



Quick Start Guide for USB-TTL Trigger Kit in cellSens Dimension

IQS016- Rev 01

Issue Date: 27/11/2023

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1 Introduction

New CoolLED Illumination Systems are not always immediately supported in cellSens software. However, on/off control is possible in cellSens Dimension when using the CoolLED USB to TTL Trigger Kit. LED intensity is controlled either through the manual control pod or the LightBridge virtual control pod GUI.

Note: This example uses the four-channel pE-400^{max} with the pE-6501, but the information can be applied to other CoolLED Illumination Systems – for example, the pE-800 with the pE-6501-8 and pE- 800^{fura} with the pE-6501-8-FR.

2 Hardware Installation

See Figure 1 for hardware setup.

- 1. Ensure CoolLED Light Source is switched on and the Control Pod is plugged in.
- 2. Connect pE-6501 to PC via USB cable (supplied with CoolLED USB to TTL Trigger Kit).
- 3. Connect CoolLED Illumination System to pE-6501 using multi-BNC cable (supplied with CoolLED USB to TTL Trigger Kit). The cables of the pE-6501 are labelled A-E. For the pE-400^{max}, connect these in wavelength order:

Cable	LED	Position on pE-400 ^{max}
А	UV – 365 or 400 nm	Тор
В	Blue – 450 nm	
С	Teal-Green-Yellow (TGY) – 550 nm	Ļ
D	Red – 635 nm	Bottom



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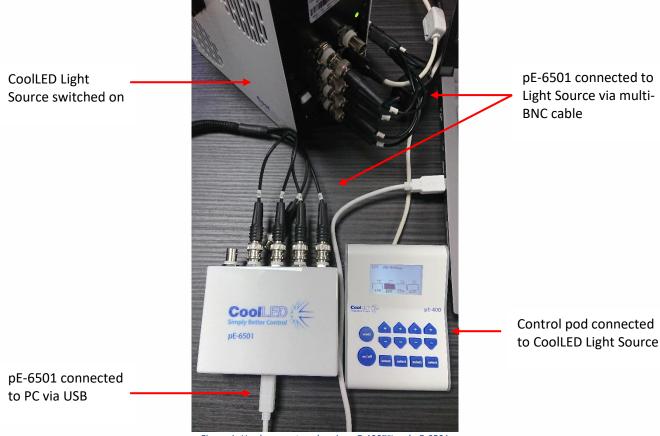


Figure 1: Hardware setup showing pE-400^{max} and pE-6501

3 Installing the Windows NI6501 driver

The National Instruments NI6501 driver is required to control the pE-6501.

- Download the National Instruments driver: <u>NI-DAQ[™]mx Download NI</u>. If installing on a PC without administrator rights or access to the internet, we recommend first saving the file to a USB memory stick, and using this to transfer the file.
- 2. Follow the instructions in the installation wizard. To test the pE-6501 is recognised in Windows, open Device Manager. The National Instruments icon should appear under 'Data Acquisition Devices' to confirm it is connected successfully (Figure 2).









Figure 2: Device Manager showing successfully installed pE-6501 under 'NI Data Acquisition Devices'

4 Install the cellSens National Instruments Driver

- 1. The pE-6501 must be added to the cellSens installation. There are two scenarios:
 - If installing cellSens for the first time, follow the installation wizard until the 'Microscope Accessories Selection' window appears.
 - If cellSens is already installed, this must be modified by running the 'Setup.exe' file found in the cellSens folder that was originally downloaded (not the cellSens Program Folder). Follow instructions until the 'Microscope Accessories Selection' window appears.
- In the 'Microscope Accessories Selection' window (Figure 3), scroll down the 'Manufacturer' list and select 'National Instruments', checking the box alongside the 'NI USB-6501'. Click 'Next>' until complete.

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Figure 3: NI devices listed in 'Microscope Accessories Selection'





5 Configure cellSens Dimension

- 1. Launch cellSens.
- 2. On the 'Start Page', select 'Device List' and select the 'Lasers/LEDs' tab.
- 3. Add the LEDs to be controlled using the pE-6501. From left to right in Figure 4:
 - a. Under 'Device', use the dropdown to select 'Single LED'.
 - b. Under 'Type', select 'NI USB-6501 Laser/LED #1'.
 - c. Under 'Description', add the LED name.
 - d. Click the '+' button to add another row and repeat this for each LED channel of the Illumination System.
 - e. Click 'OK' when finished.

Note: The LEDs occur in wavelength order on the device list. E.g., the 'NI USB-6501 Laser/LED #1' is the UV LED, 'NI USB-6501 Laser/LED #2' is the blue LED etc.

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Figure 4: How to populate the Device List for the pE-400^{max}. Add another row by clicking '+' (red box)

4. On the 'Start Page', select 'Device Settings' to test the system.

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Figure 5: 'Device Settings' page is useful for testing LED illumination by clicking 'Test'







- 5. Ensure 'Polarity' is set to 'high=on' and 'Latency' is set to '10 ms', which should be the default settings (Figure 5). Latency can later be reduced to increase imaging speed.
- 6. Test the LED triggering, by clicking the 'Test' button.
- 7. This opens the Test window, where the LEDs can be individually selected, and illumination observed (Figure 6).

Note: Ensure intensity is set above zero on the manual control pod or LightBridge.

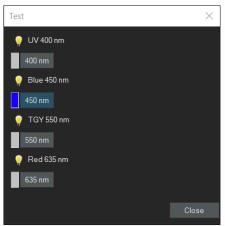


Figure 6: Test window. Selecting each LED should result in illumination - if intensity is >0% on the Control Pod or LightBridge

8. Once testing confirms the LEDs are triggering successfully, the next step is to configure Observation Methods.

5.1 Observation Methods

In this example, we consider three imaging scenarios:

- a) Manual sample observation without camera acquisition
- b) Manual sample observation in live mode while the camera is acquiring an image
- c) Automated triggering, e.g., for a time-lapse experiment

a) Manual sample observation without camera acquisition

A set of Observation Methods must be created to switch each LED on, and an Observation Method created to switch all LEDs off. This gives us a total of five observation channels for the four-channel pE-400^{max}.

- 1. Navigate to 'Device Customization' to configure Observation Methods.
- 2. Create a new observation method and enter a name, for example 'UV 400 nm ON' (Figure 7).







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🔱 General	Closed						
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Blue 450 nm (Shutter)	anne.	00 400 1111 01		_			
S TGY 550 nm (Shutter)				OK	Cancel		
S Red 635 nm (Shutter)							
						ОК	

Figure 7: Create a new Observation Method (red box) and enter a name

3. Assign a fluorophore (fluorochrome) for each Observation Method by clicking 'Fluorochromes'. Select 'Status' as 'Use' and select the relevant fluorophore from the list (Figure 8).

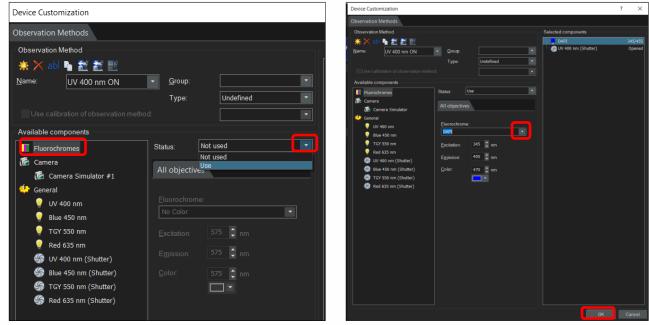


Figure 8: Assign Fluorophore. Left: Select 'Fluorochromes', change 'Status' to 'Use'. Right: select fluorophore from list and click 'OK'.





4. The shutter is used to control LED illumination. Therefore, to configure the Observation Method for the UV 400 nm LED, select the 'UV 400 nm (Shutter), change 'Status' as 'Adjust' and change the shutter to 'Opened'. The UV 400 nm LED will now illuminate when this Observation Method is selected.

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Figure 9: Configure Observation Method by selecting shutter, changing 'Status' to 'Adjust' and setting this as 'Opened'

- 5. Repeat this step for the remaining channels, assigning the corresponding fluorophore to be used in the experiment.
- 6. To switch an LED off, create an observation called 'LEDs OFF'. Do not assign a fluorochrome.
- 7. Select each shutter, change status to 'use' and 'Closed'. You will see all shutters under 'Selected components'.

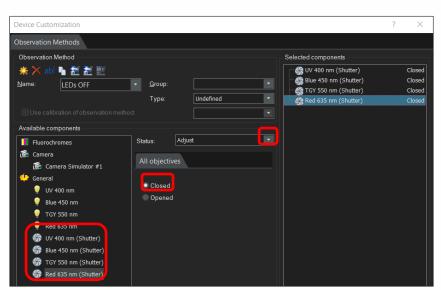


Figure 10: Creating an Observation Method to switch LEDs off, select all shutters, change 'Status' to 'Adjust' and 'Closed'







- 8. When finished creating Observation Methods, click 'OK'.
- 9. The created Observation Methods can now be accessed from the toolbar (Figure 11), or the Microscope Control window.

	Acquisition	Processing	Count and Measure	Reporting
😻 UV 400 nm ON 👋 Blue 450 nm ON	😻 TGY 550 nm ON	N 💐 RED 635	5 nm ON 😻 LEDs OFF	ا 🕵 ا
Figure 11: Obser	rvation Methods can b	e accessed from t	he toolbar	

b) Manual sample observation in live mode while the camera is acquiring an image

For manual control in live mode while the camera is acquiring an image, create an additional set of four Observation Methods where the selected LED illuminates only during camera acquisition (another 'LED off' method is not required).

- 1. Create a new Observation Method as shown in Figure 7. We will name this 'UV 400 nm Acquisition'.
- 2. Select the corresponding shutter, which is the 'UV 400 nm (Shutter)' in this example, and change 'Status' to 'Use for acquisition'. This means the UV 400 nm LED will illuminate only during camera acquisition and when this Observation Method is selected.

Note: selecting 'Use for acquisition' limits sample illumination, which is beneficial in minimising photobleaching and phototoxicity.

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GY 550 nm (Shutter)						
S Red 635 nm (Shutter)			111 I			

Figure 12: To create an Observation Method for camera acquisition, change the shutter 'Status' to 'Use for acquisition'







These extra Observation Methods now appear on the toolbar and in the Microscope Control window.

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								TGY Red	
								Device Info	▲
								UV 400 nm - 400 nm	-
								Blue 450 nm - 450 nm	-
								TGY 550 nm - 550 nm Red 635 nm - 635 nm	
								UV 400 nm (Shutter)	Closed
								Blue 450 nm (Shutter)	Closed
								TGY 550 nm (Shutter)	Closed
								Red 635 nm (Shutter)	Closed

Figure 13: All Observation Methods now appear in toolbar and Microscope Control window

a) Automated triggering, e.g., for a time-lapse experiment

It is necessary to use the Experiment Manager when running experiments which require automated illumination.

1. Navigate to the 'Experiment Manager' tab of the 'Microscope Control' window, and click 'New'.

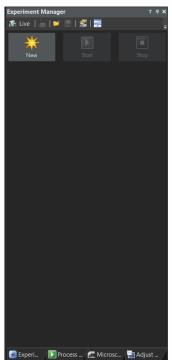


Figure 14: Create a new Experiment in the 'Experiment Manager' window







2. This opens the 'Experiment Manager' page. Click the 'Image Acquisition' dropdown to add Observation Methods from our 'Acquisition' set, where the selected LED only illuminates during camera acquisition.

4	🖻 Start Page 🗙 🧟 Experiment* 🗙
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¢	UV 400 nm ON
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4	TGY 550 nm ON
4	RED 635 nm ON
4	LEDs OFF
\$	UV 400 nm Acquisition
\$	Blue 450 nm Acquisition
\$	TGY 550 nm Acquisition
\$	Red 635 nm Acquisition

Figure 15: Adding Observation Methods to the Experiment Manager

3. The exact experimental protocol depends on individual setups, but multi-channel and timelapse LED illumination options are possible (see Figure 16).

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100 us

Figure 16: An example of a multi-channel time-lapse protocol in 'Experiment Manager'

Note: If faster imaging speeds are required, latency can be reduced in Device Settings (Figure 5).

6 ODC Configuration File – pE-400^{max} with pE-6501

To bypass configuring the settings we have explained, we have created an ODC file.

- 1. <u>Download the pE-400max-pE-6501 ODC file</u> and save to a memorable location
- 2. In cellSens, from the Start Page, select 'Device Settings' (Figure 5).
- 3. In the 'Device Settings' window, under 'Configuration', select 'open device configuration' and load our ODC file.

For further software information, please refer to the cellSens manual. For questions regarding CoolLED Illumination Systems, please contact <u>info@coolled.com</u>.

