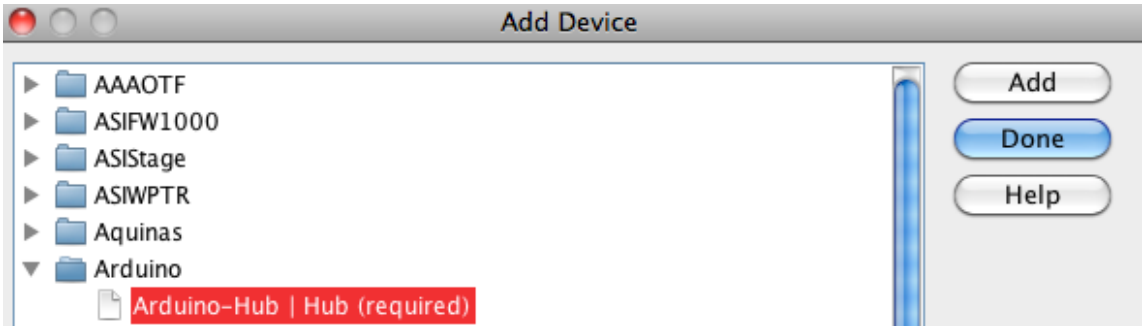
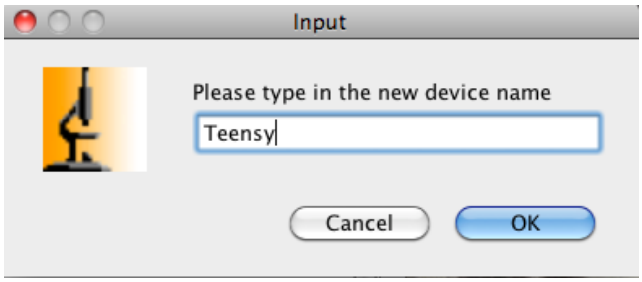
Setting up Micro-manager - additional information

1. After building the controller connect it via USB to your computer that has Micro manager installed

2. Flash the Teensy with the provided script (Check PJRC.com for further instructions)
    a. Version 5 is with physical resistors – depicted in **Figure 1B**
    b. Version 6 utilized the internal Arduino-Teensy pull-up resistors via code

3. Install Micro-manager
4. Start-up Micro-Manager
5. When prompted at start-up to select a hardware configuration choose “Create new configuration”. Alternatively, if Micro-manager is already running open the *Hardware configuration wizard* and “Create new configuration”.
6. Click Next and select Arduino hub and click done.



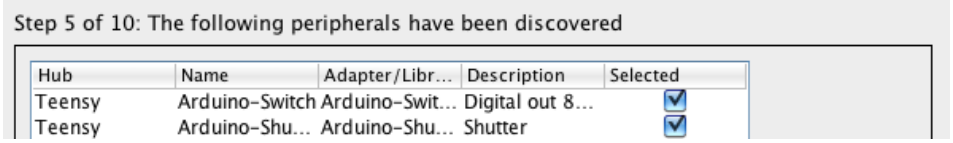


7. Add a camera that is attached to the system – and name it accordingly.

8. Click on done and then select the “Arduino“ and “scan ports”. (Step 3/10 of configuration wizard) a number of values will be returned (Step 4/10).

9. Click Next. If properly connected properties of the Arduino will show up.

10. Activate the **Arduino Switch state** and **Arduino Shutter** by ticking the box.



11. Step 6-8 click next.

12. Step 9 of 10 you can define names for the Arduino Switch states.

Switch states:

Switch State	Function	Proposed name
0	µManager off	External
1	Control LED 1 with µManager – *Continuous light*	Bright
2	Control LED 2 with µManager – *Continuous light*	Fluorescence
3	*Pulsed* LED 1 control LED 2 with foot switch	Bright - Foot Fluo
4	Alternate LED1 and LED 2 at 10 Hz, activate LED 2 with foot switch[I]	Dual overlay (10 Hz[I])
5	Alternate LED1 and LED 2 at 20 Hz, activate LED 2 with foot switch[I]	Dual overlay (20 Hz[I])

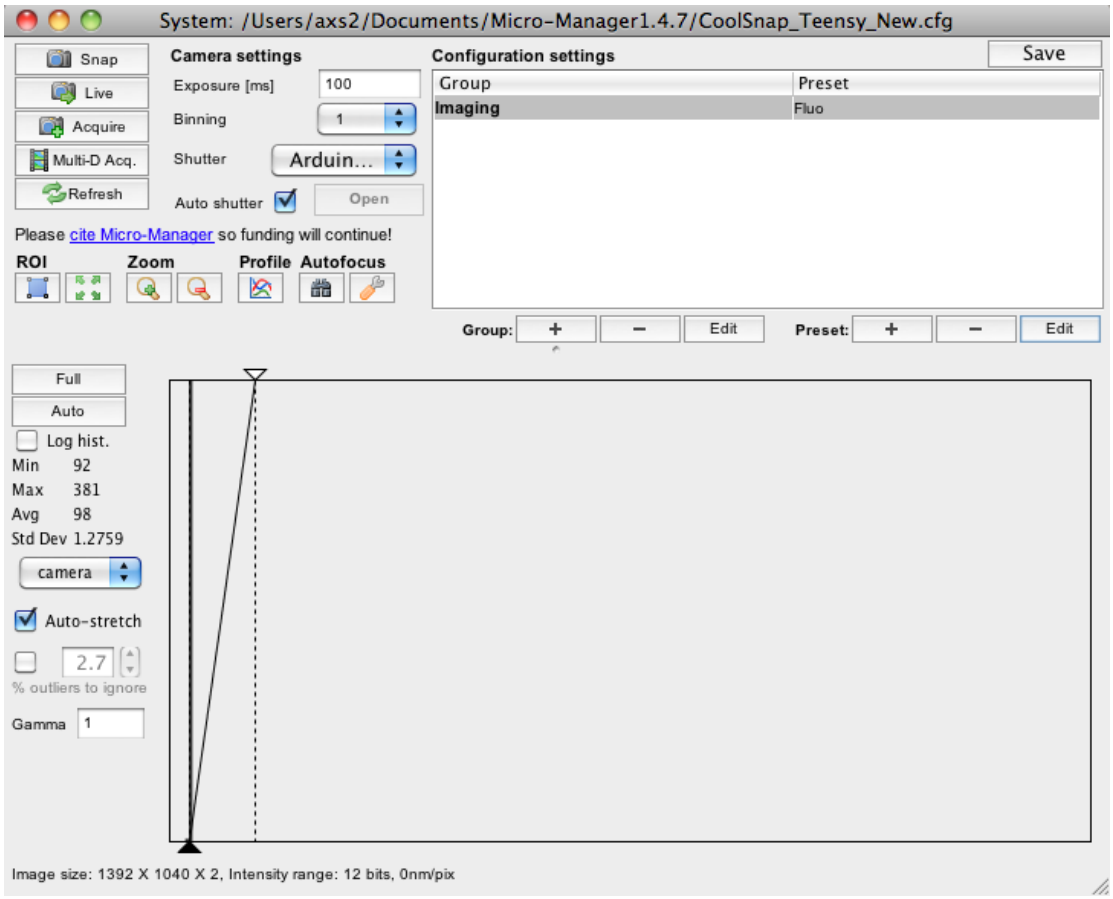
Notes:

I. State 4 and 5 require an additional bean shell script to be executed for properly assigning look up tables to the two channels.

II. Other switch states are not assigned.

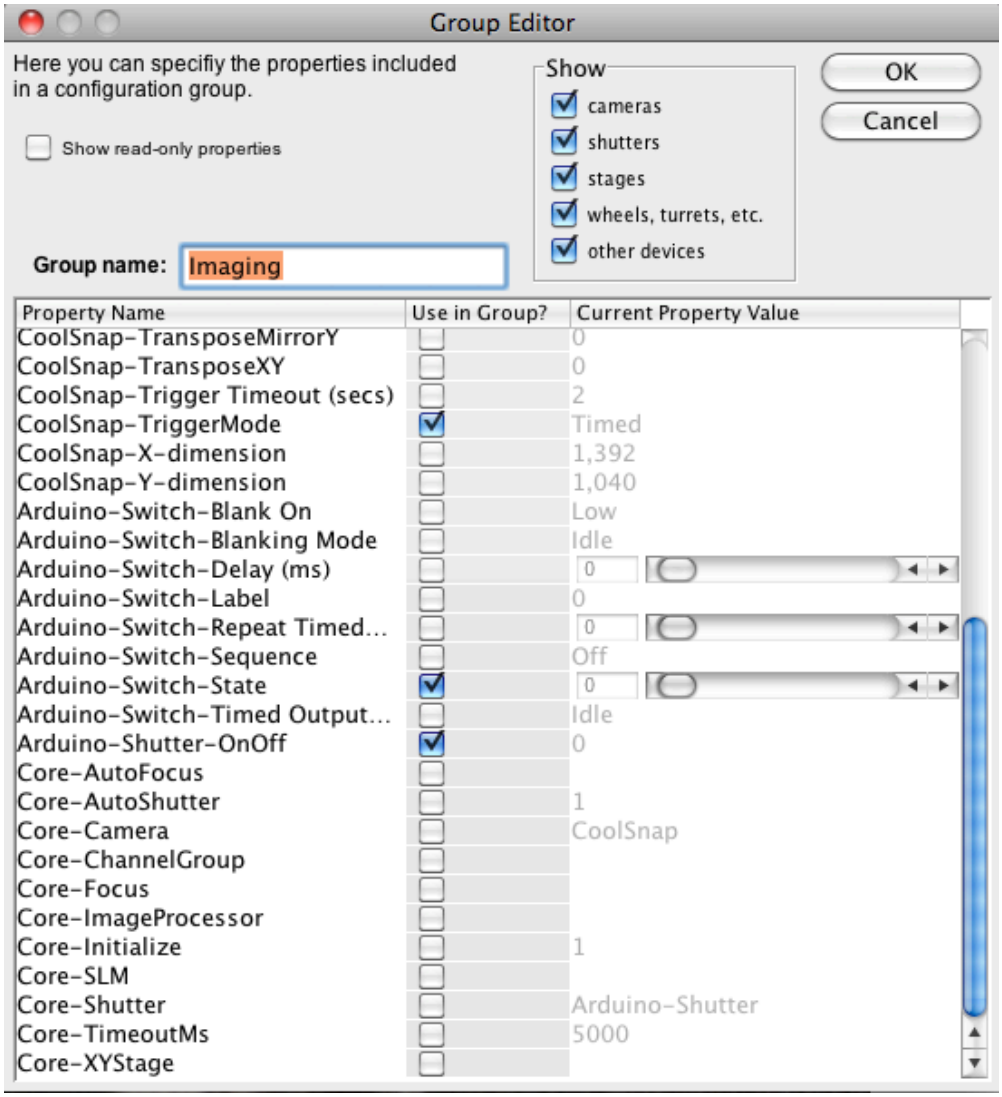
13. Step 10 of 10: Save the configuration file and click finish.

14. In the Micromanager window add a new group by clicking on the plus button - here called **Imaging.**

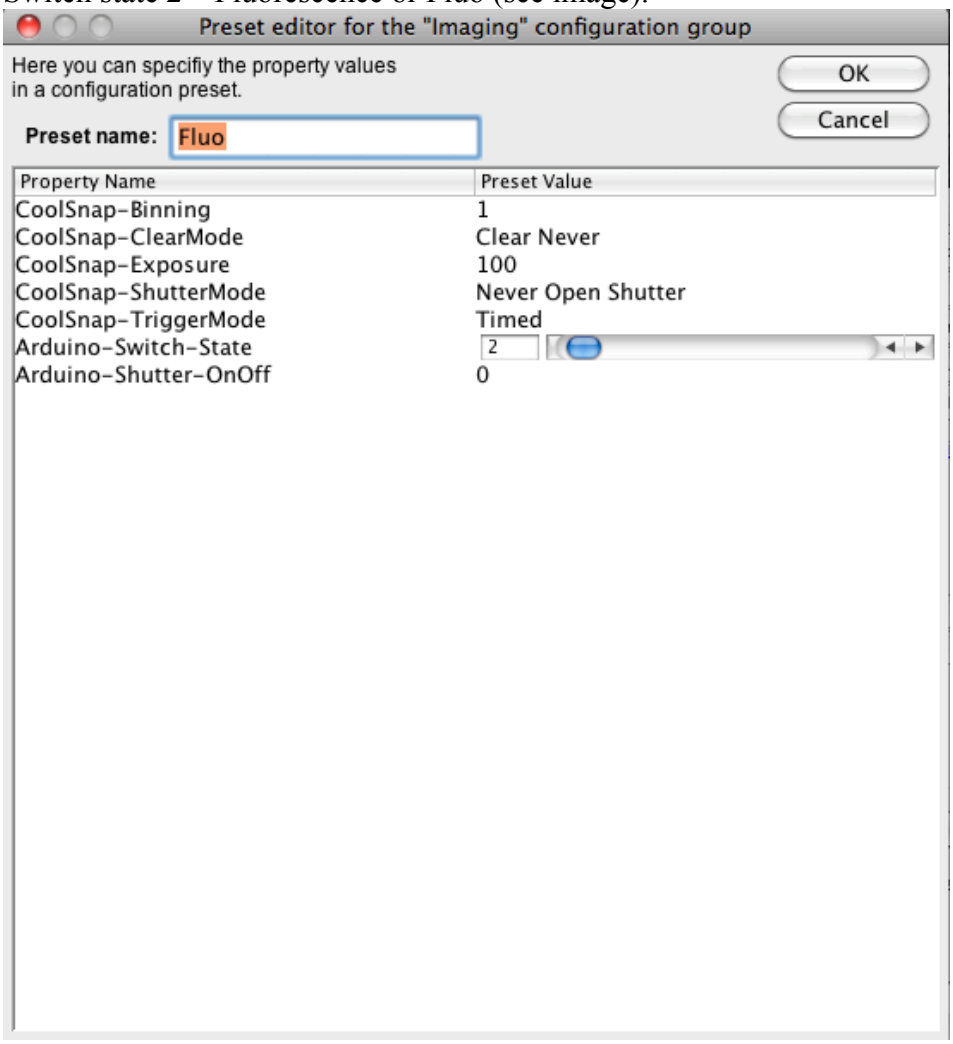


15. In the **Imaging** Group all settings are pre-defined that will be displayed in the Preset window. For other cameras than the Photometrics CoolSnap, Exposure, trigger and binning mode might be sufficient.
    Activate Camera Properties (here CoolSnap):
    a. Binning
    b. Clear Mode (needed for the PVcam/Photometrics cameras).
    c. Exposure
    d. Trigger Mode

    Activate Arduino Properties:
    e. Switch State and
    f. Arduino Shutter



16. Define the Pre-sets for a quick selection of Switch States – here exemplified for Switch state 2 – Fluorescence or Fluo (see image).



Running the Bean Shell script

- Switch State 4 and 5 require the execution of a bean shell script.
- Load the script for 10 or 20 Hz in the bean shell environment of Micro-manager and execute before starting “Live” in the Micro-manager control window.

References

Bosse, J. B., Tanneti, N. S., Hogue, I. B., and Enquist, L. W. (2015). Open LED Illuminator: A Simple and Inexpensive LED Illuminator for Fast Multicolor Particle Tracking in Neurons. PLoS ONE 10, e0143547. doi:10.1371/journal.pone.0143547.s008.