



# Quick Start Guide for $\mu$ Manager

## Versions 2.0, 1.4.23, 1.4.22

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### Contents

1	Integrated CoolLED Products .....	1
2	Setting up your CoolLED Illumination System COM Port via USB .....	1
3	Downloading Adaptor file.....	3
4	$\mu$ Manager 2.0 with Integrated CoolLED Illumination Systems .....	3
5	$\mu$ Manager 1.4 with Integrated CoolLED Illumination Systems .....	15
6	Appendix .....	24

## 1 Integrated CoolLED Products

- pE-800 Series
- pE-400 Series
- pE-4000
- pE-300<sup>white</sup>, pE-300<sup>ultra</sup>
- pE-340<sup>fura</sup>
- pE-6501, pE-6501-8, pE-6501-8-FR USB-TTL Conversion Kits
- Discontinued pE-2 (only in version 1.4)

## 2 Setting up your CoolLED Illumination System COM Port via USB

If using the pE-800 Series or pE-400 Series and Windows 10, please move directly to Downloading Adaptor file (Section 3), since the pE-Driver is not required.

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

## 2.1 Setting up the USB interface

The USB interface uses Microsoft's own built-in drivers for Virtual Serial Ports, which means that the CoolLED Illumination System behaves as an ordinary COM port on your PC and can be controlled by any application that uses Windows COM ports correctly. This means that there is a requirement to tell Windows about the CoolLED hardware via a driver .INF file.

Start by downloading the CoolLED pE-Driver from the CoolLED website:

[www.cooled.com/support/imaging-software/#cooled-pe-driver](http://www.cooled.com/support/imaging-software/#cooled-pe-driver)

When you first plug the CoolLED Illumination System into your PC via the USB interface, Windows will tell you it has found new hardware. It will ask you for the location of the driver file. Tell Windows to look in the location where you have downloaded the .INF file.

## 2.2 Setting up USB on Windows Vista and above

Navigate to: Start -> Control Panel -> System Management -> System -> Hardware -> Device Manager -> Continue

Click the [+] by Ports to see the list of COM ports. The CoolLED Illumination System ports can be identified with the name: 'CoolLED USB Virtual Serial Port (COMx)' where x is a positive number. Either COM port may 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 and allows for ease of use if there is a COM port conflict. Please note if using the pE-800 Series or pE-400 Series only one port will be available.

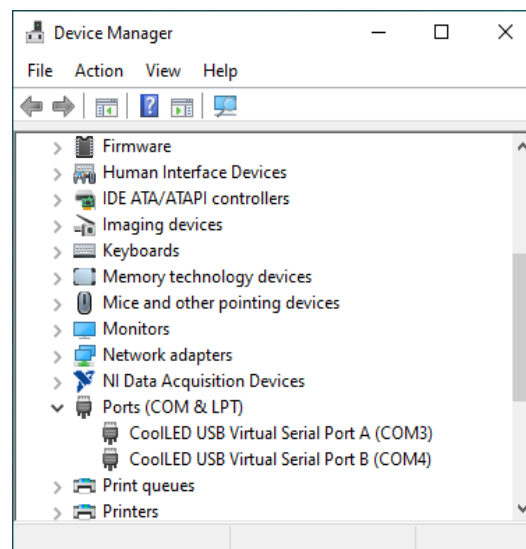


Figure 1: Example COM ports for a CoolLED Illumination



### 3 Downloading Adaptor file

In most cases, a CoolLED .dll adaptor file is required, which can be downloaded from the CoolLED website by following the relevant links in the table below:

μManager Adaptors		
Illumination System	μManager version 1.4	μManager 2.0
pE-800 Series	1.4.22 only Windows 64-bit only <a href="#">pE-800.dll</a>	<a href="#">CoolLED.dll</a> Windows 64-bit only
pE-400 Series	N/A	
pE-4000	<u>1.4.23</u> : adaptors present as standard <u>1.4.22</u> : <a href="#">pE-4000.dll</a> Windows 64-bit only	
pE-300 <sup>ultra</sup>	<u>1.4.23</u> : adaptors present as standard <u>1.4.22</u> : <a href="#">pE-300.dll</a> Windows 64-bit only	
pE-300 <sup>white</sup>		
pE-340 <sup>fura</sup>		
pE-2	Present as standard	N/A

Once downloaded, save this file in the µManager program folder (e.g., C:\Program Files\Micro-Manager-2.0)

#### 3.1 Downloading Configuration Files for µManager 2.0

We have created configuration files with basic channel on/off and intensity controls for CoolLED Illumination Systems supported in µManager 2.0.

[Download configuration files here](#)

Once downloaded, save this file in the µManager program folder (e.g., C:\Program Files\Micro-Manager-2.0)

### 4 µManager 2.0 with Integrated CoolLED Illumination Systems

#### 4.1 Hardware Configuration

1. Launch µManager and either load a hardware configuration file (if downloaded and saved as in Section 3.1) or start with '(none)' to create a new one for your individual microscope setup.

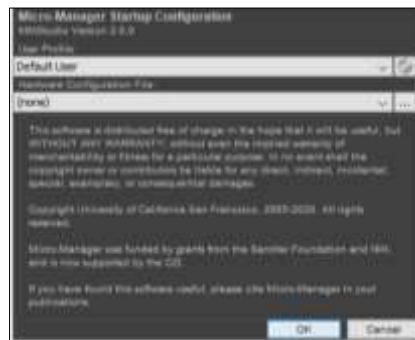


Figure 2:  $\mu$ Manager 2.0 Startup Configuration screen

2. To create a new Hardware Configuration File, navigate to 'Devices' and 'Hardware Configuration Wizard'.

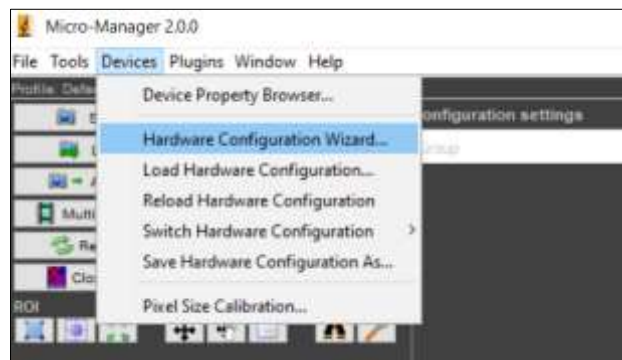


Figure 3: Navigating to the Hardware Configuration Wizard

3. At Step 1, select 'Create new configuration', and click 'Next'.

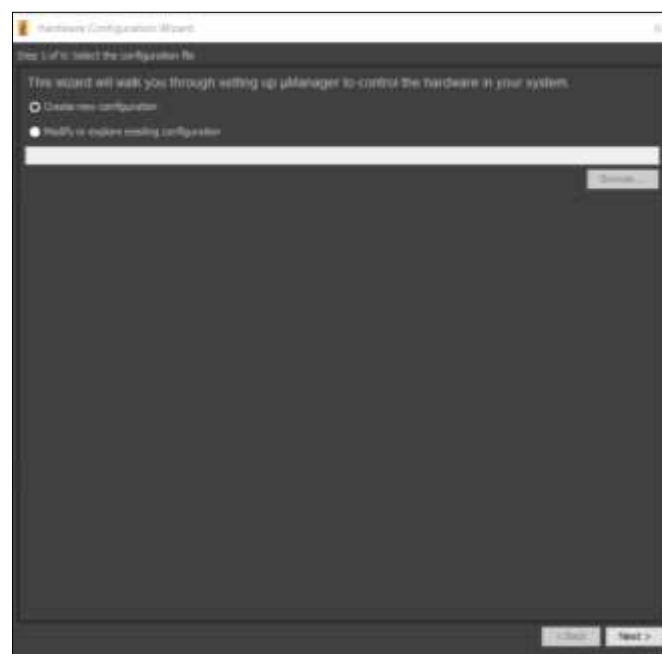


Figure 4: 'Hardware Configuration Wizard'



4. Step 2 is where devices can be added and removed from  $\mu$ Manager. To add your CoolLED Illumination System, navigate through the list and find 'CoolLED'. Expand this using the [+] icon and select the relevant product from the list:

$\mu$ Manager device name	CoolLED Illumination System
pE-300: Illumination System	pE-300 Series (pE-300 <sup>white</sup> and pE-300 <sup>ultra</sup> ), pE-340 <sup>fura</sup>
pE-400max: Illumination System	pE-400 <sup>max</sup>
pE-400: Illumination System	pE-400
pE-800: Illumination System	pE-800 Series (pE-800 and pE-800 <sup>fura</sup> )
pE-4000: Illumination System	pE-4000

5. Once chosen, click 'Add...'. This example shows the pE-400<sup>max</sup>.

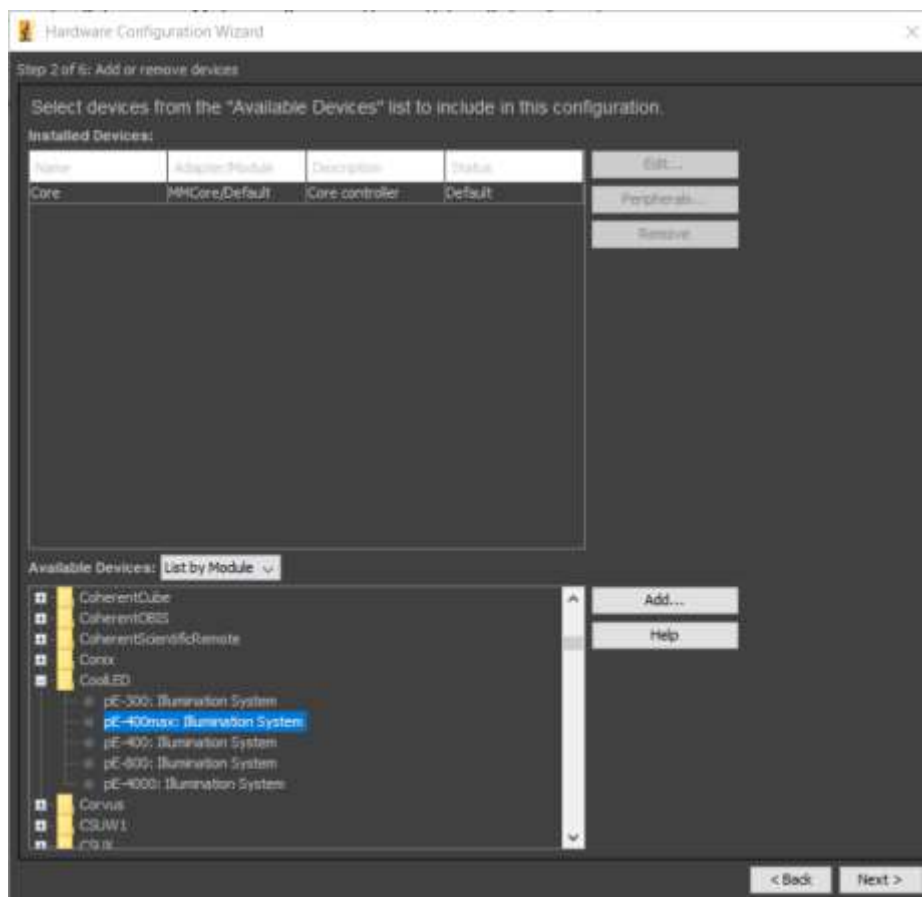


Figure 5: In step 2, integrated CoolLED products and other devices can be added or removed



- After selecting the relevant Illumination System, a pop-up screen appears. Select the correct COM port for the Illumination System, matching it to the one selected in Device Manager (see Section 2.2).

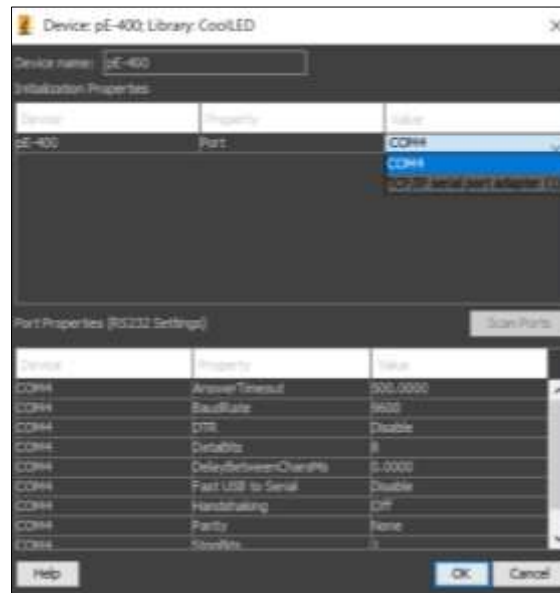


Figure 6: Selecting the COM port

- The parameters listed in 'Port Properties' (Figure 6) default to working parameters and generally should be left alone, although the BaudRate can be changed to 576000. It is possible to alter these later if required. Then click 'OK'.
- In the 'Hardware Configuration Wizard', add all relevant devices (e.g., camera, stages etc.) and click 'Next' to continue. At Step 6, name the file and save this configuration. Then click 'Finish' to close the window.

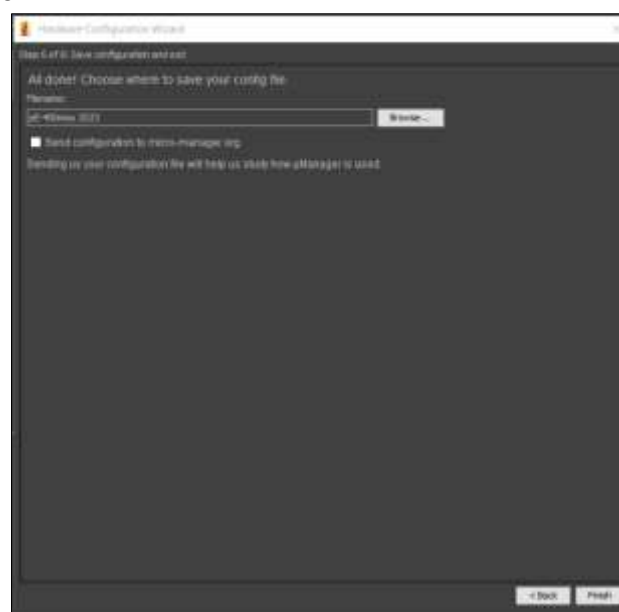


Figure 7: Final step of 'Hardware Configuration Wizard'



**Note:** The hardware configuration file can be loaded in the future by navigating to Devices > 'Load Hardware Configuration'.

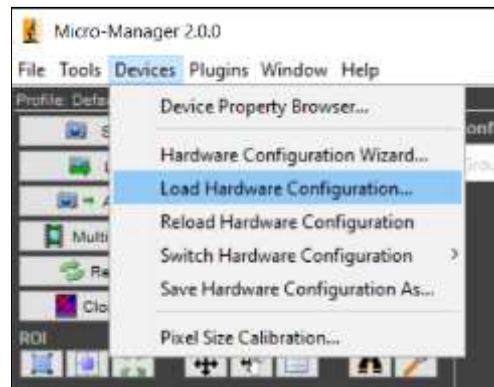


Figure 8: Load this Hardware Configuration in the future

## 4.2 View Device Properties

View the properties of your Illumination System that can be controlled in  $\mu$ Manager by navigating to 'Devices' > 'Device Property Browser' (Figure 9). The example below shows a pE-400<sup>max</sup>.

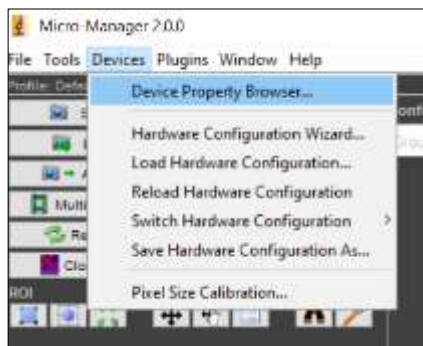


Figure 9: Navigating to Device Property Manager

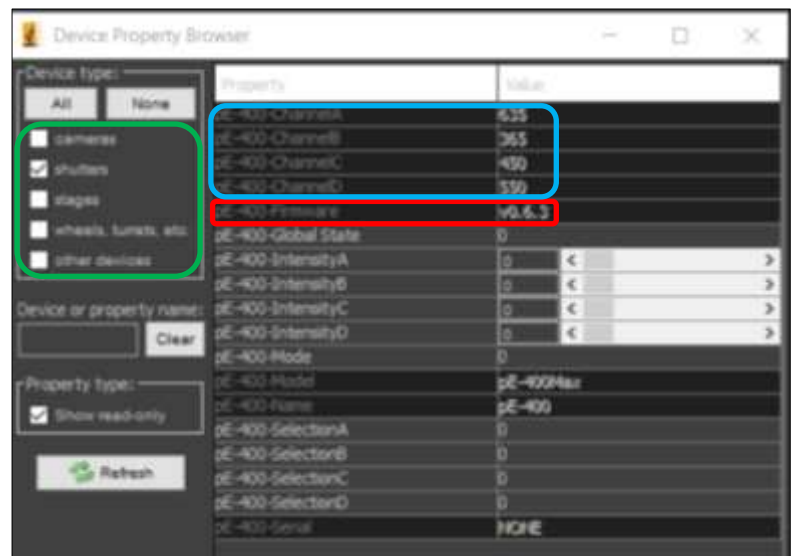


Figure 10: Device Property Browser shows all device properties  $\mu$ Manager can control, plus firmware (red) and LED channel names (blue). Limit view to relevant properties (green)

### Note:

- Unchecking all boxes under 'Device type' except shutters simplifies the view by showing only relevant properties (circled in green, Figure 10).
- The Firmware of the Illumination System can also be viewed for the pE-300 Series, pE-4000, pE-800 Series and pE-400 Series (circled in red, Figure 10).
- Pay close attention to the channel names in  $\mu$ Manager – their corresponding wavelength is listed at the top of the Device Property Browser (circled in blue, Figure 10), and in the table in 6.1 LED Labels.



### 4.3 Controlling Device Properties

µManager settings can now be tailored to suit user requirements, and controlling properties for the Illumination System is achieved using Groups and Presets.

- A Group is a user-specified selection of properties to be controlled, e.g., LED channel selection.
- A Preset is a user-specified setting (or value) for a Group, e.g., Channel A is selected.

The following sections 4.4 and 4.5 explain two examples using Groups and Presets:

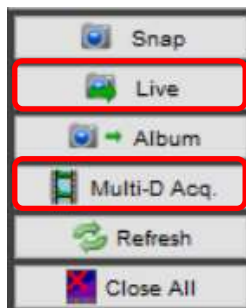


Figure 11: µManager Live and Multi-Dimensional Acquisition modes circled in red

- How to configure the pE-400<sup>max</sup> for single colour experiments or sample visualisation with live control.
- How to configure the pE-400<sup>max</sup> for a multi-dimensional acquisition using all four channels.

### 4.4 Configuring µManager for single-colour experiments with live control

The following steps explain how to achieve live control of channel selection and intensity (irradiance) during Live mode.

1. Create a 'Group' by clicking the Group '[+]' icon on the main screen (see Figure 12, circled in red).

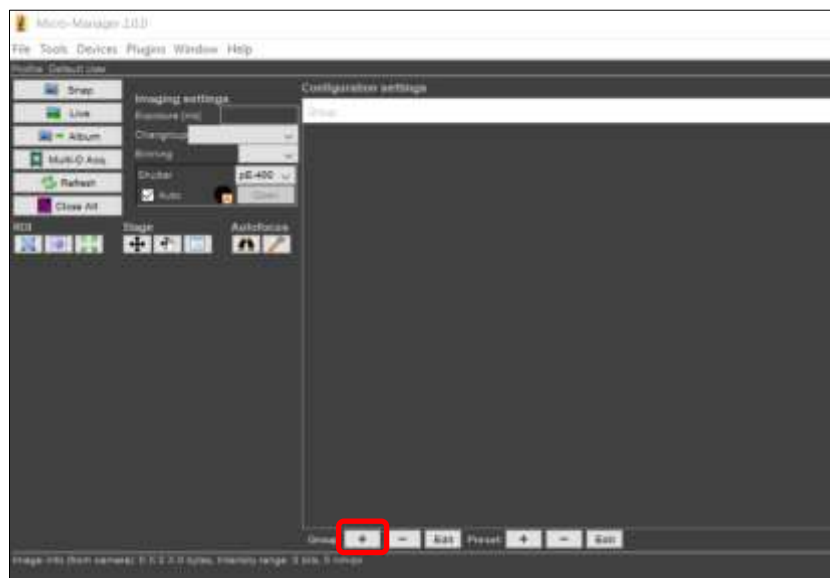


Figure 12: Adding a new Group (circled in red)



2. This brings up the 'Group Editor' Screen. Create a Group to control LED channel selection by selecting all the relevant properties. In this case, this is 'pE-400-SelectionA' through to 'pE-400-Selection D'. Enter the Group name as 'Channel selection' and click 'OK'.

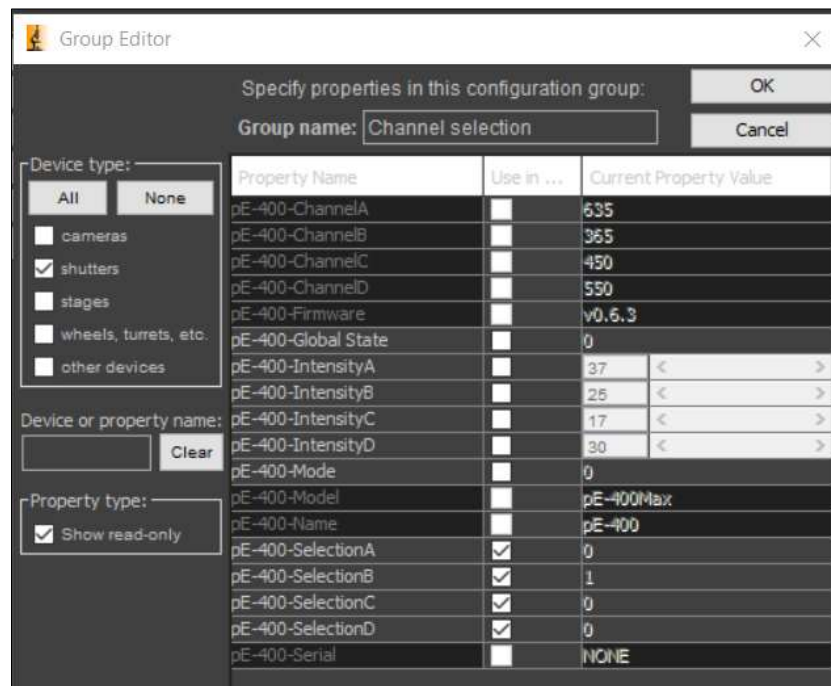


Figure 13: Creating a Channel Selection group by selecting all relevant properties

3. The 'Preset editor' opens automatically (Figure 14). This is where presets are created for the properties now selected.
  - a. Select one LED channel per preset, starting with 'pE-400-SelectionB'.
  - b. Name this by its corresponding channel (see section 6.1 LED Labels), "Channel 365 nm".
  - c. Using the dropdown menu under 'Preset Value', change '0' (unselected) to '1' (selected).
  - d. Ensure all other Preset Values are set to '0'.
  - e. Click 'OK'.

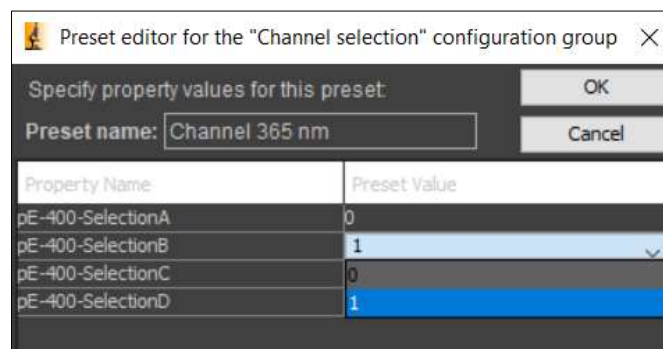


Figure 14: Creating presets with each channel selected per preset

4. Repeat Steps 3a - 3b for each item under 'Property Name'. Once complete, click the Preset dropdown on the main window to view all four presets: one per channel.

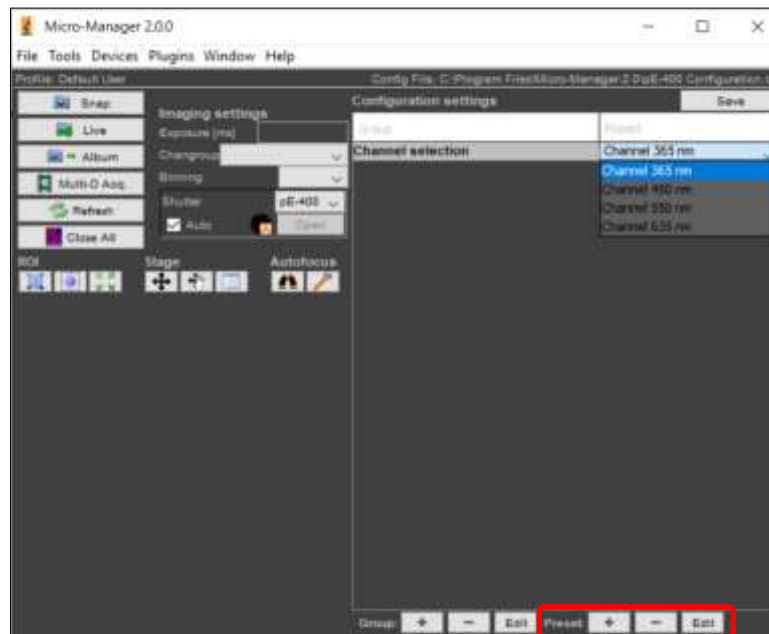


Figure 15: View and select presets in dropdown menu under 'Preset'. Add, remove, or edit presets using buttons circled in red

5. To add, remove or edit Presets, use the icons circled in red (Figure 15).
6. To control channel intensity, create a new Group by clicking the Group '+' icon on the main screen (see Figure 12, circled in red).
7. When the Group Editor appears, this time select just one value: 'pE-400-IntensityB'. Name this by its corresponding channel (see 6.1 LED Labels): "Channel 365 nm intensity" and click 'OK'.

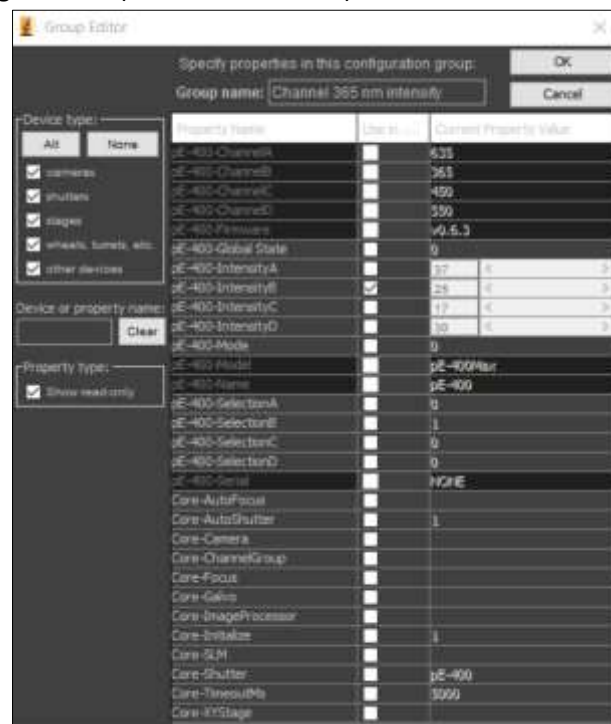


Figure 16: Group Editor, selecting just one value to control channel intensity



8. There is no requirement to add a preset in this case, since an intensity slider is available in the main screen for intensity adjustment.

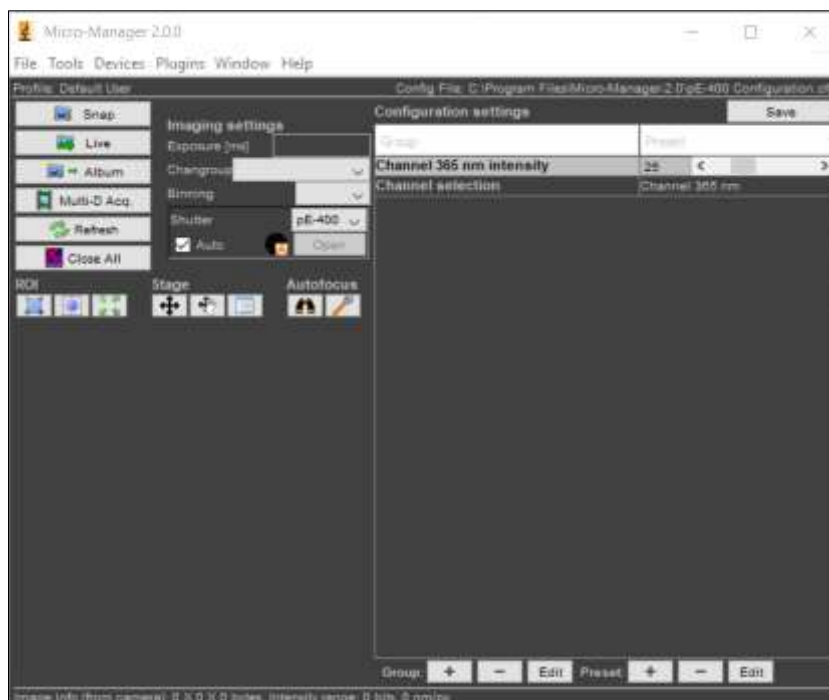


Figure 17: Main screen shows channel intensity slider

9. Repeat steps 6-7 for all remaining channels. The main screen will then look like Figure 18 and channel selection and intensity can now be controlled in real-time.



Figure 18: Main screen shows channel selection and intensity sliders for pE-400<sup>max</sup> live control

10. Select Live mode (Figure 11) to begin live image acquisition.



## 4.5 Configuring µManager for a multi-dimensional acquisition

‘Multi-Dimensional Acquisition’ makes it possible to acquire an image sequence. The following steps explain how to configure µManager for a four-colour acquisition using 365, 450, 550, 635 nm LEDs of the pE-400<sup>max</sup>, in this example using DAPI, FITC, TRITC and Cy5.

1. Create a new Group by clicking the Group ‘[+]’ icon on the main screen (see Figure 11, circled in red). To control both channel selection and intensity, select all channel selection and intensity properties, and name this Group “DAPI/FITC/TRITC/Cy5”. Click ‘OK’.

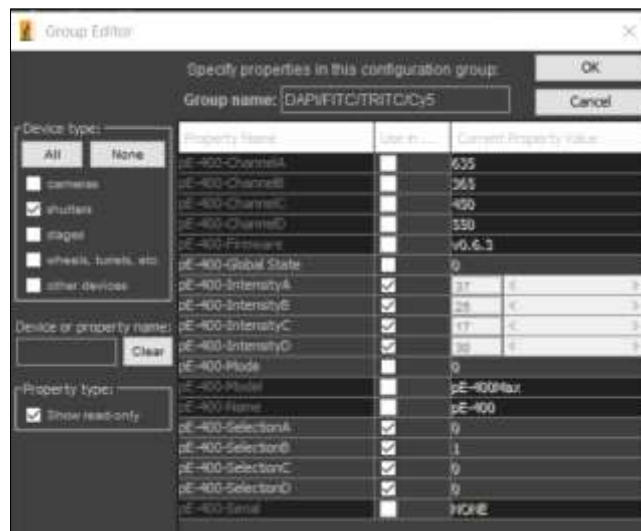


Figure 19: Group Editor for setting up a multi-dimensional acquisition

2. The ‘Preset editor’ now appears. Create a preset for each fluorophore, starting with DAPI:
  - a. Name this preset “DAPI (365 nm)”.
  - b. Select the relevant LED channel which is “pE-400-SelectionB”(see 9.1 LED Labels), using the dropdown menu to change ‘0’ (unselected) to ‘1’ (selected).
  - c. Set intensity using the ‘pE-400-IntensityB’ slider under Preset Value. Click ‘OK’ to finish.



Figure 20: Preset editor



3. Repeat steps 2a-c to add the remaining fluorophores, referencing Section 9.1 LED Labels for the corresponding channel names. When complete, the Preset dropdown menu on the main screen appears as in Figure 21.

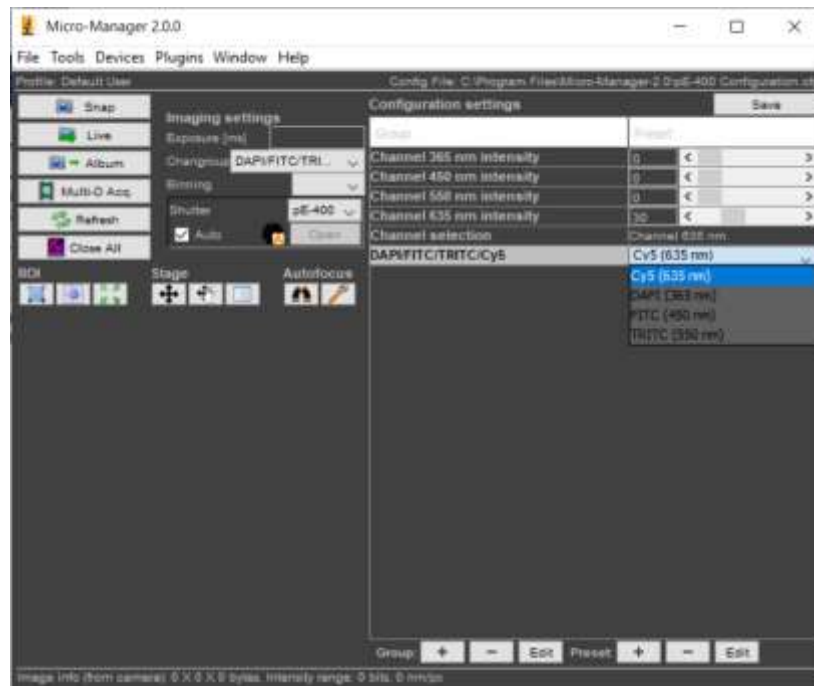


Figure 21: For a multi-dimensional acquisition, presets for channel selection and intensity are now available for each fluorophore

4. Click 'Multi-D Acq.' (Figure 11) to enter the Multi-Dimensional Acquisition setup (Figure 22).
5. As in Figure 22, you will see DAPI/FITC/TRITC/Cy5 appearing in 'channel group'. Click 'New' (circled in red) to add a new preset from this group, until DAPI, FITC, TRITC and Cy5 are listed.
6. Set your time points (circled in blue), and click 'Acquire!', and the selected wavelengths will switch sequentially.

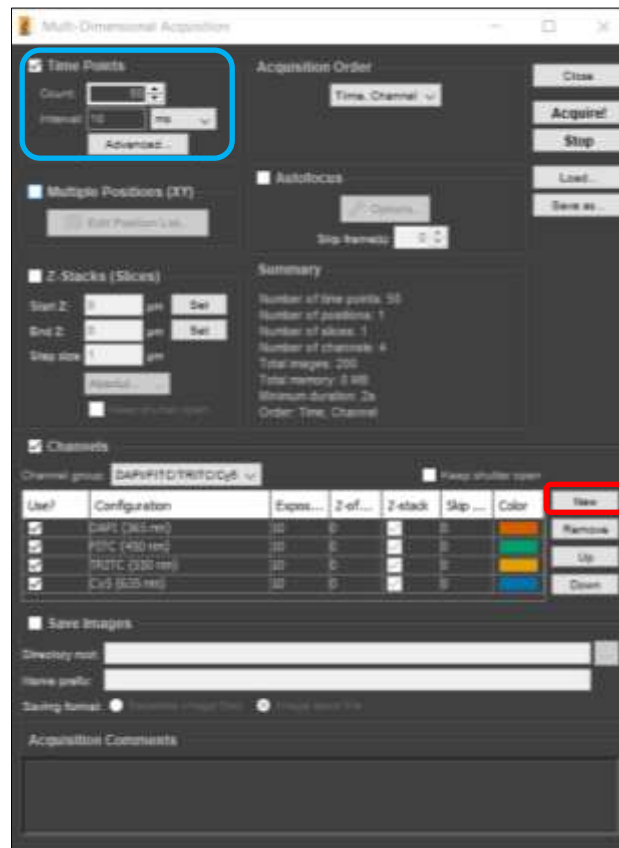


Figure 22: Multi-Dimension Acquisition setup. Add new channel configurations (circled in red) and set time points (circled in blue)



## 5 $\mu$ Manager 1.4 with Integrated CoolLED Illumination Systems

### 5.1 Hardware Configuration

When  $\mu$ Manager opens, navigate to the 'Tools' tab and then select 'Hardware Configuration Wizard'. This opens the window below:

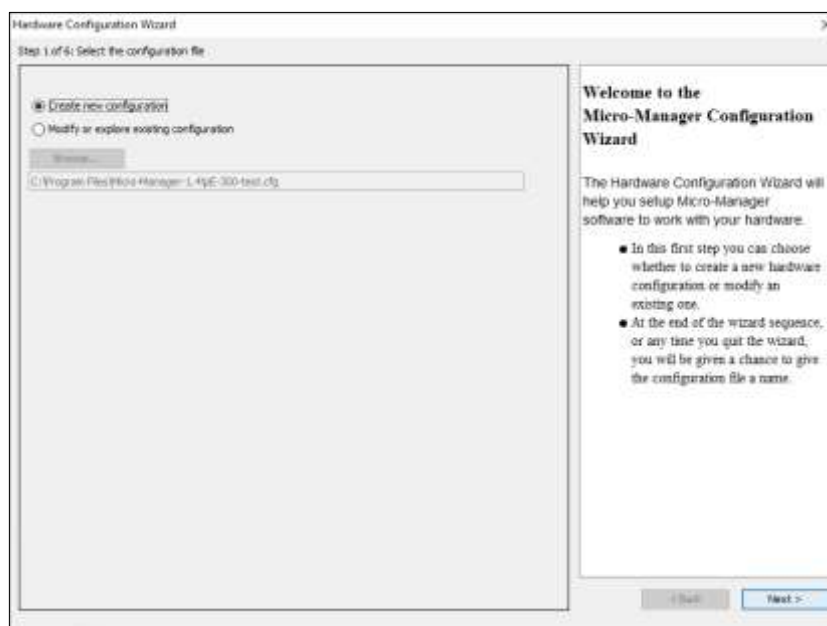


Figure 23. 'Hardware Configuration Wizard'

1. Select 'Create new configuration' and click 'Next'.
2. In Step 2 of the Hardware Configuration Wizard, you have the option to add or remove devices in addition to the core  $\mu$ Manager device. Navigate through the list and select the relevant CoolLED Illumination System. The different products can be found under different sections within  $\mu$ Manager during setup:

$\mu$ Manager device name	CoolLED Illumination System
CoolLED-pE800	pE-800 Series (pE-800 and pE-800 <sup>fura</sup> )
pE4000	pE-4000
pE300	pE-300 Series (pE-300 <sup>white</sup> and pE-300 <sup>ultra</sup> ), pE-340 <sup>fura</sup>
precisExcite	pE-2

Once chosen, select 'Add...'





Figure 24: The pE-300 & pE-4000 as listed in 'Hardware Configuration Wizard'

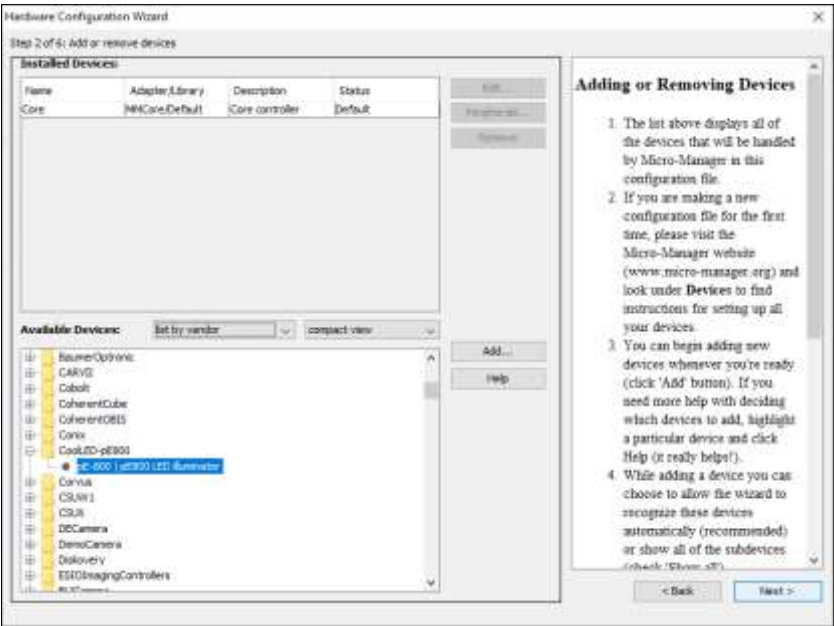
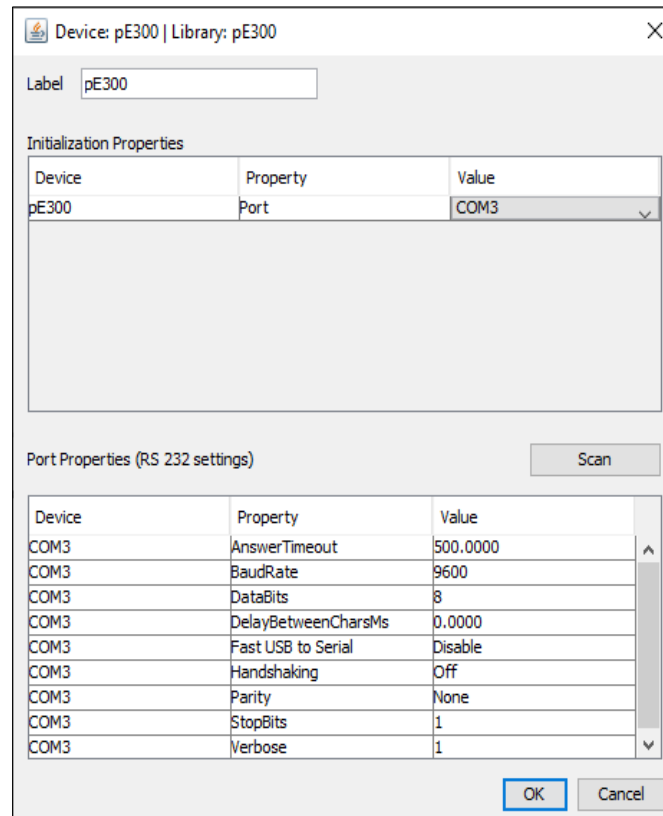


Figure 25: The pE-800 Series listed in Hardware Configuration Wizard'





3. After selecting the CoolLED Illumination System, another screen will pop up (see below). Ensure that you have selected the correct COM port for your device, matching it to the one selected in the Device Manager from earlier.



Device: pE300 | Library: pE300

Label:

Initialization Properties

Device	Property	Value
pE300	Port	COM3

Port Properties (RS 232 settings) Scan

Device	Property	Value
COM3	AnswerTimeout	500.0000
COM3	BaudRate	9600
COM3	DataBits	8
COM3	DelayBetweenCharsMs	0.0000
COM3	Fast USB to Serial	Disable
COM3	Handshaking	Off
COM3	Parity	None
COM3	StopBits	1
COM3	Verbose	1

OK Cancel

Figure 26: Device Configuration for the pE-300 Series

4. There are a number of parameters listed in 'Port Properties'. These will default to working parameters and generally should be left alone, although the BaudRate can be changed to 576000. It is possible to alter these later if required.



5. Ensure that you have added all the relevant devices for your configuration (camera, stages etc.) and continue to click 'Next' through the 'Hardware Configuration Wizard' until you reach Step 6; here you can save your configuration to be reloaded at any time. After this, click 'Finish'.

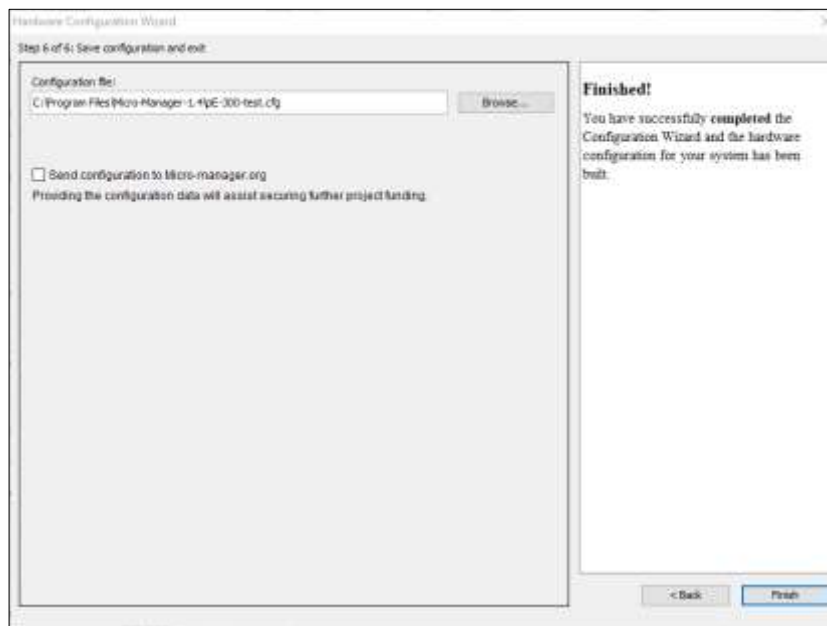


Figure 27: The final screen of the 'Hardware Configuration Wizard'

## 5.2 Configuration

1. It is possible to view the variables that can be controlled by  $\mu$ Manager in the 'Device Property Browser'. In the below example, a pE-4000 is displayed. In the 'Device Property Browser' it is also possible to view the Firmware of your unit in the 'Description' value. This feature is available on the pE-300 Series and pE-4000.

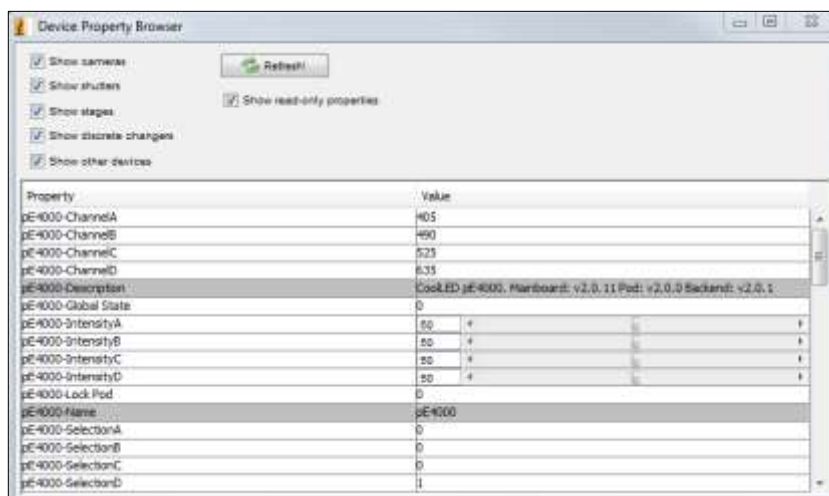


Figure 28: 'Device Property Browser'



2. You can create a ‘Group’ with any number of these values by clicking the Group ‘[+]’ icon on the main screen (see below):

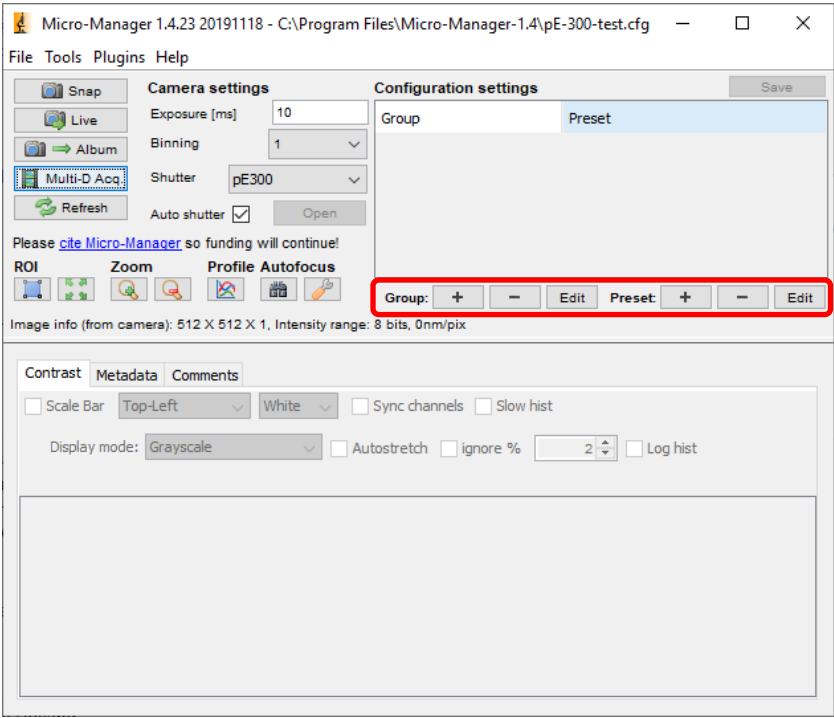


Figure 29: 'Group' and 'Preset' creation

Doing so will bring up the ‘Group Editor’ Screen (see below).

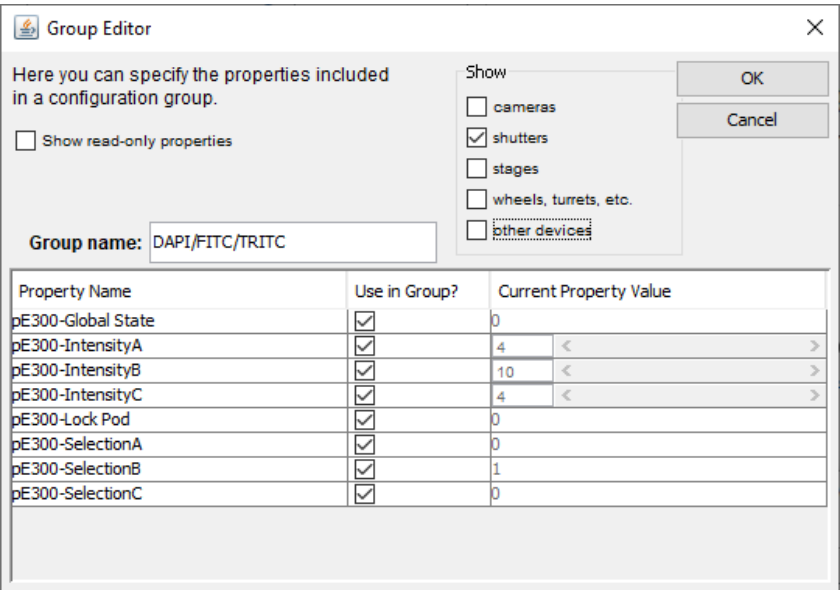


Figure 30: Group editor example for a pE-300 Series



3. For the pE-800 Series the eight LEDs correspond to the eight different labels as noted in 6.1 LED Labels.

Group Editor

Here you can specify the properties included in a configuration group.

☐ Show read-only properties

Group name: pE-800

☒ shutters  
☒ stages  
☒ wheels, turrets  
☒ other devices

Property Name	Use in ...	Current Property Value
pE-800-SelectionA	<input checked="" type="checkbox"/>	0
pE-800-SelectionB	<input checked="" type="checkbox"/>	0
pE-800-SelectionC	<input checked="" type="checkbox"/>	0
pE-800-SelectionD	<input checked="" type="checkbox"/>	1
pE-800-SelectionE	<input checked="" type="checkbox"/>	0
pE-800-SelectionF	<input checked="" type="checkbox"/>	0
pE-800-SelectionG	<input checked="" type="checkbox"/>	0
pE-800-SelectionH	<input checked="" type="checkbox"/>	0
pE-800-SequenceA	<input type="checkbox"/>	0
pE-800-SequenceB	<input type="checkbox"/>	0
pE-800-SequenceC	<input type="checkbox"/>	0
pE-800-SequenceD	<input type="checkbox"/>	0
pE-800-SequenceE	<input type="checkbox"/>	0
pE-800-SequenceF	<input type="checkbox"/>	0
pE-800-SequenceG	<input type="checkbox"/>	0

Figure 32: Group editor example for a pE-800 Series

Group Editor

Here you can specify the properties included in a configuration group.

☐ Show read-only properties

Group name: pE-800

☒ shutters  
☒ stages  
☒ wheels, turrets  
☒ other devices

Property Name	Use in ...	Current Property Value
pE-800-AnalogueH	<input type="checkbox"/>	0
pE-800-Global State	<input checked="" type="checkbox"/>	1
pE-800-IntensityA	<input checked="" type="checkbox"/>	0
pE-800-IntensityB	<input checked="" type="checkbox"/>	0
pE-800-IntensityC	<input checked="" type="checkbox"/>	0
pE-800-IntensityD	<input checked="" type="checkbox"/>	20
pE-800-IntensityE	<input checked="" type="checkbox"/>	0
pE-800-IntensityF	<input checked="" type="checkbox"/>	0
pE-800-IntensityG	<input checked="" type="checkbox"/>	0
pE-800-IntensityH	<input checked="" type="checkbox"/>	0
pE-800-Mode	<input type="checkbox"/>	0
pE-800-SelectionA	<input checked="" type="checkbox"/>	0
pE-800-SelectionB	<input checked="" type="checkbox"/>	0
pE-800-SelectionC	<input checked="" type="checkbox"/>	0
pE-800-SelectionD	<input checked="" type="checkbox"/>	1

Figure 31: Group editor example for a pE-800 Series

4. After selecting the group of variables from this screen you can create presets for it using the second '[+]' icon. In some cases, the 'Preset Editor' (see below) may open after creating a group. In doing this you could, for example, create different presets for each wavelength you would like to use, enabling you to switch between them sequentially in 'Multi-Dimensional Acquisition'.

Preset editor for the "DAPI/FITC/TRITC" configuration group

Here you can specify the property values in a configuration preset.

Preset name: DAPI

Property Name	Preset Value
pE300-Global State	0
pE300-IntensityA	100
pE300-IntensityB	0
pE300-IntensityC	0
pE300-Lock Pod	0
pE300-SelectionA	1
pE300-SelectionB	0
pE300-SelectionC	0

Figure 33: 'Preset Editor' example using the pE-300 Series



Property Name	Preset Value
pE-800-Global State	1
pE-800-IntensityA	0
pE-800-IntensityB	0
pE-800-IntensityC	0
pE-800-IntensityD	0
pE-800-IntensityE	0
pE-800-IntensityF	40
pE-800-IntensityG	0
pE-800-IntensityH	0
pE-800-SelectionA	0
pE-800-SelectionB	0
pE-800-SelectionC	0
pE-800-SelectionD	0
pE-800-SelectionE	0
pE-800-SelectionF	1
pE-800-SelectionG	0
pE-800-SelectionH	0

Figure 34: 'Preset Editor' example using the pE-800 Series for the 635 nm LED

Once all presets have been created, they will appear in the drop-down menu.

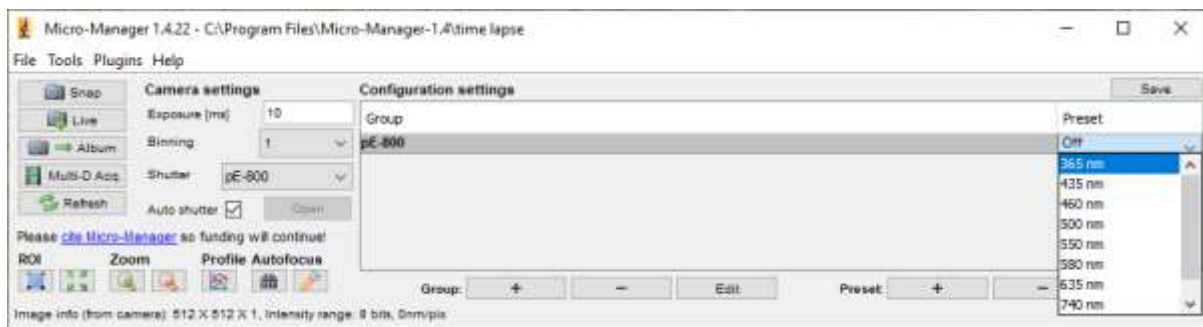


Figure 35: Drop-down list of presets created for a pE-800 Series

## 5.3 Examples of Software Functionality:

### 1. Multi-Dimensional Acquisition

In 'Multi-Dimensional Acquisition', it is possible to create a sequence of images by adding and selecting a group (in this example DAPI/FITC/TRITC) and then individually pressing 'New' to add each preset. When you then set your time parameters and click 'Acquire!', these wavelengths switch sequentially.



Multi-Dimensional Acquisition

☒ Time points

Number: 100  
Interval: 100 ms

☒ Multiple positions (XY)

Edit position list...

☐ Z-stacks (slices)

Z-start [um]: 0 Set  
Z-end [um]: 0 Set  
Z-step [um]: 1  
relative Z  
☐ Keep shutter open

Acquisition order: Time, Position, Channel

☐ Autofocus

Options...  
Skip frame(s): 0

Summary

Number of time points: 100  
Number of positions: 1  
Number of slices: 1  
Number of channels: 3  
Total images: 300  
Total memory: 75 MB  
Duration: 0h 0m 10s  
Order: Time, Position, Channel

☒ Channels

Channel group: DAPI/FITC/TRITC ☐ Keep shutter open

Use?	Configu...	Exposure	Z-offset	Z-stack	Skip Fr.	Color
<input checked="" type="checkbox"/>	DAPI	100	0	<input checked="" type="checkbox"/>	0	
<input checked="" type="checkbox"/>	FITC	100	0	<input checked="" type="checkbox"/>	0	
<input checked="" type="checkbox"/>	TRITC	100	0	<input checked="" type="checkbox"/>	0	

☐ Save images

Directory root: C:\Users\Brynn.Dachtler\AcquisitionData  
Name prefix: Untitled  
Saving format: ☒ Separate image files ☐ Image stack file

Acquisition Comments

Figure 36: 'Multi-Dimensional Acquisition' setup



2. Live Intensity Variation

For Live Intensity Variation, you can create a ‘group’ with only one value; such as intensity with a slider, as shown below:

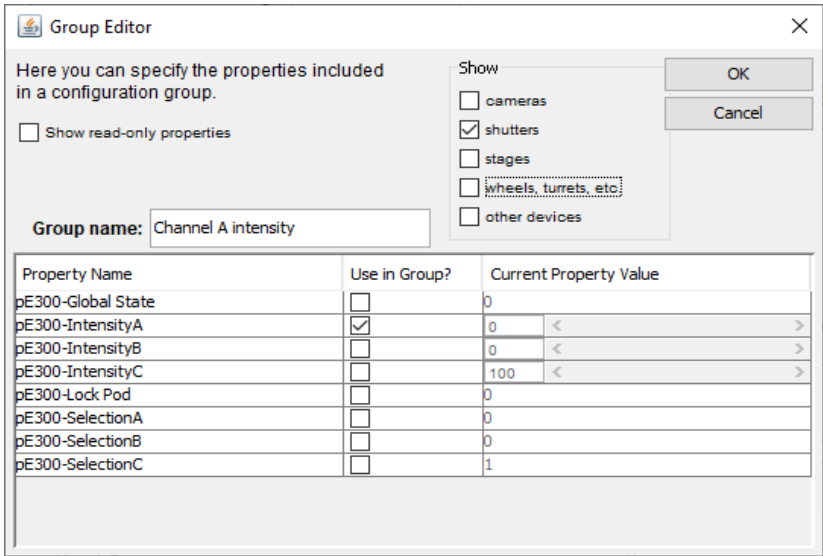


Figure 37: A possible configuration for Live Intensity Variation using a pE-300 Series

This can allow you to alter the intensity while in ‘Live’ mode, and still switch back to the values in the other group (for example the ‘DAPI/FITC/TRITC’ group shown below).

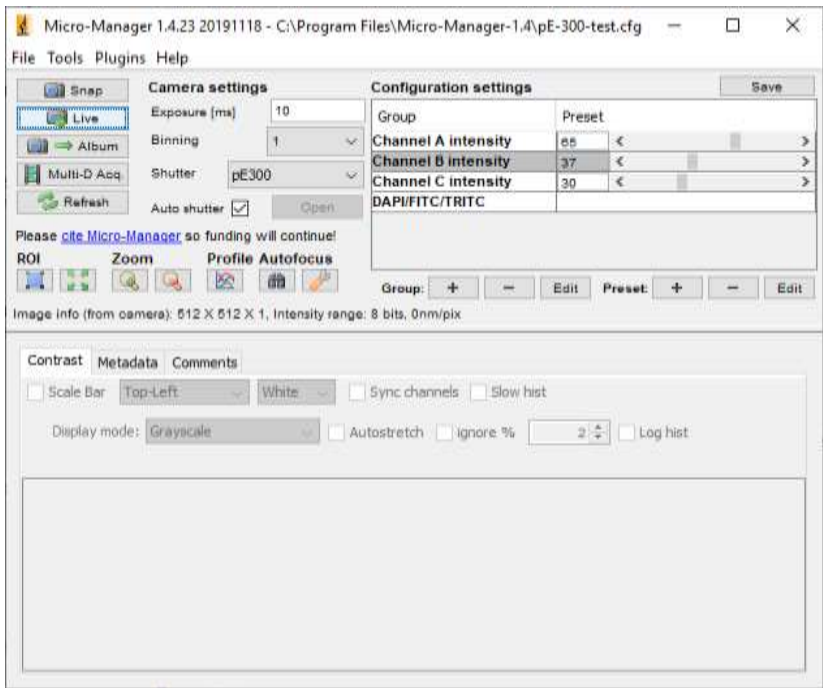


Figure 38: Live Intensity Variation examples



## 6 Appendix

### 6.1 LED Labels

When configuring  $\mu$ Manager for multi-channel LED Illumination Systems, each LED corresponds to its label shown under the Property Name columns. Please also refer to the following tables:

pE-400 <sup>max</sup>	
Property Name	LED (nm)
pE-400-ChannelA	635
pE-400-ChannelB	365/400
pE-400-ChannelC	450
pE-400-ChannelD	550

pE-4000	
Property Name	LED (nm)
pE-4000-ChannelA	365, 385, 405 or 435
pE-4000-ChannelB	460, 470, 490 or 500
pE-4000-ChannelC	525, 550, 580 or 595
pE-4000-ChannelD	635, 660, 740 or 770

pE-800	
Property Name	LED (nm)
ChannelA	365/400
ChannelB	435
ChannelC	460
ChannelD	500
ChannelE	740
ChannelF	635
ChannelG	580
ChannelH	550

pE-800 <sup>fura</sup>	
Property Name	LED (nm)
ChannelA	500
ChannelB	550
ChannelC	580
ChannelD	635
ChannelE	470
ChannelF	435
ChannelG	380
ChannelH	340

pE-300 Series	
Property Name	LED (nm)
pE-300-ChannelA	365/400
pE-300-ChannelB	450
pE-300-ChannelC	550

pE-340 <sup>fura</sup>	
Property Name	LED (nm)
pE-300-ChannelA	340
pE-300-ChannelB	380
pE-300-ChannelC	white