Quick Start Guide for NIS Elements

IQS007- Rev 06

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Contents

1 Integrated CoolLED Products

- pE-800 Series (patch required if using NIS-Elements v5.30.01 – see Summary Sheet)
- pE-4000
- pE-300^{white}, pE-300^{ultra} & pE-340^{fura}
- pE-2 (discontinued)

2 Setting up your CoolLED Illumination System COM Port via USB

If using the pE-800 Series and Windows 10, please move directly to Section 3, since the pE-Driver is not required (Windows automatically detects the hardware and installs the driver).

For Windows versions pre-dating Windows 10, or if using any other compatible Illumination System, the CoolLED pE-Driver is required and the PC configured as follows:

1. Download the CoolLED pE-Driver from the CoolLED website:

   http://www.coolled.com/product-detail/imaging-software/

   N.B. This is a general driver for Windows machines and all USB controlled CoolLED Illumination Systems (pE-2, pE-300 Series, pE-340^{fura} & pE-4000).

2. Save the files (.inf and certification files) to a preferential location (e.g. Desktop).

3. When the CoolLED Illumination System is plugged in, it will appear with a driver required warning (yellow exclamation mark). Right click and 'update' the driver, selecting the previously downloaded directory containing the .inf file.
4. Once the CoolLED Illumination System has been successfully installed into Windows, check the Virtual COM ports assigned by going into Device Manager. The CoolLED Illumination System should be listed under 'Ports' (COM & LPT):

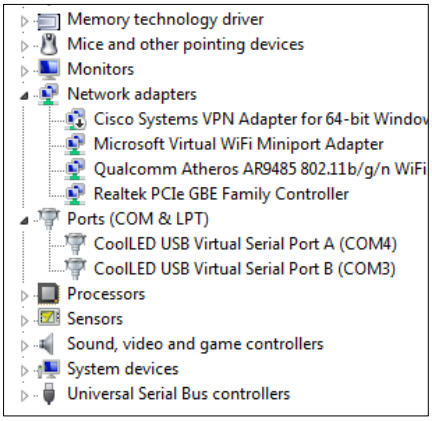


Figure 1: Device Manager with a pE-4000 installed

Either COM port can be used for control. The creation of two COM ports is to allow for multiple uses (for example: one could be used for controlling the CoolLED Illumination System whilst the other is used for command testing). Additionally, it allows for ease of use if there is a COM port conflict.

3 Installing your CoolLED Illumination System in NIS Elements

This example uses NIS-Elements AR Version 5.42.03 with the CoolLED pE-800, but the guidance can be applied to all compatible CoolLED Illumination Systems.

3.1 Add Illumination System during installation

A) If adding a new Illumination System to an existing NIS-Elements installation: The software must first be modified to select the Illumination System within the installer. In Windows settings, navigate to 'Apps & features', and select 'Modify' instead of uninstalling NIS (Figure 2). Follow the instructions and select the correct CoolLED Illumination System from 'Devices' (Figure 3).

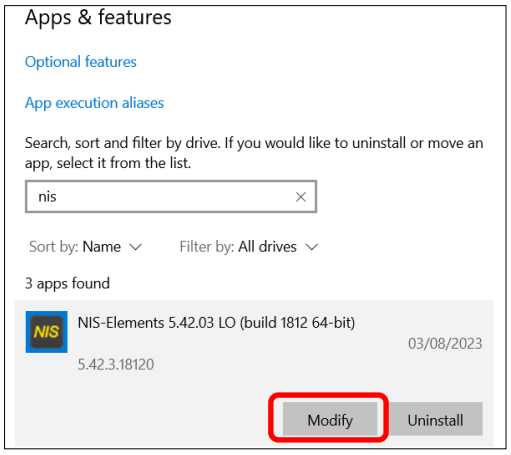


Figure 2: If modifying existing NIS-Elements installation, in Window 'Apps & features', select 'Modify' (red box) to add Illumination System

B) If installing a NIS-Elements for the first time: During NIS-Elements installation, select the relevant CoolLED Illumination System from the 'Devices' screen (Figure 3).

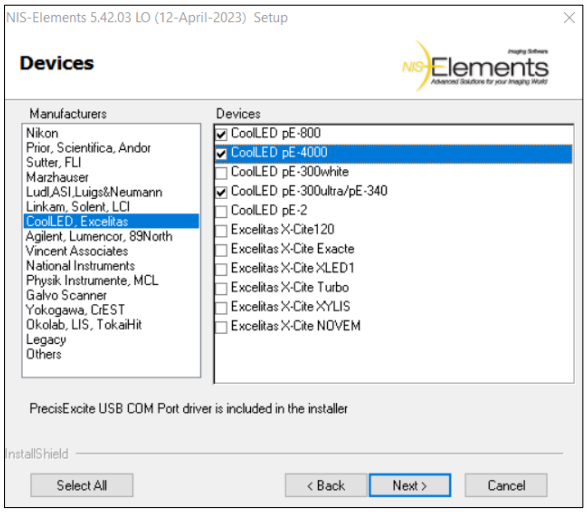


Figure 3: When 'Device' window appears during installation, select relevant CoolLED Illumination System(s)

3.2 Add Illumination System in NIS-Elements

1. Open NIS Elements and navigate to ‘Device Manager’ on the main toolbar.

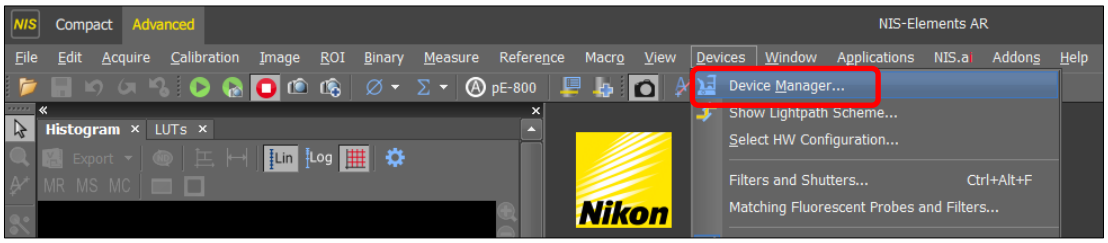


Figure 4: Navigating to 'Device Manager' (red box) from the main toolbar

2. In the ‘Device Manager’ window, click ‘+’ next to ‘Devices’ and select the relevant Illumination System from the list (in this example, the ‘CoolLED pE-800’).

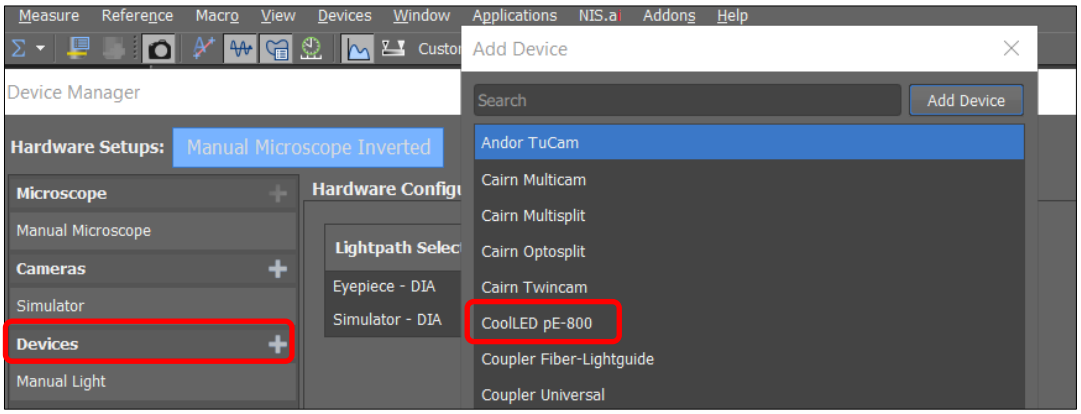


Figure 5: Select '+' by 'Devices' and select Illumination System from list, e.g., CoolLED pE-800

3. The correct COM port should show automatically (you can ensure that the COM port is correct by checking Windows Device Manager, Figure 1). If this is incorrect, it can be altered via the drop-down menu (Figure 6).

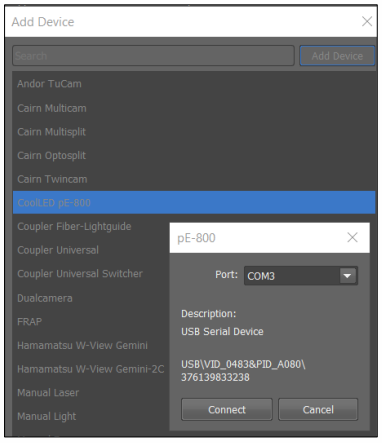


Figure 6: If COM port is incorrect, update this by selection via the drop-down menu

4. Click ‘Connect’ to return to ‘Device Manager’, where the CoolLED Illumination System now appears in the graphical representation of your setup (Figure 7). In this example, the camera simulator is selected with epifluorescence illumination (‘Simulator-EPI’).

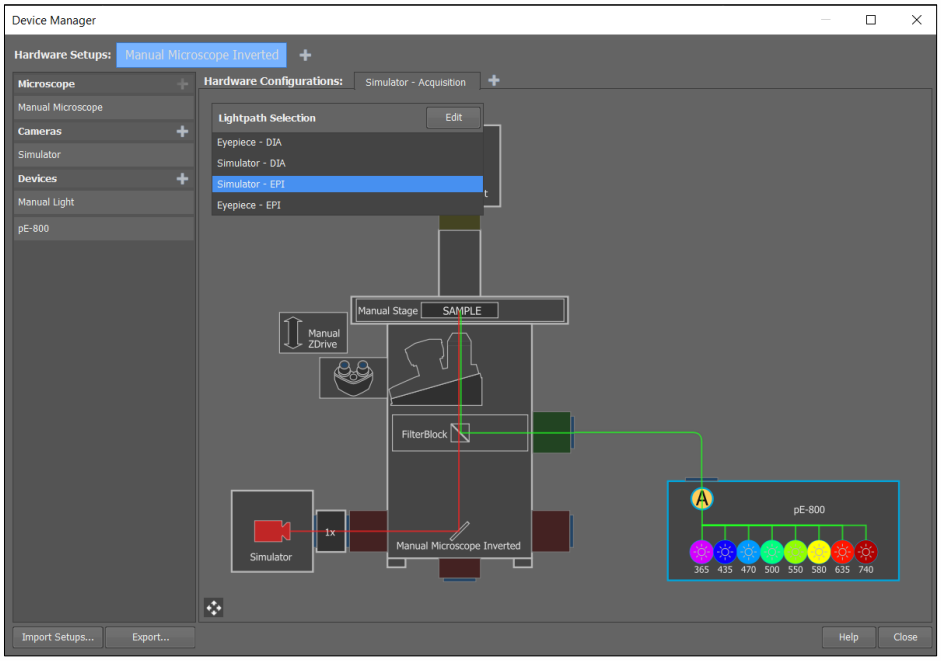


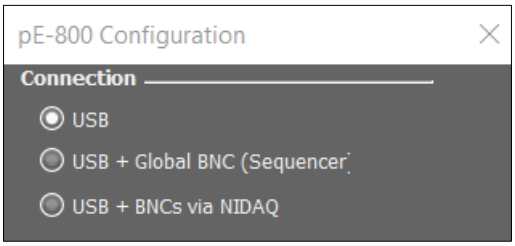
Figure 7: Device Manager shows pE-800 connected to the setup. Multiple hardware setups can be added (red box).

5. To configure the method of control, right click on the pE-800 and select ‘Configure’.

Figure 8: Configure pE-800 settings

6. In the ‘pE-800 Configuration’ pad, select the connection (Figure 9). The Configuration Pad appears differently depending on the CoolLED Illumination System in use (see the pE-800 compared to the pE-4000, Figure 9).

A



B

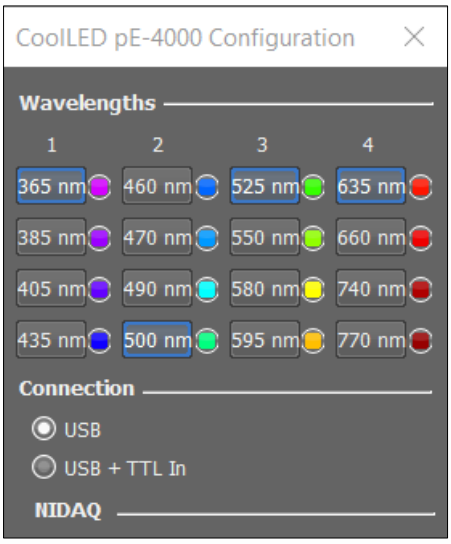


Figure 9: CoolLED pE-800 Configuration Pad (A) and pE-4000 Pad (B)

Note: The connections are as follows:

- **USB:** Standard interface.
- **USB + Global BNC (Sequencer):** This feature is also known as the CoolLED Sequence Runner. In addition to the standard USB interface, the light source accepts a TTL signal from a camera. Basic control is achieved via USB, e.g., channel selection and intensity, while the camera exposure triggers illumination, for high-speed and low-phototoxicity automation.
- **USB + BNCs via NIDAQ:** In addition to standard USB interface, TTL NIDAQ lines are used for channel selection. Basic control is achieved via USB, e.g., setting channel intensity. One NIDAQ TLL line (PFI) is connected to camera exposure signal. A NIDAQ card is also required for this configuration; please contact your local Nikon representative for more information.
- **USB + TTL In:** This is identical to above, but appears on the pE-4000 Configuration Pad.

7. Once the required connections are selected, click ‘OK’ to return to the ‘Manage Devices’ Window. The CoolLED Illumination System is now installed.

3.3 Configure Optical Filters

It is important to match optical filters to the Illumination System and assign these in NIS-Elements.

1. Navigate to ‘Filters and Shutters’ from the main menu.

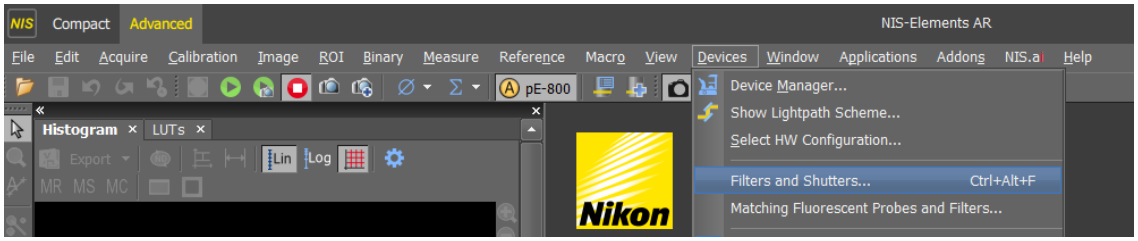


Figure 10: Adding optical filters via the main menu

2. In the ‘Filters, Shutters and Switchers Full’ window, expand the ‘FilterBlock’ and select the ‘…’ box to assign each filter.

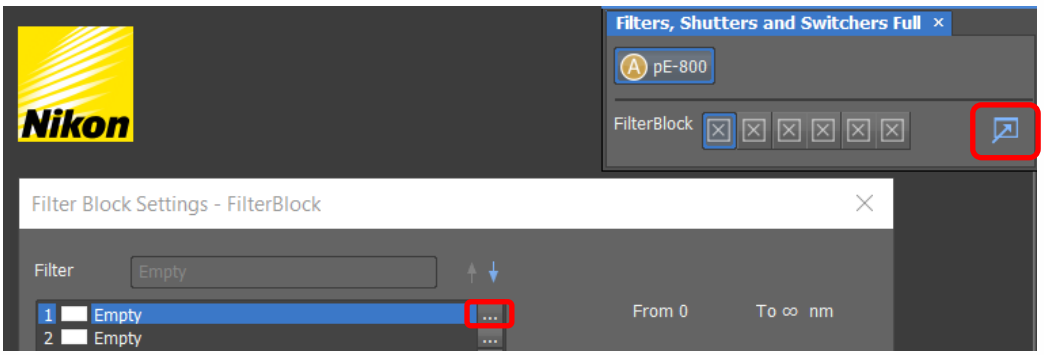


Figure 11: Assign filters by expanding selection (red), opening up ‘Filter Block Settings’ window

3. Select your filter from the list and click ‘Set’ for each position. Click ‘Close’ once complete. The filters are now assigned.

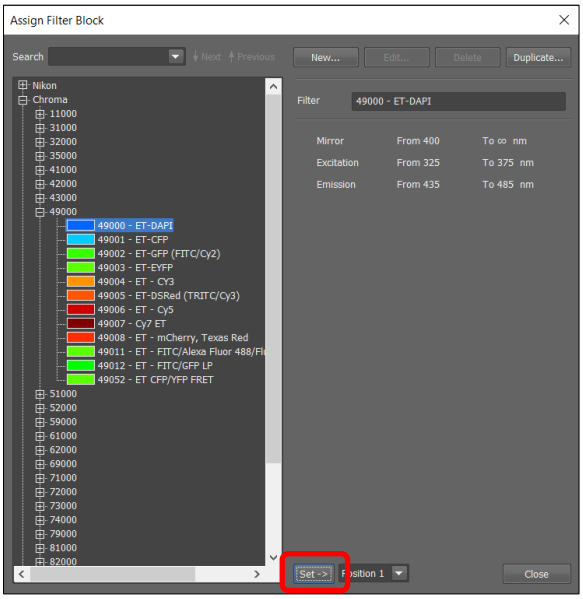


Figure 12: To assign a filter, select from the list and click 'Set ->' (red box) and ‘Close’

4 Examples of Software Functionality

4.1 Manual Control

Manual control of LED selection and intensity is achieved by using the ‘pE Pad’.

1. To add the ‘pE Pad’ to the toolbar for quick access, add this from the ‘Customize’ button on the toolbar.

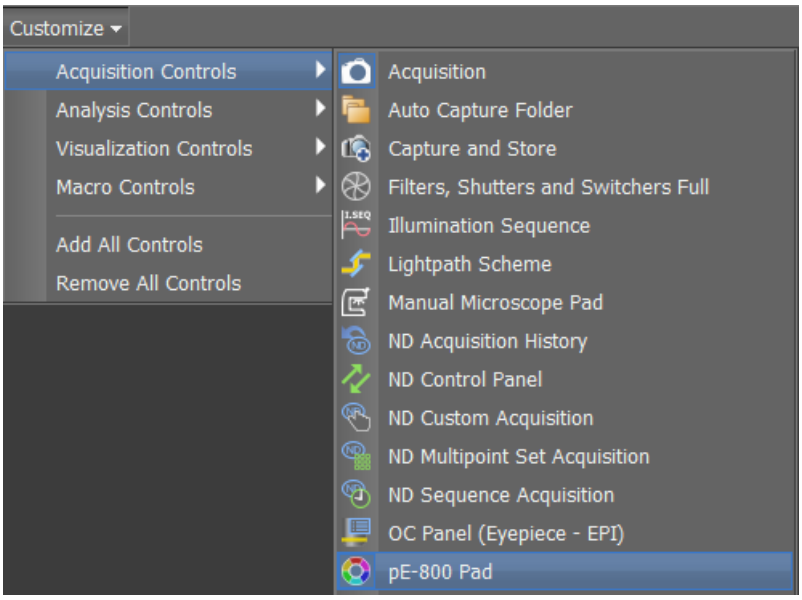
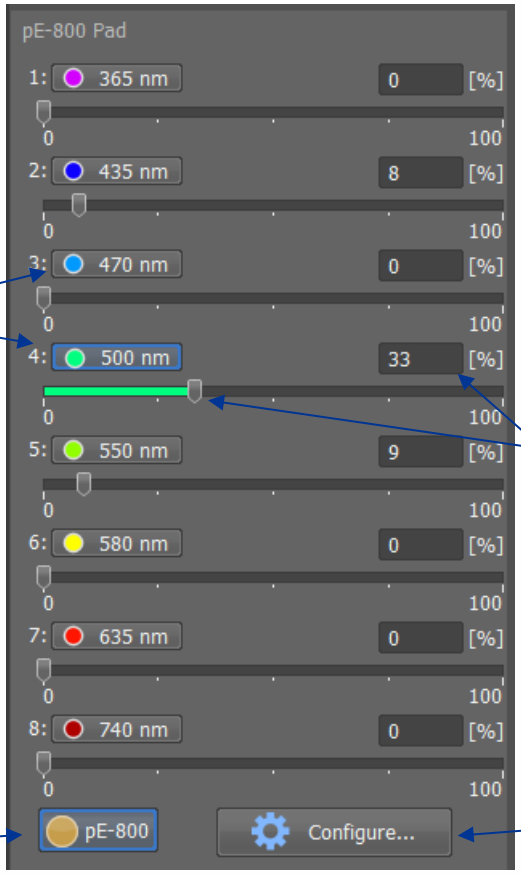


Figure 13: Adding the 'pE Pad' to the toolbar for quick access to LED controls

2. This will bring up the ‘pE Pad’ window (if not already visible), which can be used for manual LED control (Figure 14).



Select each channel by clicking these buttons. Blue outline indicates selection (i.e., 500 nm is selected).

Alter intensity from 0-100% by dragging the arrow or typing value in box.

Global shutter, which has blue outline when open.

Brings up 'Configuration' window.

Figure 14: 'pE Pad'

Note: If using the pE-4000, which has multiple LEDs per channel, use the pE-4000 Pad for channel selection and irradiance in the same way. To change the LED selection for an individual channel, select 'Configure' (Figure 15) and use the 'CoolLED pE-4000 Configuration' window.

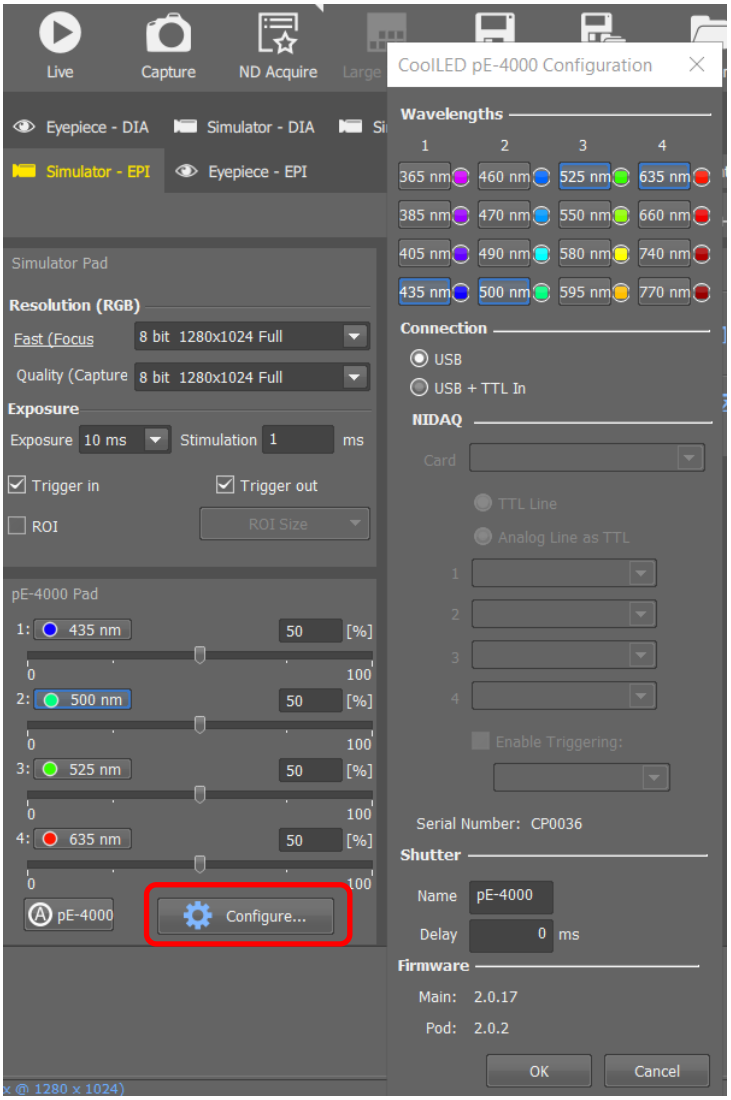


Figure 15: To change the LED within a channel of the pE-4000, select 'Configure' (red box) to access the Configuration window where all 16 LEDs are visible. Selected LEDs are outlined in blue, and these can be changed

3. For sample observation, click ‘Live’. The selected LEDs will illuminate, and the LED settings can be adjusted in real time.

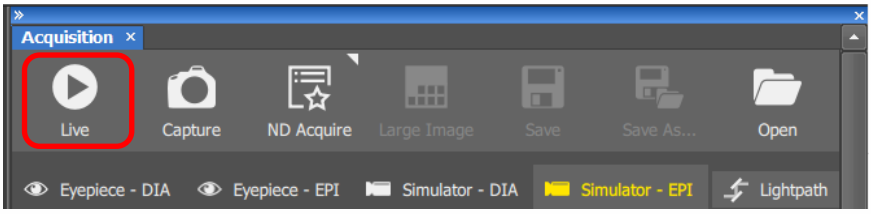


Figure 16: Access 'Live' mode (red box) at the top of the ‘Acquisition’ window

4.2 Optical Configurations

Optical Configurations can be configured to allow quick access to frequently used LED settings. For example, the following steps show how to create an Optical Configuration for two popular fluorophores – DAPI and GFP.

1. Select the required LED and intensity using the pE Pad (Figure 14). We are creating an optical configuration for DAPI in this example, and have therefore selected the 365 nm LED at 29% intensity.
2. Create a new Optical Configuration, either from the 'Calibration' menu in the toolbar or from the 'Acquisition' window.

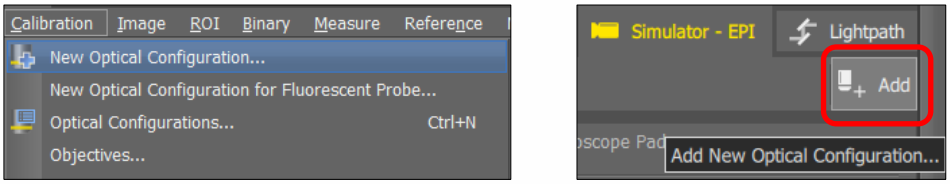


Figure 17: Add new Optical Configuration from toolbar or acquisition window (red box)

3. Name the Optical Configuration. The emission and colour tag can also be customised. Ensure Active Shutter is selected as your CoolLED Illumination System (in this example, 'pE-800, Shutter(pE-800)'. The LED settings from Step 1 are shown in the bottom right window.

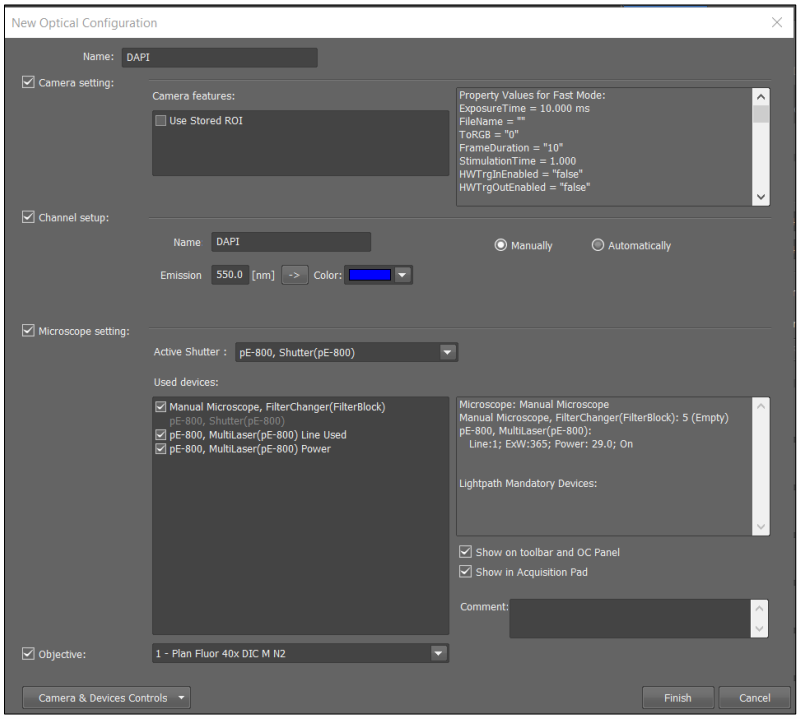


Figure 18: Configure the Optical Configuration

4. After clicking ‘Finish’, a shortcut to the DAPI Optical Configuration now appears in the toolbar and in the ‘Acquisition’ window.

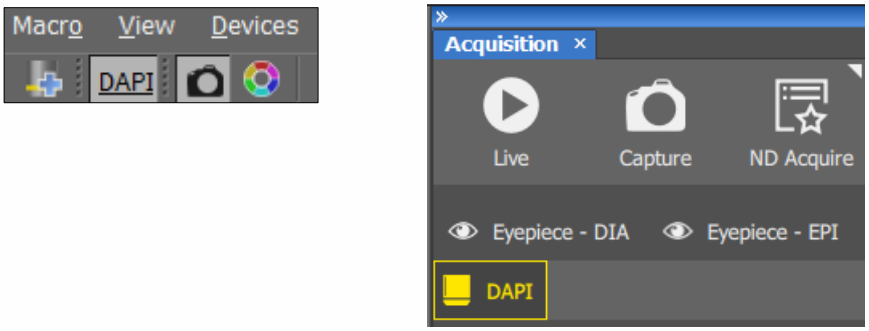


Figure 19: Optical Methods appear in the toolbar (left) and ‘Acquisition’ window (right) for easy access

Note:

- Multiple Optical Settings can be added.
- It is possible to select multiple LEDs within a single Optical Method.

4.3 Multi-channel Experiment

1. To set up a multi-channel experiment, select ‘ND Acquire’ from the ‘Acquisition’ window, which opens the ND Acquisition Wizard.

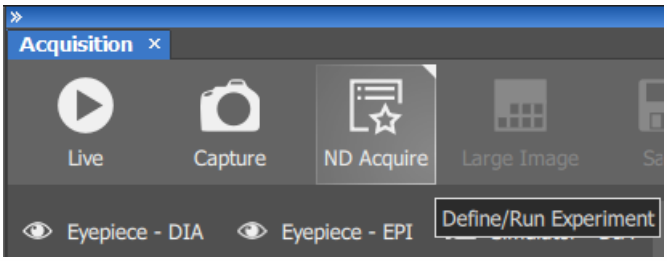


Figure 20: Selecting ND Acquire from the Acquisition window to define or run an experiment

2. Set the ‘Acquisition Parameters’, such as experiment name, multichannel or timelapse.

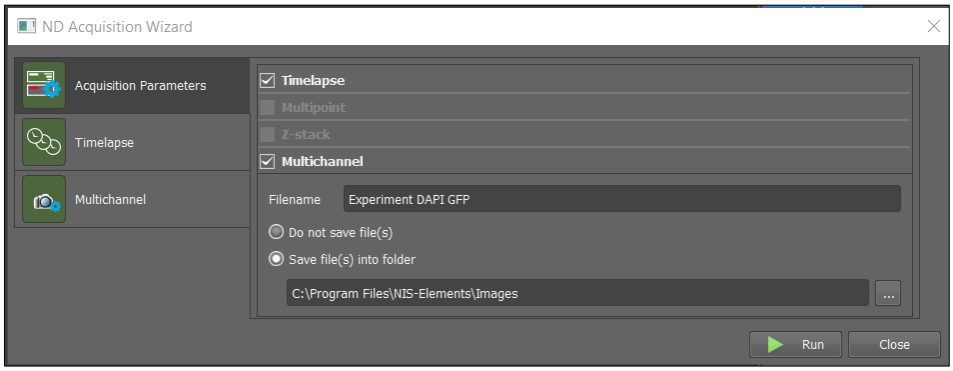


Figure 21: 'ND Acquisition Wizard' showing 'Acquisition Parameters'

Note: We are using a manual microscope in this example, where multipoint and Z-stack options are not available.

3. To set timelapse properties, select the ‘Timelapse’ window on the left. Add a ‘Time Sequence’ using the blue cross (red box, Figure 22) and enter timing parameters.

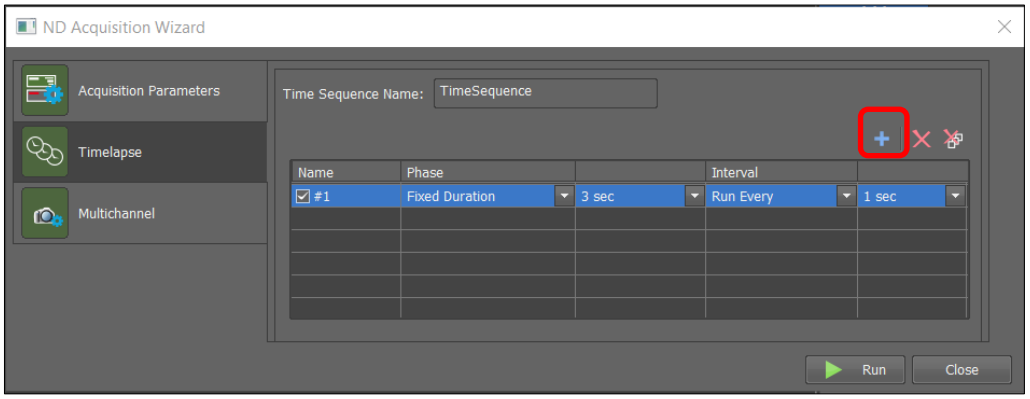


Figure 22: Setting Timelapse parameters. Add 'Time Sequence' using blue cross (red box)

4. To set multichannel properties, select the ‘Multichannel’ window from the left. Click the blue cross to add Optical Configurations previously set up (in this example, DAPI and GFP).

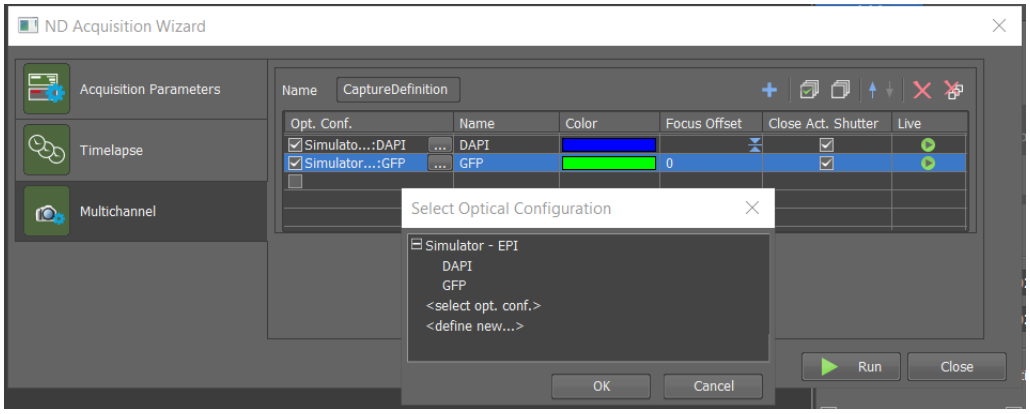


Figure 23:Select Optical Configurations for the experiment

5. Click ‘Run’ to run the experiment.

5 How the ‘pE Pad’ and pE-Control Pod interact

For compatible Illumination Systems which have a manual control pod, such as the pE-4000, the settings shown on the Control Pod replicate the ‘pE Pad’ (Figure 24).

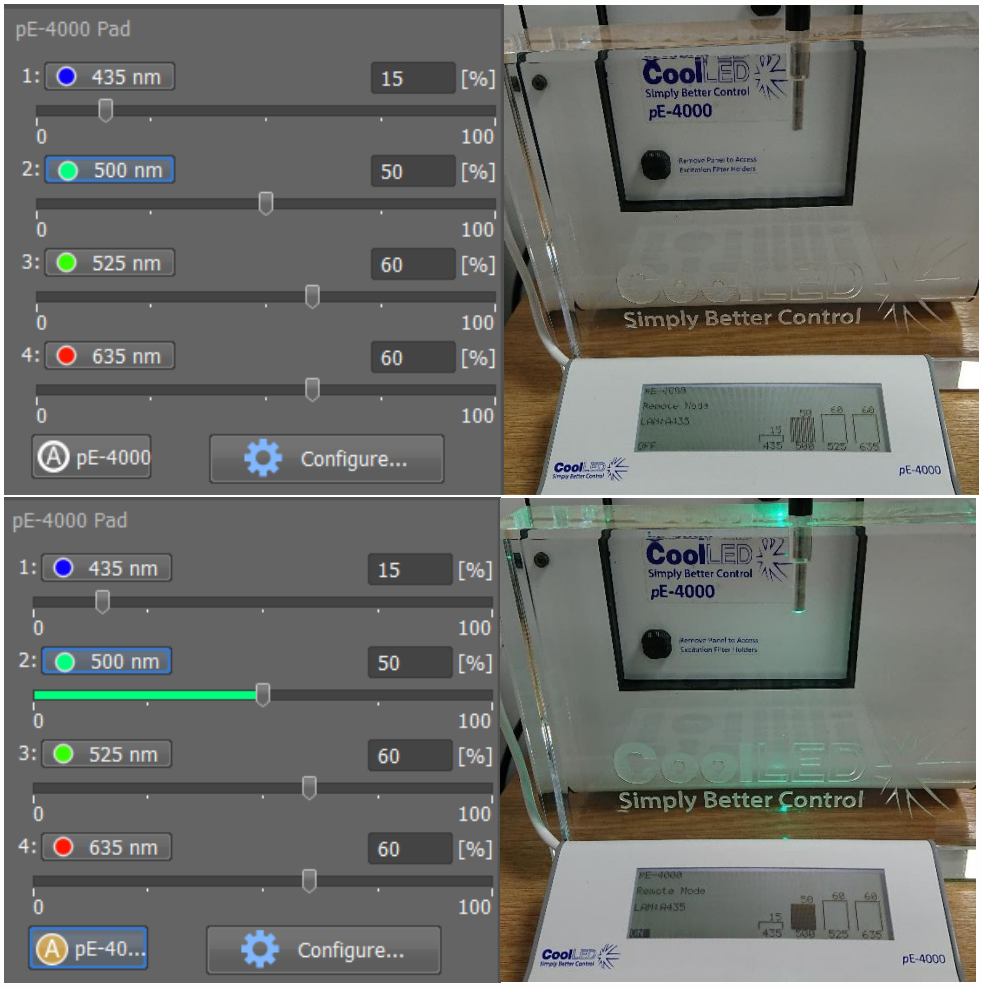


Figure 24: 'pE Pad' and pE-Control Pod replicate LED settings. A: Channel 2 is selected but shutter is closed; B: Channel 2 is selected and shutter is open