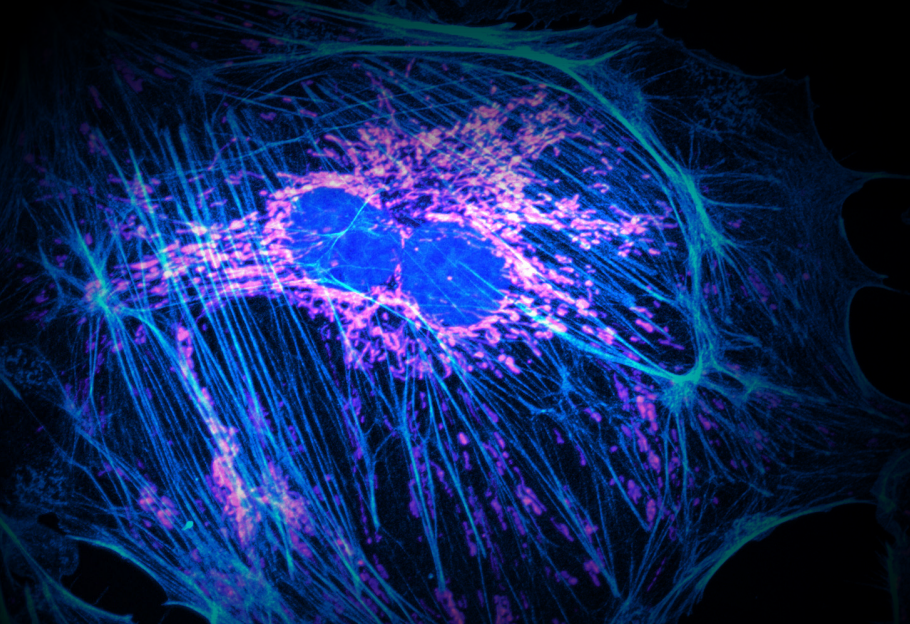
application note

pco.µManager X-Cite integration



µManager

EXCELITAS TECHNOLOGIES®

pco.
An Excelitas Technologies Brand

Excelitas PCO GmbH asks you to carefully read and follow the instructions in this document.
For any questions or comments, please feel free to contact us at any time.

pco.
An Excelitas Technologies Brand

telephone:	+49 (0) 9441 2005 50
fax:	+49 (0) 9441 2005 20
postal address:	Excelitas PCO GmbH Donaupark 11 93309 Kelheim, Germany
email:	pco@excelitas.com
web:	www.excelitas.com/pco

pco.µManager X-Cite integration application note 1.0.2

Released July 2024

©Copyright Excelitas PCO GmbH

This work is licensed under the Creative Commons Attribution-NoDerivatives 4.0 International License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nd/4.0/ or send a letter to Creative Commons, PO Box 1866, Mountain View, CA 94042, USA.

Contents

1 Introduction **4**
1.1 Prerequisites 4
1.2 Install and verify the operation of the X-Cite light source 4
1.3 Install µManager and components 4

2 Setting up a Multi-Dimensional acquisition **5**
2.1 Specify the group properties 6
2.2 Select the LED's to be used and the camera exposure 7
2.3 Create presets 8
2.4 Define the Multi-Dimensional imaging parameters 10
2.5 Acquire an image sequence 12

1 Introduction

Here we will be setting up an experiment in µManager, using a PCO camera and X-Cite® light source. In this example, the camera is a pco.panda 4.2, and the light source is the X-Cite NOVEM XT940. The configuration can be adapted to many PCO camera models and X-Cite sources.

1.1 Prerequisites

Install and verify the operation of the pco.panda 4.2

Before setting up the experiment, make sure the pco.panda 4.2 is connected and verify the camera operation using pco.camware. The driver for the pco.panda 4.2 and the latest version of pco.camware are available on our homepage.

1.2 Install and verify the operation of the X-Cite light source

Likewise, verify the operation of the X-Cite light source using the X-Cite control panel. The X-Cite illuminator will require a USB connection. The drivers and software are available on https://www.excelitas.com/product-category/x-cite-fluorescence-led-illuminators. Select your model from the ones listed on the web site to access the appropriate driver and the X-Cite control panel.

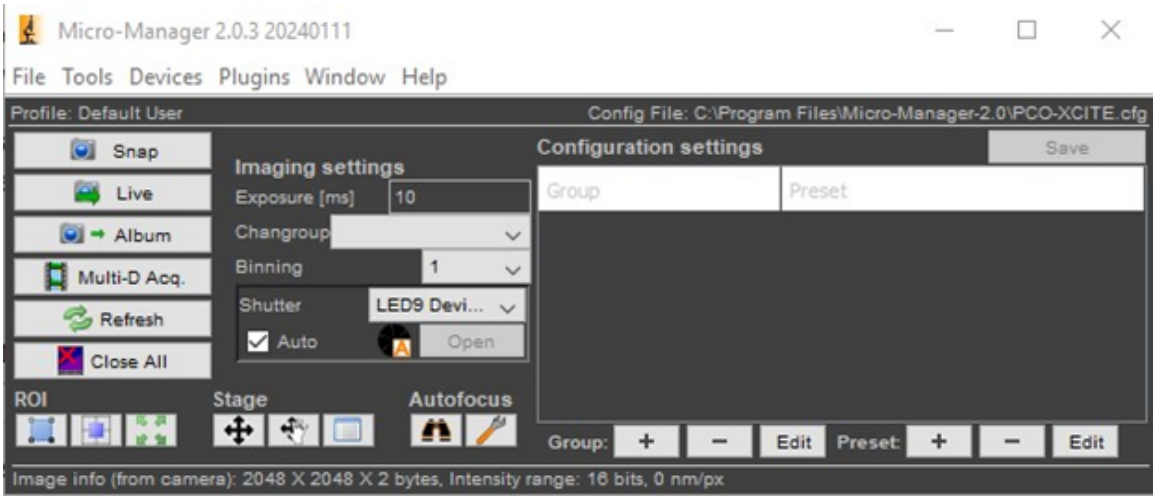
1.3 Install µManager and components

µManager is available from https://micro-manager.org/. Download and install the release version, then follow the steps in our pco.micromanager manual to setup the pco.panda in µManager. We also have a video that documents how to connect the X-Cite Novem to µManager.

2 Setting up a Multi-Dimensional acquisition

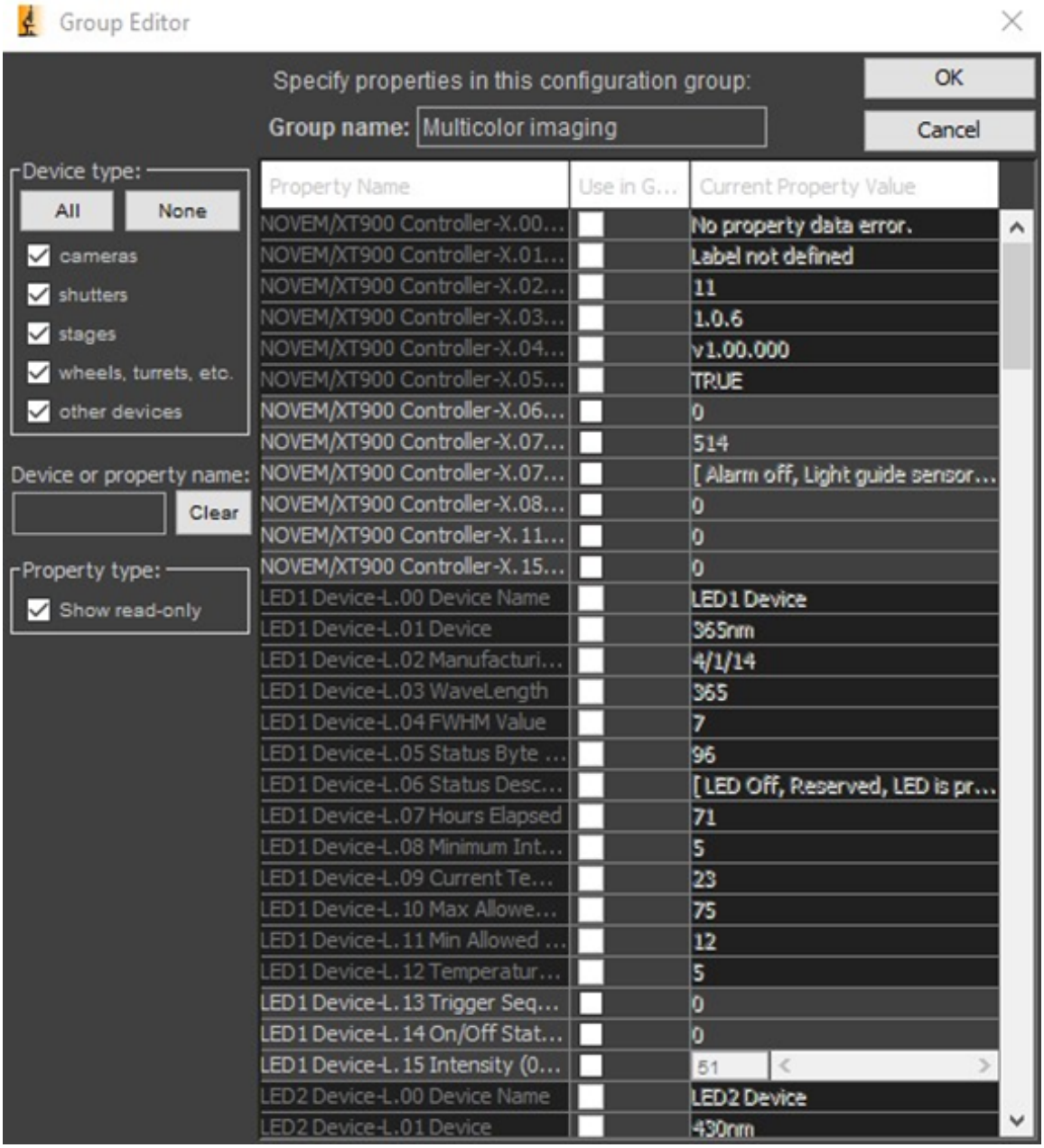
Create a new group

From the main µManager window, locate the Configuration settings. We will create a new group with our camera and illumination settings for the experiment.



Press the “+” sign beside “Group:” to create a new group. This will bring up the “Group editor” window.

2.1 Specify the group properties

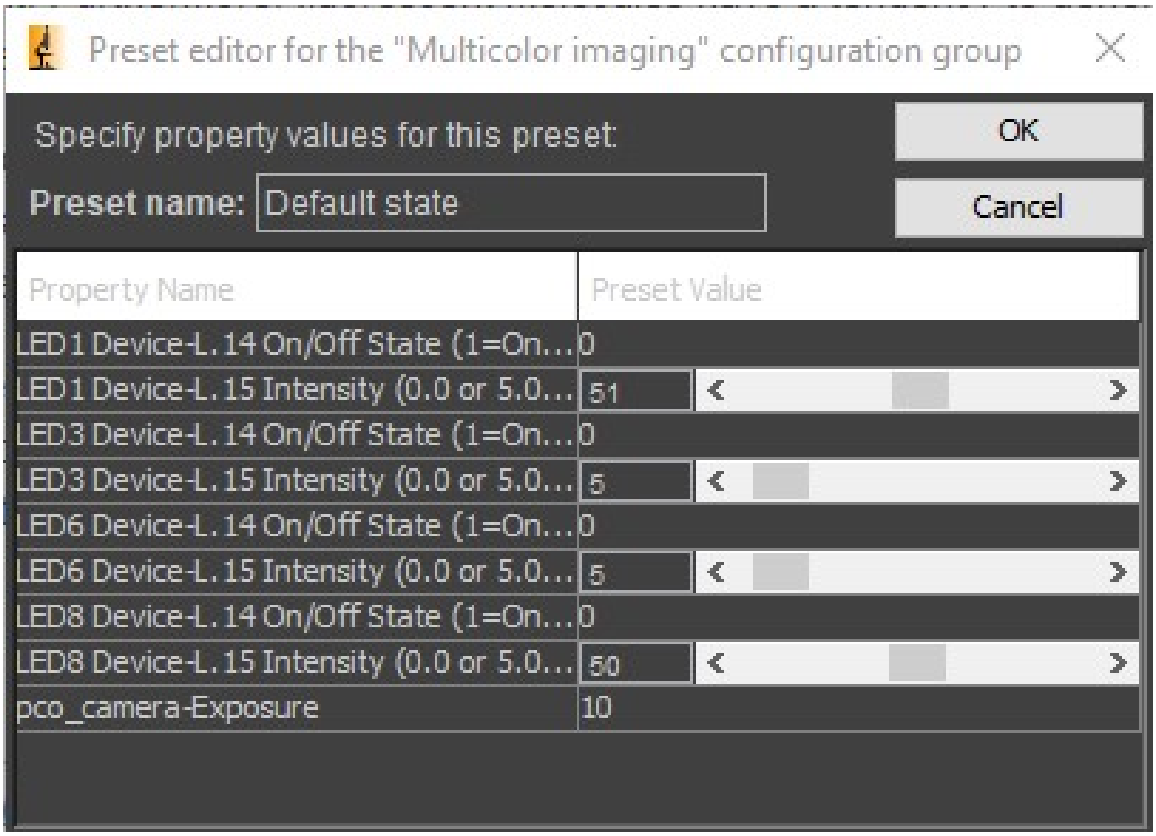


The group editor displays the properties of the devices installed by the Hardware configuration wizard. This group has been given the name “Multicolor imaging”. Now, select the devices to be used in this experiment. We want to use LED1, LED3, LED6, LED8 and the PCO camera. For each LED, we want to control the on/off state and the power level, so these properties are selected. Likewise, the camera exposure time can differ from image to image, so that property is also selected.

2.2 Select the LED's to be used and the camera exposure

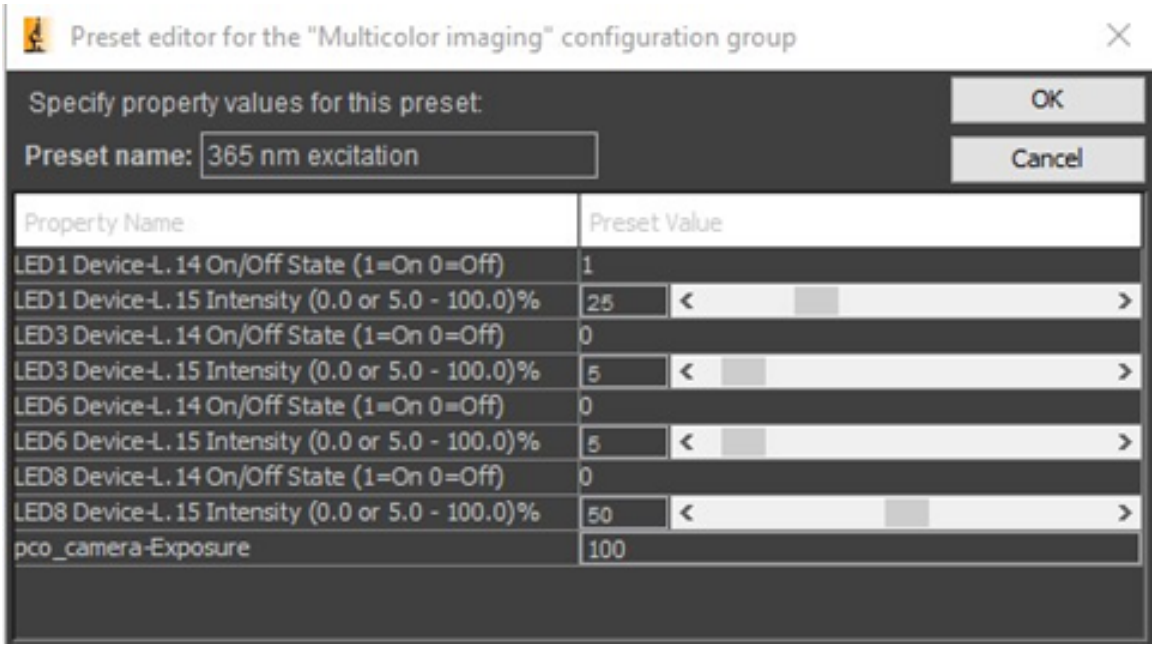
Property Name	Use in Group?	Current Property Value
NOVEM/XT900 Controller-X.11 Front Panel (1=L...	☐	0
NOVEM/XT900 Controller-X.15 Clear Alarm (1:Cl...	☐	0
LED1 Device-L.13 Trigger Sequence	☐	0
LED1 Device-L.14 On/Off State (1=On 0=Off)	☑	0
LED1 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☑	51
LED2 Device-L.13 Trigger Sequence	☐	0
LED2 Device-L.14 On/Off State (1=On 0=Off)	☐	0
LED2 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☐	29
LED3 Device-L.13 Trigger Sequence	☐	0
LED3 Device-L.14 On/Off State (1=On 0=Off)	☑	0
LED3 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☑	5
LED4 Device-L.13 Trigger Sequence	☐	0
LED4 Device-L.14 On/Off State (1=On 0=Off)	☐	0
LED4 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☐	5
LED5 Device-L.13 Trigger Sequence	☐	0
LED5 Device-L.14 On/Off State (1=On 0=Off)	☐	0
LED5 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☐	5
LED6 Device-L.13 Trigger Sequence	☐	0
LED6 Device-L.14 On/Off State (1=On 0=Off)	☑	0
LED6 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☑	5
LED7 Device-L.13 Trigger Sequence	☐	0
LED7 Device-L.14 On/Off State (1=On 0=Off)	☐	0
LED7 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☐	5
LED8 Device-L.13 Trigger Sequence	☐	0
LED8 Device-L.14 On/Off State (1=On 0=Off)	☑	0
LED8 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☑	50
LED9 Device-L.13 Trigger Sequence	☐	0
LED9 Device-L.14 On/Off State (1=On 0=Off)	☐	0
LED9 Device-L.15 Intensity (0.0 or 5.0 - 100.0)%	☐	100
pco_camera-Acquiremode	☐	Internal
pco_camera-Binning	☐	1
pco_camera-Exposure	☑	10

On exiting the Group Editor, a preset will be created for the elements of the group. Here, we have the default state with all LED's off and the camera exposure at 10 ms.

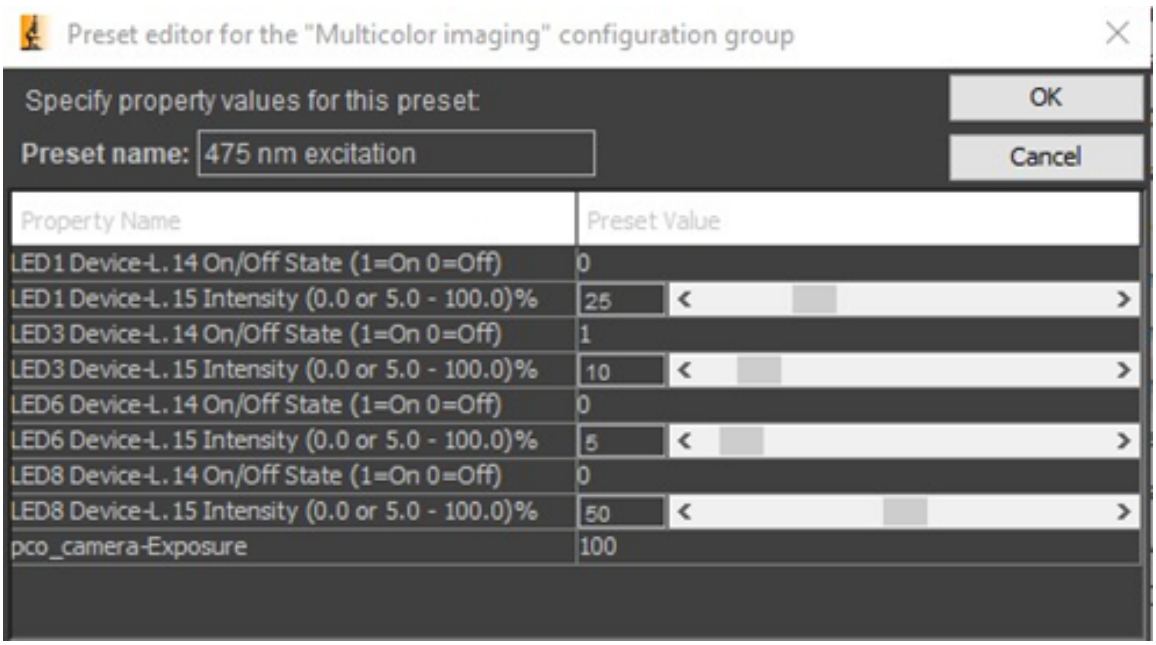


2.3 Create presets

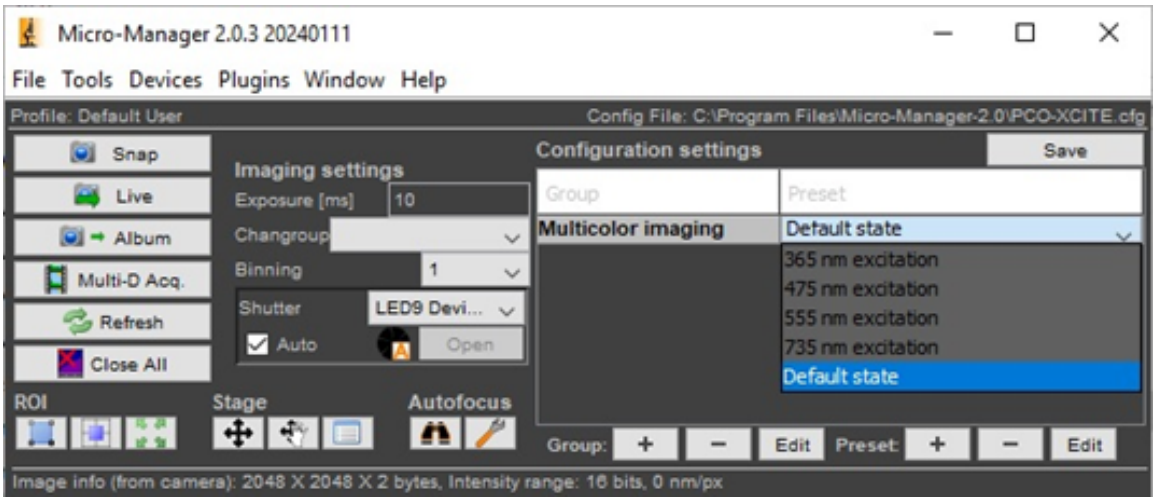
"OK" brings us back to the main menu, which now displays the "Multicolor imaging" in the "Configuration settings". Now we can add specific presets for the stages of our experiment. Press "+" beside "Presets" to launch the preset editor. For the first image in the experiment, we will illuminate at 365 nm with LED1 at 25% power, and image for 100 ms. The other LED's in the group remain off.



For the second image, we will illuminate at 475 nm with LED2 at 10% power and expose the camera for 100 ms. Select the “Multicolor imaging“ group by clicking on this in the “Configuration settings”, then press the “+” beside “Presets to create a preset for this setup.

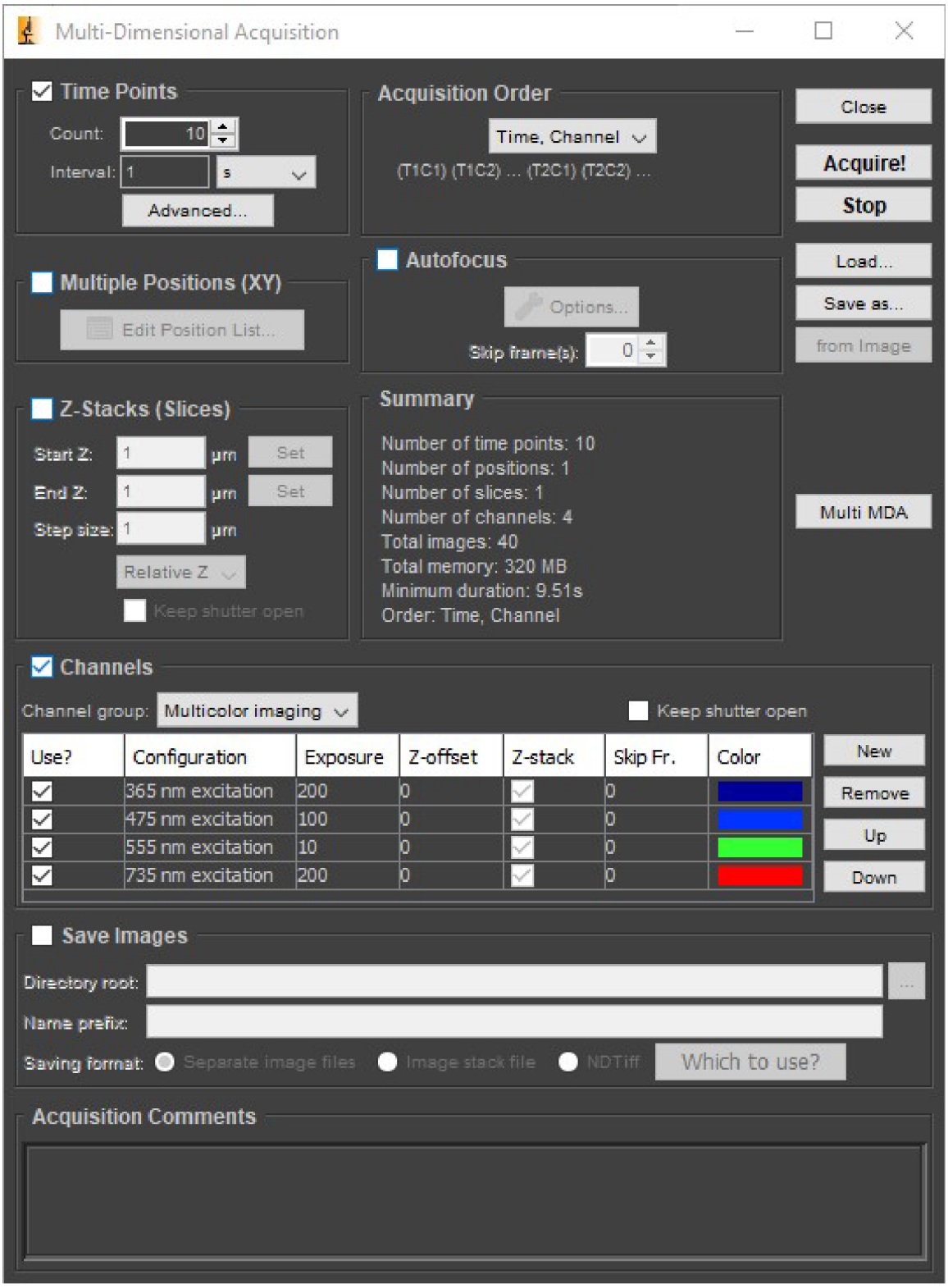


Repeat the process for LED6 and LED8. You can then see a list of presets by clicking on the selection list in the “Configuration settings”.



2.4 Define the Multi-Dimensional imaging parameters

To use these presets, start a multi-dimension imaging sequence by pressing the “Multi-D Acq.” button. We will cycle through the LED’s 10 times, with each sequence separated by 1 s. Select “Time points” and “Channels”. For “Time points”, enter 10 as the count and 1 s as the interval. Under “Channels”, select “Multicolor imaging” as the channel group, then click the “New” button to add a preset. Click “New” until all the presets are added. The color can be adjusted to match the expected emission color that will be imaged, and the time of each exposure can also be adjusted. The final setup will look something like this:


Multi-Dimensional Acquisition
Time Points
Count: 10
Interval: 1 s
Advanced...
Acquisition Order
Time, Channel
(T1C1) (T1C2) ... (T2C1) (T2C2) ...
Close
Acquire!
Stop
Load...
Save as...
from Image
Multiple Positions (XY)
Edit Position List...
Autofocus
Options...
Skip frame(s): 0
Z-Stacks (Slices)
Start Z: 1 µm Set
End Z: 1 µm Set
Step size: 1 µm
Relative Z
Keep shutter open
Summary
Number of time points: 10
Number of positions: 1
Number of slices: 1
Number of channels: 4
Total images: 40
Total memory: 320 MB
Minimum duration: 9.51s
Order: Time, Channel
Multi MDA
Channels
Channel group: Multicolor imaging
Keep shutter open
Use? Configuration Exposure Z-offset Z-stack Skip Fr. Color
365 nm excitation 200 0 0
475 nm excitation 100 0 0
555 nm excitation 10 0 0
735 nm excitation 200 0 0
New
Remove
Up
Down
Save Images
Directory root:
Name prefix:
Saving format: Separate image files Image stack file NDTiff
Which to use?
Acquisition Comments

2.5 Acquire an image sequence

The “Acquire” button will start the sequence. µManager will step through each selected LED, acquiring and displaying an image in the chosen color. This channel sequence will repeat after a 1 second delay, until all images are captured. For more information on PCO cameras and X-Cite illuminators, visit www.excelitas.com.

pco.

An Excelitas Technologies Brand

postal address:	Excelitas PCO GmbH Donaupark 11 93309 Kelheim, Germany
telephone:	+49 (0) 9441 2005 0
e-mail:	pco@excelitas.com
web:	www.excelitas.com/pco

EXCELITAS TECHNOLOGIES®