



Quick Start Guide for NIS Elements

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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).

Apps & features						
Optional features						
App execution aliases						
Search, sort and filter by drive. If you would like to us app, select it from the list.	ninstall or move an					
nis ×						
Sort by: Name V Filter by: All drives V 3 apps found						
NIS NIS-Elements 5.42.03 LO (build 1812 64-bit) 03/08/2023						
5.42.3.18120						
Modify	Uninstall					

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).

Devices		NIS-Elements
Manufacturers Nikon Prior, Scientifica, Andor Sutter, FLI Marzhauser Lucl, ASI, LuigisMeumann Linkam, Solent, LCI Erool, ESP, Exocitates Agilent, Lumencor, S8North Vincert Associates National Instruments Matomal Instruments Matomal Kastuments Matomal Resociates National Instruments Matomal Resociates National Instruments Matomatication Control (LIS), Tokaihit Legacy Others	Devices Cool.ED pE-800 Cool.ED pE-4000 Cool.ED pE-300white Cool.ED pE-300white Cool.ED pE-300white Cool.ED pE-2 Exceltas X-Cite 120 Exceltas X-Cite 120 Exceltas X-Cite XLED1 Exceltas X-Cite YLIS Exceltas X-Cite XYLIS Exceltas X-Cite NOVEM	
PrecisExcite USB COM Port driv InstallShield Select All	ver is included in the installer	Next > Cancel

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.

NIS	Compact Advanced	NIS-Elements AR
<u>F</u> ile	Edit <u>A</u> cquire <u>C</u> alibration Image <u>R</u> OI <u>B</u> inary <u>M</u> easure Refere <u>n</u> ce Macr <u>o V</u> iew	Devices Window Applications NIS.a Addon <u>s H</u> elp
1	🔚 🗠 🕼 💫 🚱 🖸 🛍 🕼 🖉 τ Σ τ 🙆 pE-800 📮 👆 🖸 🖗	🚂 Device <u>M</u> anager
4	K X Histogram X LUTs X	Show Lightpath Scheme Select HW Configuration
*		Filters and Shutters Ctrl+Alt+F Matching Fluorescent Probes and Filters

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).

Add Device	×
	Add Device
Andor TuCam	
CoolLED pE-800	
	nE-800 ×
	pc 000
	Port: COM3 🖵
	Description:
	OSB Sendi Device
	USB\VID_0483&PID_A080\ 376139833238
	Connect Cancel

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).

Α										
	pE-800 Configuration X									
	Connection									
	© USB									
	O USB + Global BNC (Sequencer									
	USB + BNCs via NIDAQ									
В										
	CoolLED pE-4000 Configuration \times									
	Wavelengths									
	1 2 3 4									
	365 nm 460 nm 525 nm 635 nm									
	385 nm 470 nm 550 nm 660 nm									
	405 nm 🔵 490 nm 🥃 580 nm 💭 740 nm 😑									
	435 nm 500 nm 5595 nm 770 nm									
	Connection									
	O USB									
	O USB + TTL In									
	NIDAQ									
	Figure Q. Cooll ED nE 800 Configuration Red (A) and nE 4000 De-									

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).









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.







0 0		Cooll ED	
Live Captu	Ire ND Acquire La	Wavelen	gths —
		1	
Simulator - EPI	Eyepiece - EPI	365 nm	3 460 nm 🔵 525 nm 🗧 635 nm 🖲
		385 nm) 470 nm 🔵 550 nm 🔵 660 nm 🖲
Simulator Pad		405 nm) 490 nm 🔵 580 nm 🔵 740 nm 🖲
Resolution (RGB)		435 nm	; 500 nm 💽 595 nm 💭 770 nm 🖲
Fast (Focus 8 bit	1280x1024 Full	, Connecti	on
Quality (Capture 8 hit	1280x1024 Full	O USB	
Exposure			+ TTL In
Exposure 10 ms 💌	Stimulation 1 m	s	
Trigger in	🗹 Trigger out		
pE-4000 Pad			
1: • 435 nm	50 [4	2 %]	
	0	3	
2: 5 00 nm	50 [4	60 %] 4	
		00	
3: 🔵 525 nm	50 [4	%]	
		00	
4: • 635 nm	50 [9	Serial N [%]	lumber: CP0036
· · _	<u> </u>	00	-5 4000
(A) pE-4000	🔅 Configure	Name	pE-4000
		Delay	0 ms
		Main:	2.0.17
		Pod:	2.0.2
			OK Cancel
x @ 1280 x 1024)			

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.





www.CoolLED.com





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.

New Optical Configuration	n		×
Name: DAPI			
Camera setting:	Camera features:	Property Values for Fast Mode:	^
	Use Stored ROI	ExposureTime = 110.000 ms FileName = "" TORGB = "0" FrameDuration = "10" StimulationTime = 1.000 HWTrgInEnabled = "false" HWTrgDutEnabled = "false"	Ĵ
Channel setup:			
	Name: DAPI	Manually	ally
	Emission 550.0 [nm] -> Color:		
Microscope setting:	Artive Shutter: nE-200 Shutter(nE-200)	7	
	Used devices:		
	Manual Microscope, FilterChanger(FilterBlock) pE=800, Shutter(pE=800) Ø pE=800, MultiLaser(pE=800) Line Used Ø pE=800, MultiLaser(pE=800) Power	Microscope: Manual Microscope Manual Microscope, FilerChanger(Filer8k p=630, Millusar(p=630); Line:1; ExW/365; Power: 29.0; On Lightpath Mandatory Devices:	ock): 5 (Empty)
		Show on toolbar and OC Panel	
		Comment:	<u></u>
Objective:	1 - Plan Fluor 40x DIC M N2		
Camera & Devices Cont	trols -		

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.

Macr <u>o V</u> iew <u>D</u> evices	* Acquisition × Live	Capture	ND Acquire
	Eyepiece -	DIA 👁	Eyepiece - EPI

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.

ND Acquisition Wizard	X
Acquisition Parameters	Timelapse Multipoint
Timelapse	Z-stack
Multichannel	Filename Experiment DAPI GFP O Do not save file(s) Save file(s) into folder
	C:\Program Files\NUS-Elements\Images

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.

ND Acquisition Wizard								×
Acquisition Parameters	Time Sequence N	iame: TimeSequence				(+)×	*	
	Name	Phase Fixed Duration		Interval Run Every		1 sec		
Multichannel								
					•	Run	Close	

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).

						\times
Name CaptureDefinition				+ 00	× &	
Opt. Conf. ☑ Simulato:DAPI	Name DAPI	Color	Focus Offset	Close Act. Shutter	Live	
Simulator:GFP	GFP		0			
Select	: Optical Confi ulator - EPI	guration	×			
D/ Gi	API FP					
<sel< th=""><th>ect opt. conf.> fine new></th><th></th><th></th><th></th><th></th><th></th></sel<>	ect opt. conf.> fine new>					
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	Name CaptureDefinition Opt. Conf. Simulator:CAPI Simulator:GFP Select D G G < <ee <det< th=""><th>Name CaptureDefinition Opt. Conf. Name Simulator:OAPI Simulator:GFP Select Optical Confit GFP GFP GFP GFP Gefine new></th><th>Name CaptureDefinition Opt. Conf. Name Color Simulator;GFP GFP Select Optical Configuration Select Optical Configuration GFP GFP <select conf.="" opt.=""> <define new=""> OK</define></select></th><th>Name CaptureDefinition Opt. Conf. Name Color Focus Offset Simulato:DAPI DAPI Simulator:GFP 0 Select Optical Configuration Select opt. conf.> <define new=""> OK Cancel</define></th><th>Name CaptureDefinition Opt. Conf. Name Color Focus Offset Close Act. Shutter Simulato:DAPI Simulato:CGP Opt. Simulato:CGP Simulator:CGP Opt. Simulator:CGP Simulator:CGP Opt. Simulator:CGP Opt. Simulator:CGP Opt. Copy Opt. Copy Opt. Copy Opt. Copy Opt. Copy Copy <</th><th>Name CaptureDefinition + Image: Color Focus Offset Close Act. Shutter Live Opt. Conf. DAPI DAPI Image: Color Focus Offset Close Act. Shutter Live Simulator:DAPI DAPI Image: Color Image: Color Image: Color Image: Color Image: Color Simulator:GFP Image: Color Image: Color Image: Color Image: Color Image: Color Simulator:GFP Image: Color Image: Color Image: Color Image: Color Image: Color Simulator:GFP Image: Color Image: Color Image: Color Image: Color Image: Color Simulator> Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Ima</th></det<></ee 	Name CaptureDefinition Opt. Conf. Name Simulator:OAPI Simulator:GFP Select Optical Confit GFP GFP GFP GFP Gefine new>	Name CaptureDefinition Opt. Conf. Name Color Simulator;GFP GFP Select Optical Configuration Select Optical Configuration GFP GFP <select conf.="" opt.=""> <define new=""> OK</define></select>	Name CaptureDefinition Opt. Conf. Name Color Focus Offset Simulato:DAPI DAPI Simulator:GFP 0 Select Optical Configuration Select opt. conf.> <define new=""> OK Cancel</define>	Name CaptureDefinition Opt. Conf. Name Color Focus Offset Close Act. Shutter Simulato:DAPI Simulato:CGP Opt. Simulato:CGP Simulator:CGP Opt. Simulator:CGP Simulator:CGP Opt. Simulator:CGP Opt. Simulator:CGP Opt. Copy Opt. Copy Opt. Copy Opt. Copy Opt. Copy Copy <	Name CaptureDefinition + Image: Color Focus Offset Close Act. Shutter Live Opt. Conf. DAPI DAPI Image: Color Focus Offset Close Act. Shutter Live Simulator:DAPI DAPI Image: Color Image: Color Image: Color Image: Color Image: Color Simulator:GFP Image: Color Image: Color Image: Color Image: Color Image: Color Simulator:GFP Image: Color Image: Color Image: Color Image: Color Image: Color Simulator:GFP Image: Color Image: Color Image: Color Image: Color Image: Color Simulator> Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Image: Color Ima

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

