

Behavior Camera: Synchronization and Recording

Application Note

Version 1.0.0

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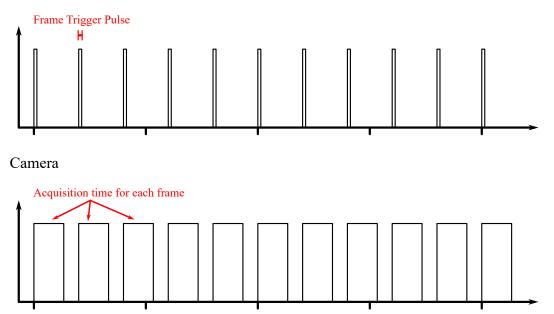
Introduction

1

To synchronize behavior imaging with cellular activity modulation and/or recording, it is highly recommended to control both modalities with the same device. When you define one of your devices as a master device, behavior and fluorescence recording are aligned on the clock of this device, which reduces the risks of desynchronization and drift over time.

In this configuration, the behavior camera is set in external trigger mode (slave) and is triggered frame by frame by the master device. This master device can be, for example, an Optogenetics TTL Pulse Generators (OTPG), a Fiber Photometry Console (FPC), or a Fluorescence Microscope Driver (FMD). A TTL pulse train, defined in the interface of the master device, is sent to the camera which starts recording a frame at each TTL pulse (Fig. 1.1).

The present application note explains how to configure the camera in external trigger mode, and how to synchronize it with other Doric hardwares (e.g. OTPG, FPC, FMD, ...).







Configuration for a Behavior Camera and an Optogenetics TTL Pulse Generator

Many experiments require the synchronization of the Optogenetic TTL Pulse Generator (OTPG) with a Behavior Camera. This chapter explains the simplest case where the OTPG is the master device that drives the camera.

2.1 Connections (Fig 2.1)

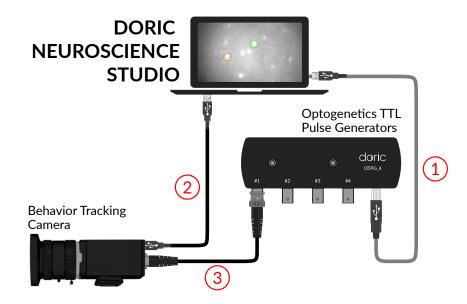


Figure 2.1: Electronic connections necessary to trigger the Behavior Camera using the OTPG.

1 - Connect the OTPG to the computer

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

2 - Connect the Camera to the computer

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

3 - Connect the Camera to the OTPG

Connect the triggering cable of the camera to one of the **BNC Port** of the OTPG to synchronize the Behavior Camera with it.

2.2 Configuration Example

This section explains how to setup the different Tabs in Doric Neuroscience Studio for an example of an experiment involving a triggered Behavior Camera monitoring. The parameters are the following (fig 2.2):

Behavior Camera

- Camera Trigger connected to the OTPG Port #1.
- Frame rate of 30 FPS.
- Continuous image recording.

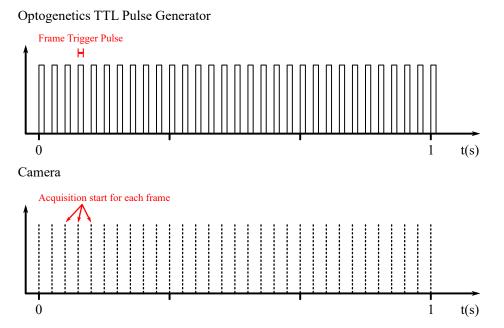


Figure 2.2: Schematic of the camera input signal. The Camera frame rate is triggered by a train of pulses from the OTPG whose frequency matches the Camera frame rate.

2.2.1 Configure the Camera Tab (Fig. 2.3)

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.
- 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.

Capture	Synchronization	View	Settings		
Live	Snap 6	Record	Saving Options	Target file: C:/Users/ing32	2/D1-Camera0000.avi
O Saving	Menu				? ×
Genera	I Encoding	iera	File Setting	<u>15</u>	.avi 🔻
File	Index : 0 🌲]		1/	
Capture	Synchronization	View	Settings	2	
Device		olution	FPS	Exposure	0.033 s
DMK 33	0X290 640)x480 🔻	30	Sain	27.3 dB
Capture	Synchronization	4 ^{View}	Settings		
Trigger me External	D2: Mic				

Figure 2.3: Camera Configuration in Doric Neuroscience Studio.

6. Select the *Capture* Tab and click *Record* to enable the acquisition. The recording of the camera will start only when pulses are received by the camera from the OTPG. Once the OTPG finished its acquisition, the Camera is still in record mode in the Camera tab to continue recording images if another trigger is received from the OTPG. When the acquisition is finished, click *Stop* to exit record mode and save the video.

2.2.2 Configure the OTPG Tab (Fig. 2.4)

The OTPG needs to be configured to send trigger pulses to the Behavior Camera.

- 1. On the OTPG Main Tab, select the Configuration Tab.
- 2. Click on Add Channel. An external window will open.
- 3. Set the following parameters in OTPG Options:
 - Channel: OTPG | Ch1. (Channel used to trigger the Behavior Camera).
 - Mode: Square.
- 4. Set the following parameters in Sequences Options:
 - Starting Delay: 00:00:00:000.
 - Frequency: 30 Hz.
 - Time ON: 5 ms.
 - Pulse per Sequence: 0. The train of pulse will run continuously as long as the OTPG is running.
- 5. Click OK to save the selection.
- 6. Set the other OTPG channels for your experiment. For more information on the OTPG configuration, please refer to the OTPG user manual.

Acquisition Configuration	on View					
Cc	Clear Onfiguration	Save Configuration	Standar Mode	rd Record T	ampling Frequency : igger Source :	1 kHz 🔹 Manual 🔹
O Channel(s) Configurat	tion					? ×
		<u>Channel Options</u>		<u>Sequence</u>	' <u>s) Options</u>	
OTPG		OTPG Options				.
Ciri C		Channel : OTPG Ch. 1		Starting Delay : (hh:mm:ss:zzz)	00:00:00:000 🗘	1
		Mode : Square		Frequency 🔻	30.003 Hz 🗘	17
				Time ON 🔻	5.00 ms 🗘	
		Trigger Options		THE ON	5.00 ms 👻	
		Source : Manual				
		Mode : Triggered (Normal)		Pulse(s) per Sequence :		
				Number of Sequence(s) :		
				Delay Between Sequences :		
				Total Duration : (d:hh:mm:ss:zzz)		
			<u>Sequence</u>	<u>Preview</u>		
		OFF 00:00:00:00	00:0	00:00:0	7:500	
					<u>ok</u>	Cancel Add
Acquisition Configuration	on View	0 0				
		7 Target File —				
		C:/Users/ing32	D2-OTPG_8_	_0.csv		
Start All Star	rt Recording	Saving Options * Only used in rec				

Figure 2.4: OTPG Configuration in Doric Neuroscience Studio.

Save and Start

- 7. 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.
- 8. To save the recording of the OTPG¹, click *Saving Options* in the *Acquisition* tab. An external window will open. Set the saving Filename and Path and click OK.
- 9. 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 camera to start recording images. When the acquisition is finished, <u>do not forget to stop the acquisition of the OTPG (in the OTPG Tab) as well as</u> the camera (in the Camera Tab) to save the OTPG signal and the behavior recording.

¹Record Mode is available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher. The sampling rate of the OTPG recording can be adjusted to with the drop-down menu on the right of the Record Mode button. We suggest a sampling rate at least 10 times the camera sampling rate.

Configuration for a Behavior Camera and a Fiber Photometry Console

This chapter explains how to synchronize the Behavior Camera with a photometry system where the Fiber Photometry Console is the master device that drives the camera.

3.1 Connections (Fig 3.1)

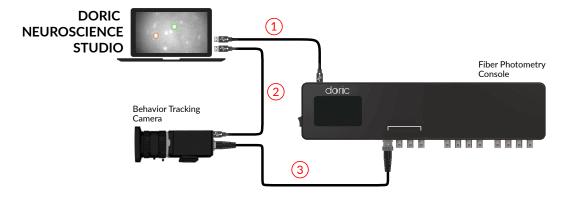


Figure 3.1: Electronic connections necessary to perform photometry using the Fiber Photometry Console combined with a behavior recording of the subject.

1 - Connect the Fiber Photometry Console to the computer

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

2 - Connect the Camera to the computer

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

3 - Connect the Camera to the Fiber Photometry Console

Connect the triggering cable of the camera to one of the **Digital I/O** of the Fiber Photometry Console to synchronize the Behavior Camera with the console.

3.2 Configuration Example

This section explains how to setup the different Tabs in Doric Neuroscience Studio for an example of an experiment involving photometry recording with a Behavior Camera monitoring. The parameters are the following (fig 3.2):

3

Behavior Camera

- Camera Trigger connected to the Fiber Photometry Console Digital I/O #1.
- Frame rate of 30 FPS.
- Continuous image recording.

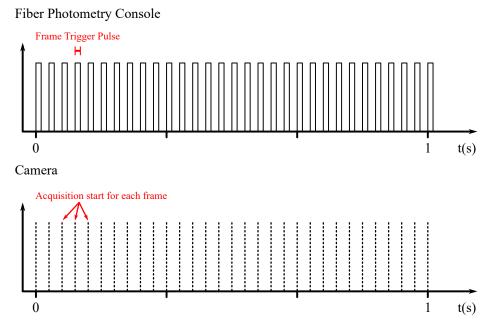


Figure 3.2: Schematic of the camera input signal. The Camera frame rate is triggered by a train of pulses from the FPC whose frequency matches the Camera frame rate.

3.2.1 Configure the Camera Tab (Fig. 3.3)

The camera will be triggered by the Fiber Photometry Console 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 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 recording of the camera will start only when pulses are received by the camera from the Fiber Photometry Console. Once the console finished its acquisition, the Camera is still in record mode to continue recording images if another trigger is received from the console. When the acquisition is finished, click *Stop* to exit record mode and save the video.

3.2.2 Configure the FPConsole Tab (Fig. 3.4)

The camera triggering and photometry experiment is configured via this Tab.

- 1. On the FPConsole Main Tab, select the Configuration Tab.
- 2. Click on Add Channel. An external window will open.

Capture	Synchronization	View	Settings			
Live	Snap 6	Record	Saving Options	Target file: C:/Users/ing32	2/D1-Camera00	100.avi
O Saving	Menu				?	×
Genera	I Encoding					
	name: D1-Cam Index: 0 ‡	era	File Setting	s 1	.avi 🔻	
Capture	Synchronization	View				
Device DMK 33		olution)x480 -	FPS 30	Exposure Bain	0.033 s	-
Capture	Synchronization	View	Settings			
Trigger m External *When us	D2: Mic					ra FPS s

Figure 3.3: Camera Configuration in Doric Neuroscience Studio.

- 3. Set the following parameters in *Digital I/O Options*:
 - Channel: Digital I/O | Ch1. (Channel used to trigger the Behavior Camera).
 - Mode: Square.
- 4. Set the following parameters in Sequences Options:
 - Starting Delay: 00:00:00:000.
 - Frequency: 30 Hz.
 - Time ON: 5 ms.
 - Pulse per Sequence: 0. The train of pulse will run continuously as long as the console is running.
- 5. Click OK to save the selection.
- 6. Set the other Fiber Photometry Console channels for your photometry experiment. For more information on the Fiber Photometry Console configuration, please refer to the user manual.

D1: FPConsole						
Controls & Settings						
Acquisition Configuration	View					
Add Channel 2 Clea						
O Channel(s) Configuration		? >				
☐ Digital I/0	2					
	Channel Options	Sequence(s) Options				
Digital I/O	Digital-I/O Options Channel : Digital I/O Ch.1 -	Starting Delay : 00:00:00:00 +				
	Mode: Square *	Frequency - 30.000 Hz				
	Triager Options	Time ON 👻 5.00 ms				
Analog Output	Source : Manual -					
	Mode : Triggered (Normal) *					
Analog Input						
		Sequences : 00:00:00:00 (d.hh.mm:ss:zzz)				
		Total Duration : co (d:hh:mm:ss.zzz)				
	<u>Seque</u>	nce Preview				
	5					
	optrage 2					
	0.00.00.000 00:00:02:500 0	0.00.05.000 00.00.07:500				
		5 Cancel Ad				
Acquisition Configuration View						
View						
	0 <mark>。 🖹 。</mark> 🖉	Target File C:/Users/ing32/Desktop/D1-FPConsole_0000.csv				
Live Record	Time Series Export Data Options					

Figure 3.4: Fiber Photometry Console Configuration in Doric Neuroscience Studio.

Save and Start

- 7. 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.
- 8. To save the recording of the experiment, click *Saving Options* in the *Acquisition* tab. An external window will open. Set the saving Filename and Path and click OK.
- 9. When ready to start the experiment, click *Live*, *Record* or *Time Series*, depending of your recording requirements. This will also sent the triggers to the camera to start recording images. When the acquisition is finished, <u>do not</u> forget to stop the acquisition of the camera in the *Camera* Tab to save the behavior recording.

Configuration for a Behavior Camera and a Fiber Photometry Console triggered by an external source

This chapter explains how to synchronize the Behavior Camera with the photometry system where the recordings start at the reception of an external trigger.

4.1 Connections (Fig 4.1)

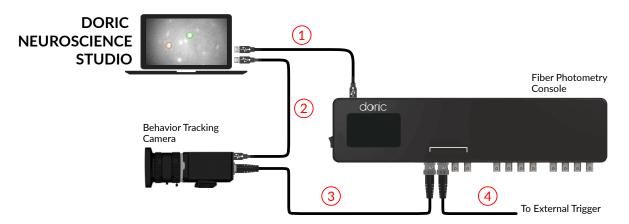


Figure 4.1: Electronic connections necessary to perform photometry using the Fiber Photometry Console combined with a behavior recording of the subject triggered by an external source.

1 - Connect the Fiber Photometry Console to the computer

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

2 - Connect the Camera to the computer

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

3 - Connect the Camera to the Fiber Photometry Console

Connect the triggering cable of the camera to one of the **Digital I/O** of the Fiber Photometry Console to synchronize the Behavior Camera with the console.

4

4 - Connect the External Trigger to the Fiber Photometry Console

Connect the triggering cable of the external trigger to one of the **Digital I/O** of the Fiber Photometry Console to enable the external source to trigger the experiment.

4.2 Configuration Example

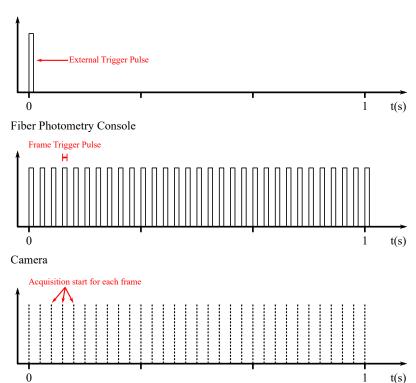
This section explains how to setup the different Tabs in Doric Neuroscience Studio for an example of an experiment involving photometry recording with a Behavior Camera monitoring triggered by an external source. The parameters are the following (fig 4.2):

Behavior Camera

- Camera Trigger connected to the Fiber Photometry Console Digital I/O #1.
- Frame rate of 30 FPS.
- Continuous image recording.

External trigger

- Trigger cable connected to the Fiber Photometry Console Digital I/O #2.
- One TTL pulse is sent at the start of the event.



External Trigger



Capture	Synchronization	View	Settings		
Live	Snap 6	Record	Saving Options	Target file: C:/Users/ing32	!/D1-Camera0000.avi
O Saving	Menu				? ×
Genera	Encoding		File Setting	76	
			<u>File Setting</u>	<u> </u>	
	name : D1-Cam Index : 0			1	.avi 🔻
Capture	Synchronization	View			
Device DMK 33		olution Ix480 -	FPS 30	Exposure	0.033 s
				San	27.3 UB
Capture	Synchronization	View	Settings		
Trigger me External		ource roscope 👻			
*When us					

Figure 4.3: Camera Configuration in Doric Neuroscience Studio.

4.2.1 Configure the Camera Tab (Fig. 4.3)

The camera will be triggered by the Fiber Photometry Console 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 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 recording of the camera will start only when pulses are received by the camera from the Fiber Photometry Console. Once the console finished its acquisition, the Camera is still in record mode to continue recording images if another trigger is received from the console. Click *Stop* to exit record mode and save the video.

4.2.2 Configure the FPConsole Tab (Fig. 4.4)

The camera triggering and photometry experiment is configured via this Tab.

- 1. On the FPConsole Main Tab, select the Configuration Tab.
- 2. Click on Add Channel. An external window will open.

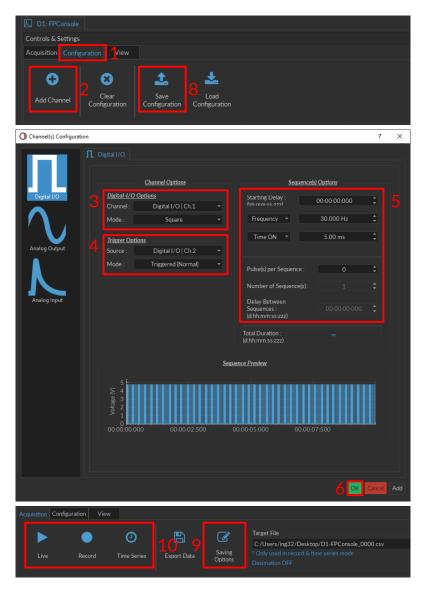


Figure 4.4: Fiber Photometry Console Configuration in Doric Neuroscience Studio.

- 3. Set the following parameters in Digital I/O Options:
 - Channel: Digital I/O | Ch. 1. (Channel used to trigger the Behavior Camera)
 - Mode: Square.
- 4. Set the following parameters in *Trigger Option*:
 - Source: Digital I/O | Ch. 2. (The external trigger channel)
 - Mode: Triggered
- 5. Set the following parameters in Sequences Options:
 - Starting Delay: 00:00:00:000.
 - Frequency: 30 Hz.
 - Time ON: 5 ms.
 - Pulse per Sequence: 0. The train of pulse will run continuously as long as the console is running.
- 6. Click OK to save the selection.

7. Set the other Fiber Photometry Console channels for your photometry experiment. It is not explained as it is outside the scope of this application note but the channels should also be set to be triggered by the external trigger. The Fiber Photometry Console will use the trigger parameters of the last channel added as global. Always use the same trigger parameters for all channels to avoid misconfigurations. For more information on the Fiber Photometry Console configuration, please refer to the user manual.

Save and Start

- 8. 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.
- 9. To save the recording of the experiment, click *Saving Options* in the *Acquisition* tab. An external window will open. Set the saving Filename and Path and click OK.
- 10. When ready to start the experiment, click *Live, Record* or *Time Series*, depending of your recording requirements. The system will wait for the external trigger pulse to start recording. This will also sent the triggers to the camera to start recording images. When the acquisition is finished, <u>do not forget to stop the acquisition of the camera</u> in the *Camera* Tab to save the behavior recording.

Configuration for a Behavior Camera and a Microscope with the Same Frame Rate

This chapter 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. For a configuration where the microscope and the camera have a different framerate, please see the chapter 6.

5.1 Connections (Fig 5.1)

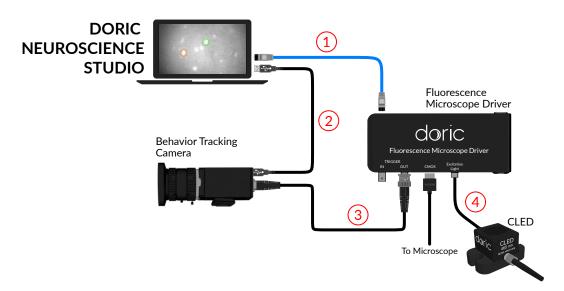


Figure 5.1: Electronic connections necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with a behavior recording of the subject.

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 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.

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 5.2):

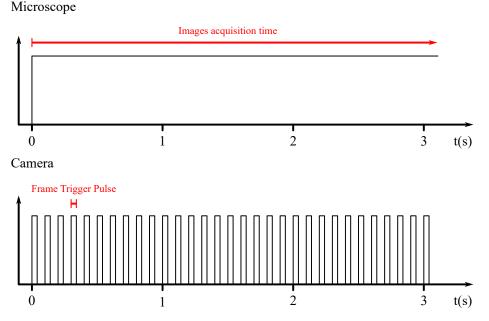


Figure 5.2: Schematic of the microscope acquisition and camera input signal. The microscope is manually triggered, and the Camera frame acquisition is triggered by the microscope output signal.

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 frame rate of the microscope).

5.2.1 Configure the Camera Tab (Fig. 5.3)

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.

Capture	Synchronization	View	Settings	
Live	Snap 6	Record	Saving Options	Target file: C:/Users/ing32/D1-Camera0000.avi
O Saving	Menu			? ×
Genera	Encoding			
			File Setting	35
	name : D1-Cam Index : 0			avi 🔹
Capture	Synchronization	View		
Device DMK 33		olution 0x480 -	FPS 10	Exposure 0.083 s
Capture		View	Settings	
Trigger m External *When us	D2: Mic			

Figure 5.3: Camera Configuration in Doric Neuroscience Studio.

- 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 recording of the camera will start only when pulses are received by the camera from the Microscope Driver Trigger Out. 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.

5.2.2 Configure the Microscope Tab (Fig. 5.4)

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.

Capture	Microscope settings	Image settings	View	Ethernet		
Exposu 100	🗘 Gain (0-2)	0 power (%) 20		П Trigger Options	Save Save	Load Configuration
O Trigge	ring Options					? ×
Trigger	IN Trigger OUT					
Mod	e :	Trigge	ered w/ ea	ch frame		-
Capture	Microscope settings	Image settings	View	Ethernet		
		o 🗈) E	গু	¢°	Target file:
Live	Record 7	Snap Albur	n Time	Series	Saving options	C:/Users/ing32/D
O Savin	g Menu					? ×
Gener						
		Savin	ig File Setti	ngs		
Filer	name : D1-Micro	scope				.tif 🔻
Inde	x 0 ‡					
Targ	et File :					
C:/						
* To re	ecord files bigger th	an 4 GB, use th	e .doric ext	tension.		OK Cancel

Figure 5.4: Microscope Configuration in Doric Neuroscience Studio.

- 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.

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

The simplest case of synchronizing the Behavior Camera with the Miniature Fluorescence Microscope is presented in chapter 5 but presents the drawback of having to match the Camera frame rate with the Microscope frame rate. This chapter explains how to synchronize the Behavior Camera with the microscope using a different frame rate for the Camera. In this configuration, the Optogenetics TTL Pulse Generators (OTPG) is the master device and drives the Behavior Camera and the Microscope Driver.

6.1 Connections (Fig. 6.1)

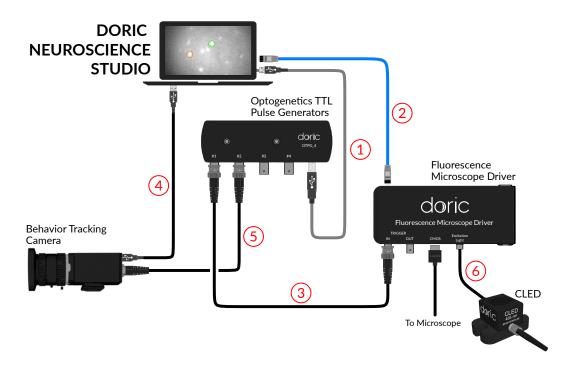


Figure 6.1: Electronic connection necessary to perform Miniature Fluorescence Microscopy of GFP-like fluorophore combined with a behavior recording of the subject.

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 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

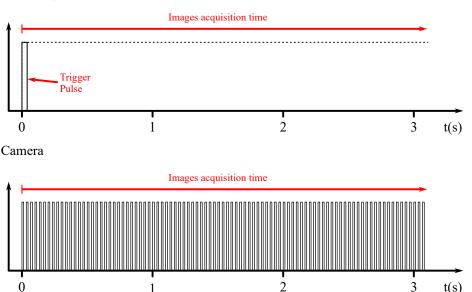
Connect the triggering cable of the camera to the **BNC Output Port** of the OTPG. The Behavior Camera will start at the trigger of the OTPG.

6 - Connect the LED to the Microscope Driver

Connect the **LED to the M8 output** of the Fluorescence Microscope Driver.

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 with a Behavior Camera monitoring. The parameters are the following (fig 6.2):



Microscope

Figure 6.2: 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.

6.2.1 Configure the Camera Tab (Fig. 6.3)

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

Capture	Synchronization	View	Settings		
Live	Snap 6	Record	Saving Options	Target file: C:/Users/ing32	2/D1-Camera0000.avi
O Saving	Menu				? ×
Genera	Encoding				
			File Setting		
Filer	name: D1-Can	nera			
File	Index: 0 🗘				
Capture	Synchronization	View	Settings	2	
Device DMK 33		olution)x480 、	FPS 30	Exposure Bain	0.033 s
Capture	Synchronization	View	Settings		
Trigger m External *When us	D2: Mic				

Figure 6.3: Camera Configuration in Doric Neuroscience Studio.

- 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 recording of the camera will start only when pulses are received by the camera from 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.

Capture Microscope settings Image settings View Ethernet	
Exposure (ms) SENSOR 1 Image: Configuration figuration 100 Image: Hold matrix 0 Image: Configuration Configuration	
Triggering Options ? X	
Trigger IN Trigger OUT	
Target File : C:/Users/ing32/Desktop/temp/D1-Microscope_000.tif	
Gated Mode : Gated	
6 🧣 Start 0%	
Saving Menu ? ×	
General	
Saving File Settings	
Filename : D1-Microscopetif •	
Index 0	
C:/Users/ing32/Desktop/temp/D1-Microscope_000.tif	

6.2.2 Configure the Microscope Tab (Fig. 6.4)

Figure 6.4: Microscope Configuration in Doric Neuroscience Studio.

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).
- 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.

6.2.3 Configure the OTPG Tab (Fig. 6.5)

Acquisition Configuration View	Acquisition Configuration View
Add Channel Clear Configuration Ead Configuration Configuration Save Configuration O Standard Mode Sampling Frequency: 1 kHz	Start All Start Recording Saving Option Only used in record & time series made
Channel(s) Configuration ?	X O Channel(s) Configuration ? X
Chemical Outcome Source (Normal) Chemical Outcome Starting Delay: Other Outcome Other Outcome Other Outcome Starting Delay: Outcome Other Outcome Inverted Outcome Time ON: Source: Manual Mode: Triggered (Normal)	Channel Options Channel Options Channel: Ch
Total Duration: 0.00.00.00.0040	Total Duration: (dth/mmrsszz2) 0.00.14.59.910
Securace Preview 5 5 5 6 7 7 9 9 9 9 9 9 9 9 9 9 9 9 9	Sequence Preview Sequence Pre
and 100	Add

Figure 6.5: OTPG Tab Configuration in Doric Neuroscience Studio.

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

Microscope Trigger Configuration

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

Camera Configuration

- 5. Set the following parameters in OTPG Options:
 - Channel: Channel OTPG|Ch2. (Channel used to trigger the Behavior Camera).
 - Mode: Square.
- 6. Set the following parameters in Sequences Options:
 - Starting Delay: 00:00:00:000.
 - Frequency: 30 Hz.
 - Time ON: 10 ms.

- Pulse per Sequence: 27000 (30 frames/seconds for 15 minutes).
- Number of Sequences: 1.
- Delay between sequences: 00:00:00:000
- 7. Click OK to save the selection.

Save and Start

- 8. 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.
- 9. To save the OTPG signal¹, select the desired path and filename in the *Saving Option* menu in the *Acquisition* tab. An external window will open. Set the saving Filename and Path and click OK.
- 10. 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 microscopes and the camera to start recording images. When the acquisition is finished, <u>do not forget to stop the acquisition of the OTPG (in the OTPG Tab)</u>, the triggering of the microscope (in the Triggering Options external window), as well as the camera (in the Camera Tab) to save the OTPG signal and the behavior recording.

¹Record Mode is available for OTPG with the firmware version 4.4 or higher and Doric Neuroscience Studio version 5.4.0.0 or higher. The sampling rate of the OTPG recording can be adjusted to with the drop-down menu on the right of the Record Mode button. We suggest a sampling rate at least 10 times the camera sampling rate.

7

Support

7.1 Contact us

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

Phone 1-418-877-5600

Email sales@doriclenses.com



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357 rue Franquet - Quebec, (Quebec) G1P 4N7, Canada Phone: 1-418-877-5600 - Fax: 1-418-877-1008 www.doriclenses.com