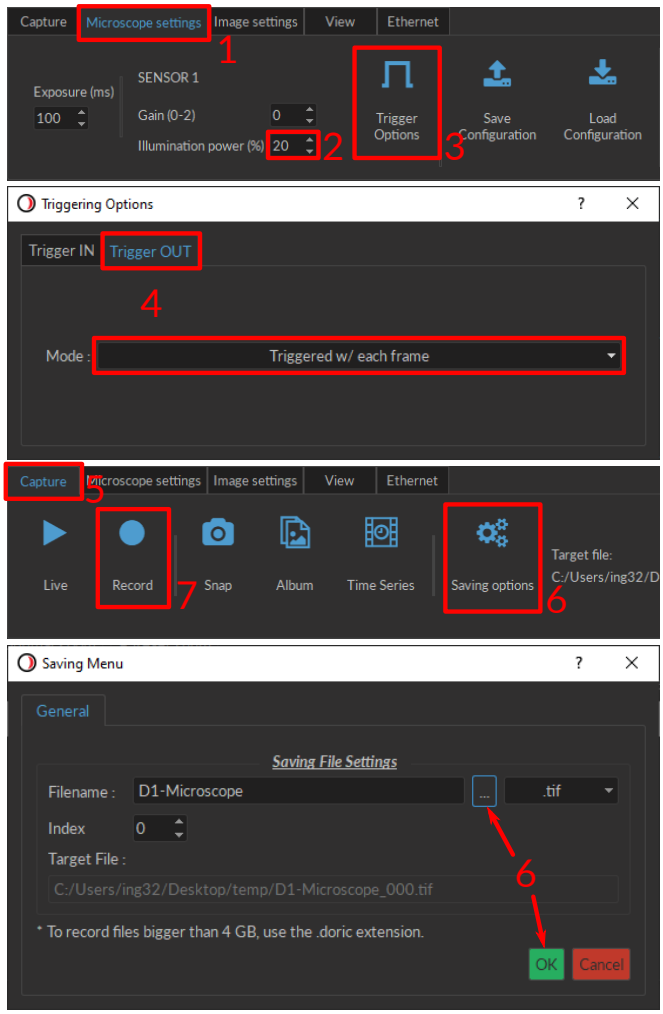

Figure 2.16: *Microscope Configuration in Doric Neuroscience Studio.*

2.5 Configuration for a Behavior Camera and a Microscope with a Different Frame Rate

The synchronization of the Behavior Tracking Camera with the Miniature Fluorescence Microscope as presented in section 2.4 is the simplest case but presents the drawback of having to match the camera frame rate with the microscope frame rate. This section explains how to synchronize the Behavior Tracking Camera with the microscope with a different frame rate. In this configuration, the OTPG is the master device and drives the Behavior Camera and the Microscope Driver.

2.5.1 Connections

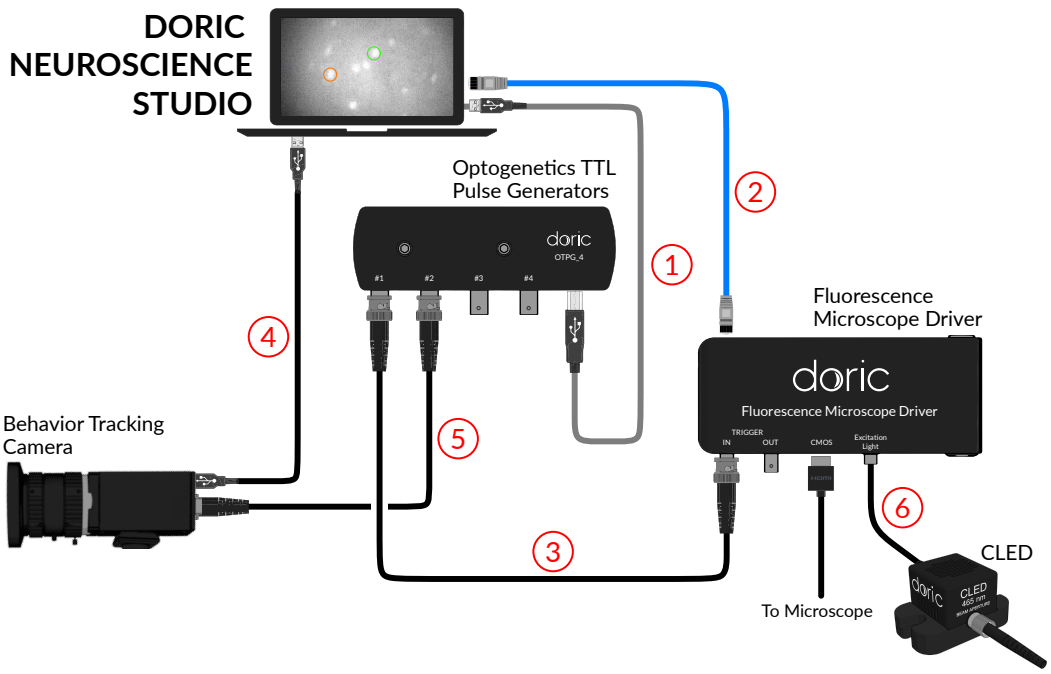

Figure 2.17: *Electronic connection necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with a behavior recording of the subject.*

1 - Connect the OTPG to the computer

Connect the OTPG to the computer using the provided **USB-A - USB-B connection cable**. This allows the OTPG to be configured and controlled with the Doric Neuroscience Studio software.

2 - Connect the Microscope Driver to the computer

Connect the Microscope Driver to the computer using the provided **Ethernet cable**. This allows the Microscope Driver to be configured and controlled with the Doric Neuroscience Studio software.

3 - Connect the Microscope Driver to the OTPG

Connect the Microscope Driver **Trigger IN BNC port** to a **BNC Output Port** of the OTPG. The Microscope will be triggered by the OTPG.

4 - Connect the Camera to the computer

Connect the provided USB 3.0 cable of the camera to a **USB3 port of the computer**. To install the camera driver, please refer to the Behavior Tracking Camera user manual. The Behavior Camera can then be configured by the computer and images can be acquired.

5 - Connect the Camera to the OTPG

Connect the triggering cable of the camera to the **BNC Output Port** of the OTPG. The Behavior Tracking Camera will start at the trigger of the OTPG.

6 - Connect the LED to the Microscope Driver

Connect the **LED to the M8 output** of the Fluorescence Microscope Driver. As no optogenetic stimulation is necessary, the fluorescence illumination will be produced via a standalone LED connected to the Microscope Driver.

2.5.2 Configuration Example

This section explains how to setup the different Tabs in Doric Neuroscience Studio for an example of an experiment involving GFP-like fluorophore with a Behavior Camera monitoring. The parameters are the following (fig 2.18):

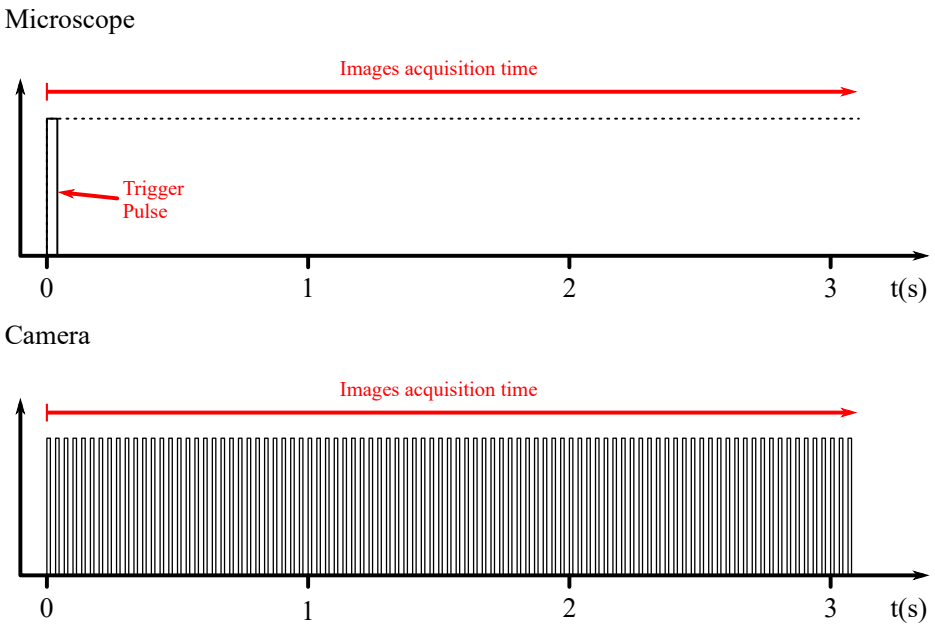

Figure 2.18: *Schematic of the Microscope and Camera input signal generated by the OTPG. The microscope is triggered by a single pulse, and the Camera frame acquisition is triggered by a train of pulses whose frequency matches the Camera frame rate.*

Microscope

- Continuous image recording for 15 minutes.
- Microscope exposure at 100 ms (10 images/second).
- LED light source with an intensity of 20 %.
- Microscope Trigger IN connected to the OTPG Channel #1.

Behavior Camera

- Continuous image recording for 15 minutes.
- Frame rate of 30 FPS.
- External Trigger connected to the OTPG Channel #2.

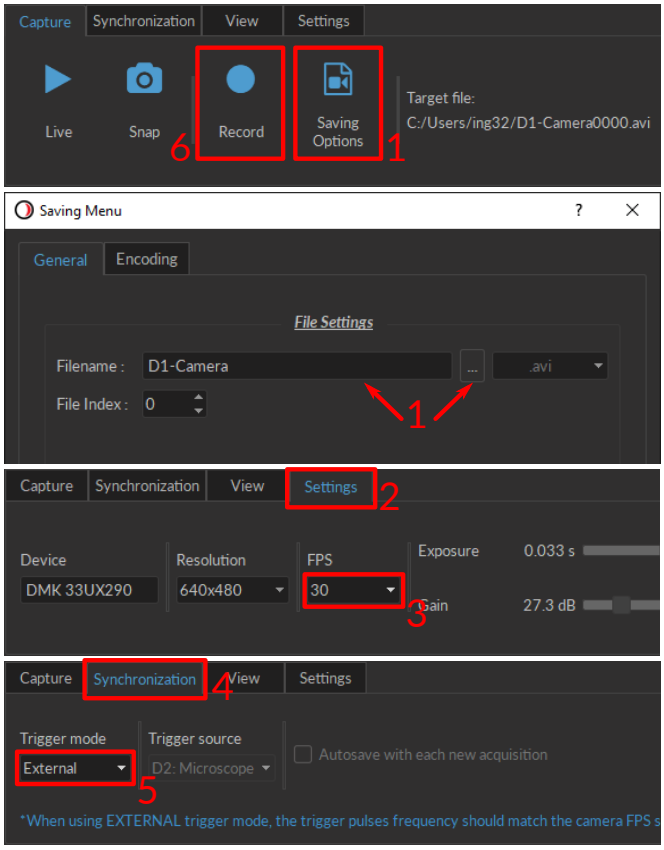

Figure 2.19: *Camera Configuration in Doric Neuroscience Studio.*

Configure the Camera Tab (Fig. 2.19)

The camera will be triggered by the OTPG and needs to be configured in External Trigger mode.

1. In the Capture Tab, click *Saving options*. An external window will open. Set the saving Filename and Path. Click *OK* to close the saving menu window.
2. Select the *Settings* Tab.
3. In the *FPS* field, select 30. This sets the camera at 30 frames/seconds. The other camera settings in this tab can be set to optimize the image settings at the user's preference.
4. Select the *Synchronization* Tab.
5. In the *Trigger mode* field, select *External*.
6. Select the *Capture* Tab and click *Record* to enable the acquisition. The camera recording will start only when a trigger is received by the OTPG. Once the OTPG finished its sequence, the Camera is still in record mode to continue recording images if another trigger is received from the OTPG. Click *Stop* to exit record mode and save the video.

Configure the Microscope Tab (Fig. 2.20)

The microscope and the LED illumination power is configured via this Tab.

1. On the Microscope Main Tab, select the *Microscope Settings* Tab.
2. In the *Illumination Power* field, type 20. This will provide a 20 % LED illumination power to the microscope.
3. Click on *Trigger Options*. An external window will open.
4. Set number of *Image per Trig* to 9,000 (10 images/second for 15 minutes).

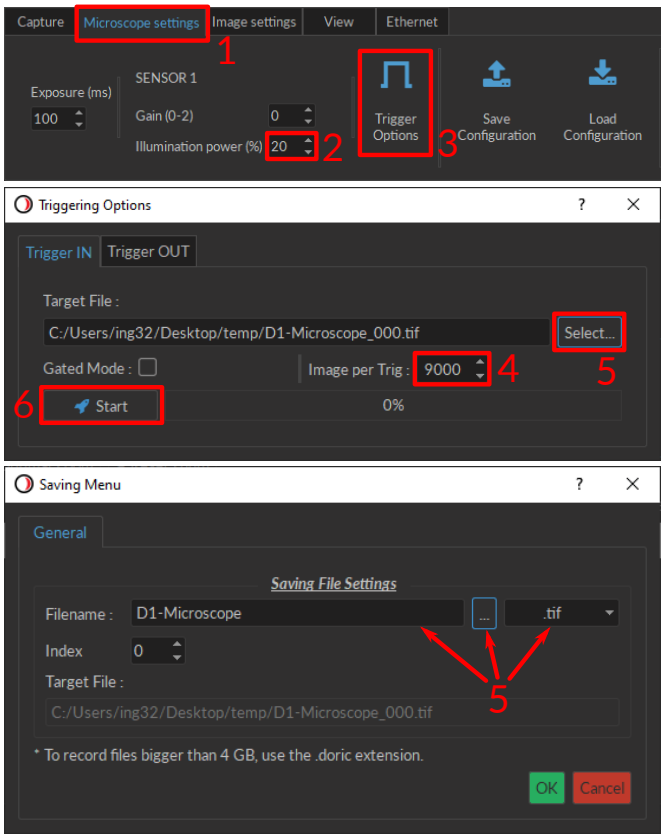

Figure 2.20: *Microscope Configuration in Doric Neuroscience Studio.*

5. Click *Select...* and set the saving Filename and Path. For files larger than 4GB, it is recommended to use the .doric extension file (HDF5 based file format) to save the microscope images. Click *OK* to close the saving menu.
6. On the Triggering Options window, click *Start*. The microscope is now ready to start acquiring images. No images will be acquired until a trigger is sent from the OTPG.

Configure the OTPG Tab (Fig. 2.21)

The OTPG needs to be configured to send trigger pulses to the Behavior Tracking Camera and the Microscope Driver.

1. If you wish to record the Input/Output signals of the OTPG, set the OTPG in *Record Mode* in the Configuration Tab[4].

Microscope Trigger Configuration

2. In the *Configuration* tab, click *Add Channel*.
3. Set the following parameters in the *OTPG Options*:
   - Channel: Channel OTPG|Ch.1 (Channel used for the Microscope Trigger IN).
   - Mode: CW.
4. Set the following parameters in *Sequences Options*:
   - Starting Delay: 00:00:00:000.
   - Time ON: 00:00:00:040.
5. Click *Add* to save the current channel and add a new channel.

[4] Record Mode is only available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher.

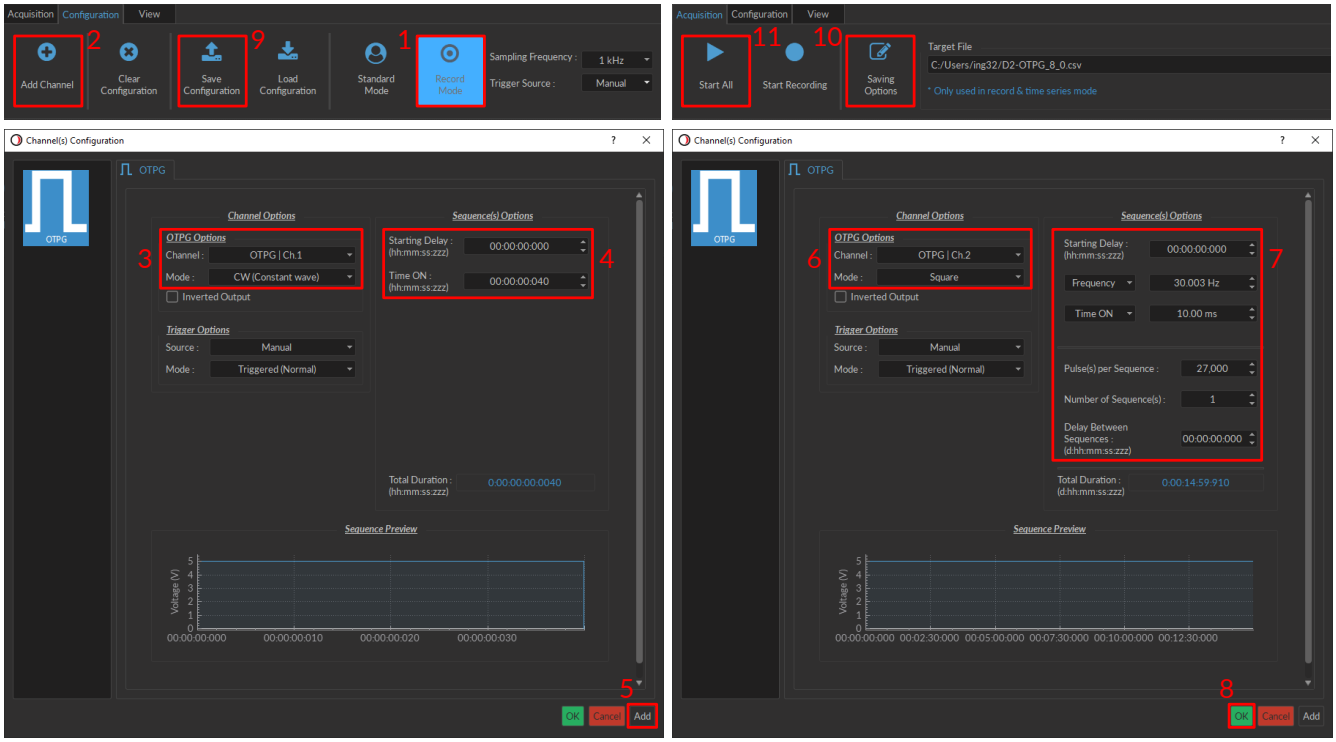

Figure 2.21: *OTPG Tab Configuration in Doric Neuroscience Studio.*

Camera Configuration

6. Set the following parameters in *OTPG Options*:
    - Channel: Channel OTPG|Ch2. (Channel used to trigger the Behavior Tracking Camera).
    - Mode: Square.
7. Set the following parameters in *Sequences Options*:
    - Starting Delay: 00:00:00:000.
    - Frequency: 30 Hz.
    - Time ON: 10 ms.
    - Pulse per Sequence: 27000.
    - Number of Sequences: 1.
    - Delay between sequences: 00:00:00:000
8. Click *OK* to save the selection.

Save and Start

9. To save the configuration for future use, click *Save Configuration* and save the file with the desired filename. The configuration can be loaded in Doric Neuroscience Studio using the *Load Configuration* button.
10. To save the OTPG signal, select the desired path and filename in the *Saving Option* menu in the *Acquisition* tab.
11. When ready to start the imaging session, click *Start Recording* to record the OPTG signal or *Start All* to start the OTPG without recording the signal. This will also sent the triggers to the microscope and the camera to start recording images. When the acquisition is finished, do not forget to stop the acquisition of the camera in the *Camera* Tab to save the behavior recording and to stop the triggering of the microscope in the *Triggering Options* external window.

2.6 Configuration for a Behavior Camera and a Microscope with Optogenetics Stimulation

The Behavior Tracking Camera can be synchronized with the Miniature Fluorescence Microscope while optogenetics stimulation is performed. This section explains the configuration necessary to perform miniature fluorescence imaging of GFP-like fluorophore combined with NpHR3 opsin activation and synchronized with a Behavior Tracking Camera acquiring at a different frame rate of the microscope. In this configuration, the OTPG is the master device and drives the other devices.

2.6.1 Connections

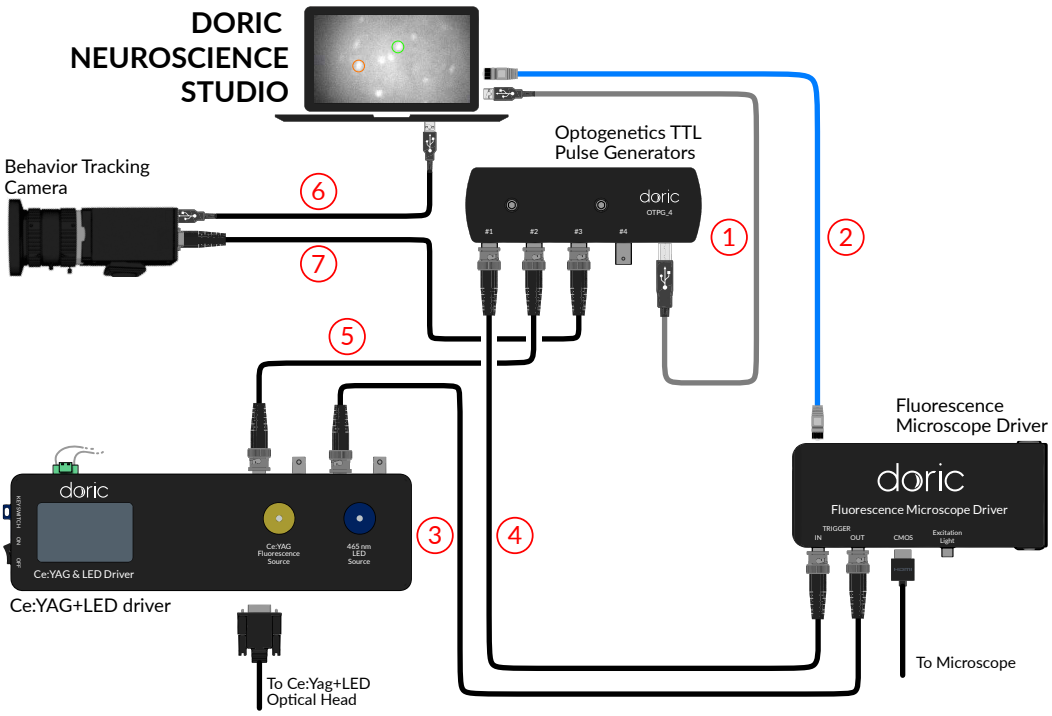

Figure 2.22: *Electronic connection necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with NpHR3 opsin activation and behavior recording of the subject.*

1 - Connect the OTPG to the computer

Connect the OTPG to the computer using the provided **USB-A - USB-B connection cable**. This allows the OTPG to be configured and controlled with the Doric Neuroscience Studio software.

2 - Connect the Microscope Driver to the computer

Connect the Microscope Driver to the computer using the provided **Ethernet cable**. This allows the Microscope Driver to be configured and controlled with the Doric Neuroscience Studio software.

3 - Connect the LED Light Source to the Microscope Driver

Connect the **Trigger OUT** BNC port of the Microscope Driver to the **LED IN** BNC port of the Ce:YAG + LED Driver with a BNC cable. This allows the blue LED of the Ce:YAG + LED Optical Head to be in function when the microscope is recording.

4 - Connect the Microscope Trigger IN to the OTPG

Connect the **Trigger IN** BNC port of the Microscope Driver to one of the **BNC Output Port** of the OTPG. The OTPG can then generates a pulse that will trigger the microscope.

5 - Connect the Ce:Yag Light Source to the OTPG

Connect the **Ce:YAG IN** BNC port of the Ce:YAG + LED Driver to a **BNC Output Port** of the OTPG. The Ce:YAG light source, used for opsin activation, can than be controlled with the OTPG to produce pulse patterns.

6 - Connect the Camera to the computer

Connect the provided USB 3.0 cable of the camera to a **USB3 port of the computer**. To install the camera driver, please refer to the Behavior Tracking Camera user manual. The Behavior Camera can then be configured by the computer and images can be acquired.

7 - Connect the Camera to the OTPG

Connect the triggering cable of the camera to the **BNC Output Port** of the OTPG. The Behavior Tracking Camera will start at the trigger of the OTPG.

2.6.2 Configuration Example

This section explains how to setup the different Tabs in Doric Neuroscience Studio for an example of an experiment involving GFP-like fluorophore imaging with NpHR3 opsin activation and a Behavior Camera monitoring. The parameters are the following (fig 2.23):

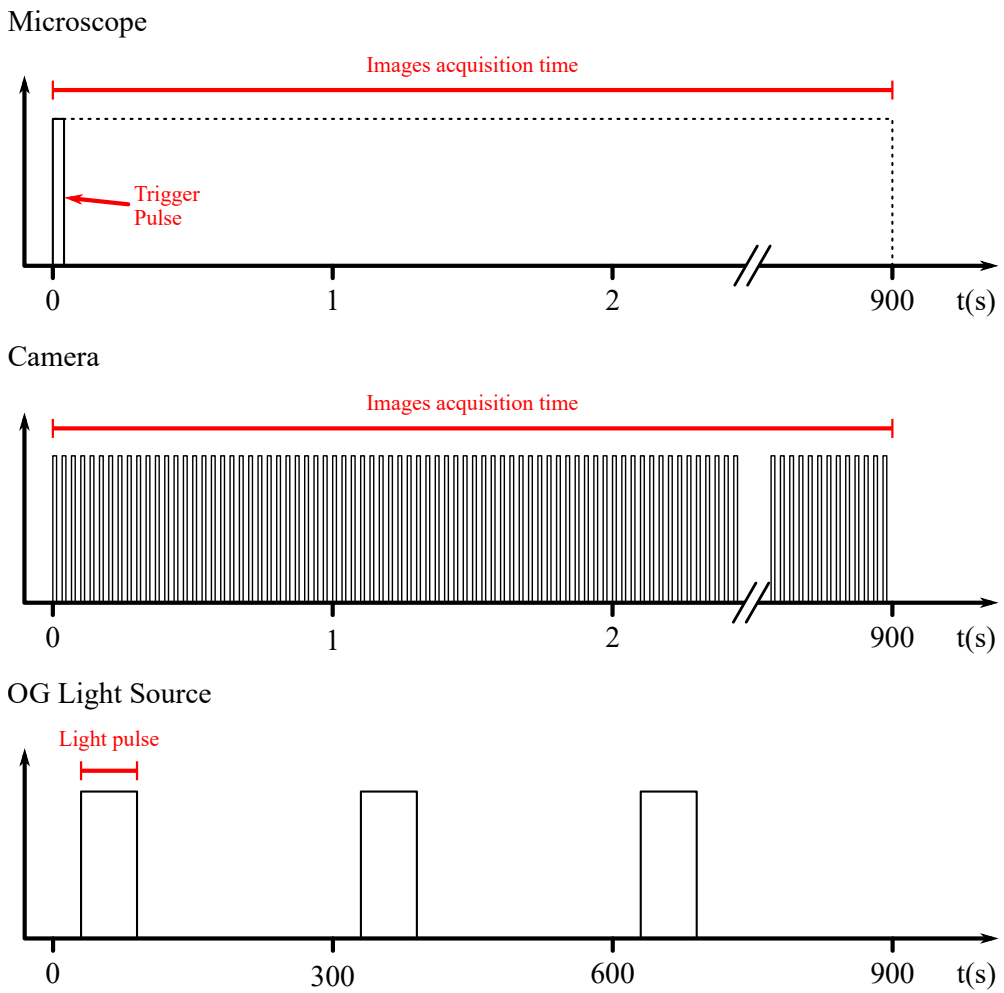

Figure 2.23: *Schematic of the Microscope, Camera, and OG Light Source input signal from the OTPG. The microscope is triggered by a single pulse from the OTPG, the Camera frame acquisition is triggered by a train of pulses whose frequency matches the Camera frame rate, and the OG Light Source is enabled by pulses of 60 seconds every 5 minutes, with an initial delay of 30 seconds.*

Microscope

- Continuous image recording for 15 minutes.
- Microscope exposure at 100 ms (10 images/second).
- Light source with an intensity of 20 %.
- Microscope Trigger IN connected to the OTPG Channel #1.

OG stimulation

- OG stimulation every 5 minutes starting 30 seconds after recording for a total of 3 OG stimulations.
- A 60 seconds continuous OG light exposition each OG stimulation.
- OG light source (i.e. Ce:YAG light source) at half the maximum intensity (>25 mW/mm^2).
- Ce:YAG Trigger IN connected to the OTPG Channel #2.

Behavior Camera

- Continuous image recording for 15 minutes.
- Frame rate of 30 FPS.
- External Trigger connected to the OTPG Channel #3.

Configure the Ce:YAG + LED Driver

For the light sources to be driven by the OTPG and the Microscope Driver, they need to be set in TTL mode.

1. Turn ON the Ce:YAG + LED Driver.
2. Push the *Ce:YAG Control Knob* a few time until the *TTL Mode* is displayed on the screen.
3. Turn the *Control Knob* to increase the current applied to the Ce:YAG light source. A maximum of 1200 mA can be applied on the light source. To have half the maximum intensity, set the current at 600 mA.
4. Push the *LED Control Knob* a few time until the *TTL Mode* is displayed on the screen.
5. Turn the *Control Knob* to increase the current applied to the LED light source to reach the desired output power. A maximum of 1000 mA can be applied on the light source. For a 20 % illumination, a current of 200 mA should be applied. It is recommended to not use the LED at maximum power as it could photobleach the fluorophores and/or saturate the imaging detector.
6. Ensure that the *Safey Key* is in the Driver and that the *Interlock* is in a closed loop.
7. The driver is ready to be triggered by the OTPG or the Microscope Driver.

Configure the Camera Tab (Fig. 2.24)

The camera will be triggered by the OTPG and needs to be configured in External Trigger mode.

1. In the Capture Tab, click *Saving options*. An external window will open. Set the saving Filename and Path. Click *OK* to close the saving menu window.
2. Select the *Settings* Tab.
3. In the *FPS* field, select 30. This sets the camera at 30 frames/seconds. The other camera settings in this tab can be set to optimize the image settings at the user's preference.
4. Select the *Synchronization* Tab.
5. In the *Trigger mode* field, select *External*.
6. Select the *Capture* Tab and click *Record* to enable the acquisition. The camera recording will start only when a trigger is received by the OTPG. Once the OTPG finished its sequence, the Camera is still in record mode to continue recording images if another trigger is received from the OTPG. Click *Stop* to exit record mode and save the video.

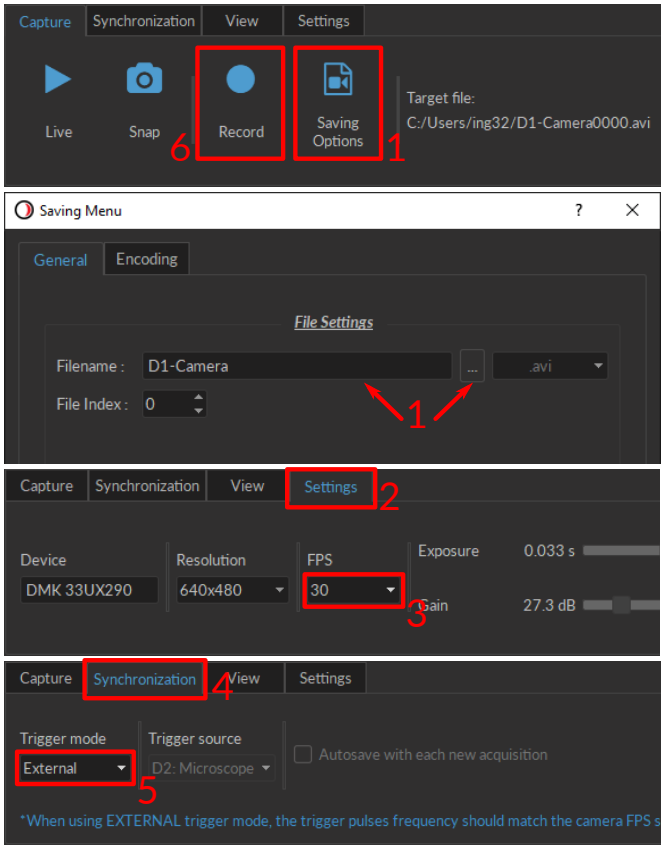

Figure 2.24: *Camera Tab Configuration in Doric Neuroscience Studio.*

Configure the Microscope Tab (Fig. 2.25)

For the microscope to be driven by the OTPG, it needs to be set in trigger mode.

1. On the Microscope Main Tab, select the *Microscope Settings* Tab.
2. Click on *Trigger Options*. An external window will open.
3. Set number of *Image per Trig* to 9,000 (10 images/second for 15 minutes).
4. Click *Select...* and set the saving Filename and Path. For files larger than 4GB, it is recommended to use the .doric extension file (HDF5 based file format) to save the microscope images. Click *OK* to close the saving menu.
5. On the *Triggering Options* window, click *Start*. The microscope is now ready to start acquiring images. No images will be acquired until a trigger is sent from the OTPG.

Configure the OTPG Tab (Fig. 2.26)

The OTPG needs to be configured to send trigger pulses to the Microscope, the OG Light Source and the Behavior Tracking Camera.

1. If you wish to record the Input/Output signals of the OTPG, set the OTPG in *Record Mode* in the *Configuration* Tab[5].

Microscope Trigger Configuration

2. In the *Configuration* Tab, click *Add Channel*.
3. Set the following parameters in the *OTPG Options*:

[5] Record Mode is only available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher.

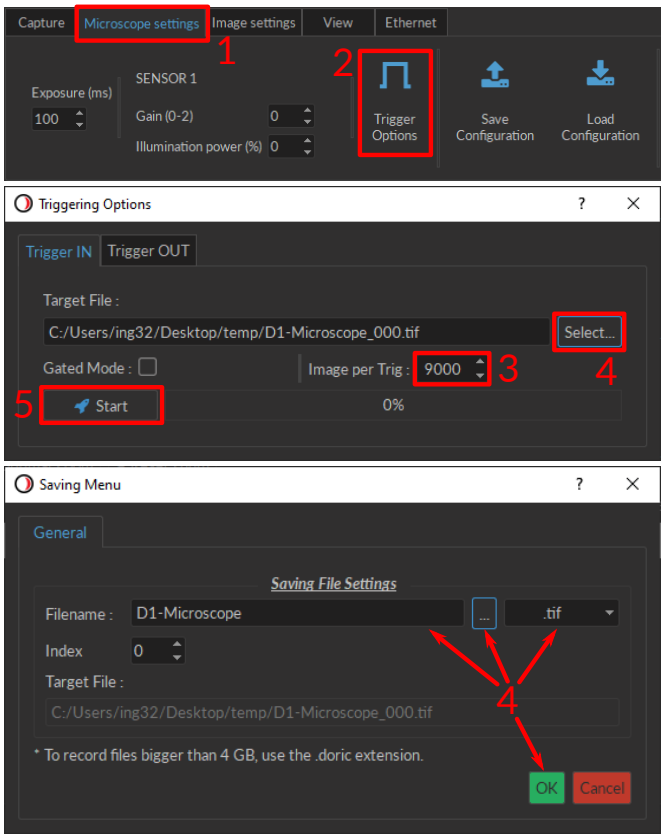

Figure 2.25: *Microscope Tab Configuration in Doric Neuroscience Studio.*

- Channel: Channel OTPG|Ch.1 (Channel used for the Microscope Trigger IN).
- Mode: CW.

4. Set the following parameters in *Sequences Options*:
   - Starting Delay: 00:00:00:000.
   - Time ON: 00:00:00:040.
5. Click *Add* to save the current channel and add a new channel.

OG Light source Configuration

6. Set the following parameters in *OTPG Options*:
   - Channel: Channel OTPG|Ch2. (Channel used to trigger the OG Ce:YAG Light source).
   - Mode: Square.
7. Set the following parameters in *Sequences Options*:
   - Starting Delay: 00:00:30:000.
   - Frequency: change to Period: 300,000.00 ms.
   - Time ON: 60,000.00 ms.
   - Pulse per Sequence: 1.
   - Number of Sequences: 3.
   - Delay between sequences: 00:00:00:000
8. Click *Add* to save the current channel and add a new channel.

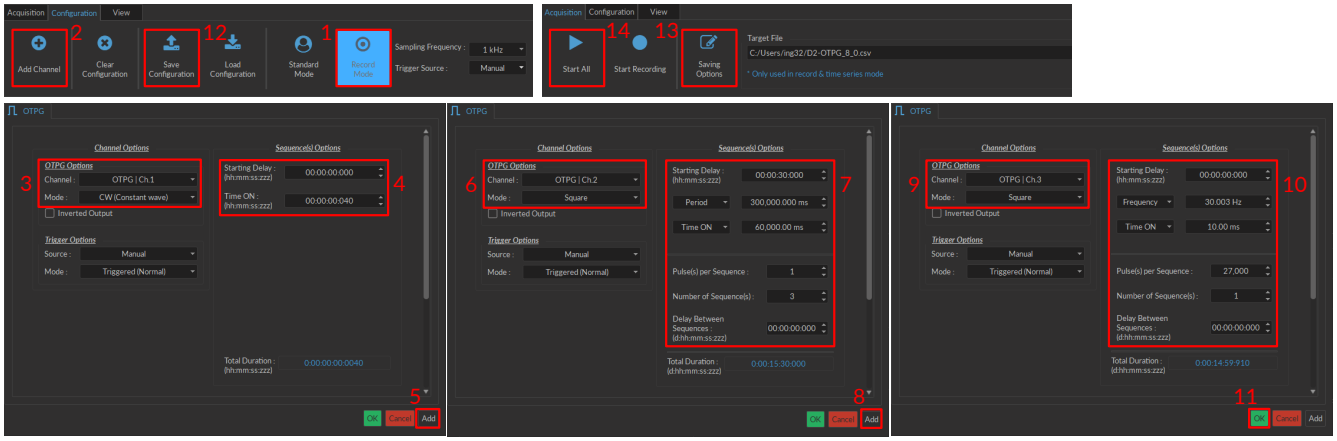

Figure 2.26: *OTPG Tab Configuration in Doric Neuroscience Studio.*

Camera Configuration

9. Set the following parameters in *OTPG Options*:
    - Channel: Channel OTPG|Ch3. (Channel used to trigger the Behavior Tracking Camera).
    - Mode: Square.
10. Set the following parameters in *Sequences Options*:
    - Starting Delay: 00:00:00:000.
    - Frequency: 30 Hz.
    - Time ON: 10 ms.
    - Pulse per Sequence: 27000.
    - Number of Sequences: 1.
    - Delay between sequences: 00:00:00:000
11. Click *OK* to save the selection.

Save and Start

12. To save the configuration for future use, click *Save Configuration* and save the file with the desired filename. The configuration can be loaded in Doric Neuroscience Studio using the *Load Configuration* button.
13. To save the OTPG signal, select the desired path and filename in the *Saving Option* menu in the *Acquisition* tab.
14. When ready to start the imaging session, click *Start Recording* to record the OPTG signal or *Start All* to start the OTPG without recording the signal. This will also sent the triggers to the microscope and the camera to start recording images and start the illumination pattern of the OG light source (as shown in Fig. 2.23). When the acquisition is finished, do not forget to stop the acquisition of the camera in the *Camera* Tab to save the behavior recording and to stop the triggering of the microscope in the *Triggering Options* external window.

3

Support

3.1 Contact us

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

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