doric

Patch Cord Photobleaching

Application Note

Version 1.0.0

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Introduction

In tethered fiber photometry systems, the optical fiber patch cord (sample as well as rotary joint patch cords) can increase the fluorescence signal baseline because of its intrinsic fluorescence property, also called auto-fluorescence. This effect is particularly pronounced in optical fibers that use polymer cladding to achieve higher numerical apertures (e.g., NA 0.48 or NA 0.57). (Note: Patch cords with lower NA (e.g., NA 0.37, 0.22) typically use silica cladding, which naturally exhibits low auto-fluorescence and are not discussed here.)

Such auto-fluorescence is stronger in the green spectrum and can interfere with photometry signals recorded in the green range, such as GCaMP-based recordings, by distorting the true photometry signal, as shown in figure 1.1.

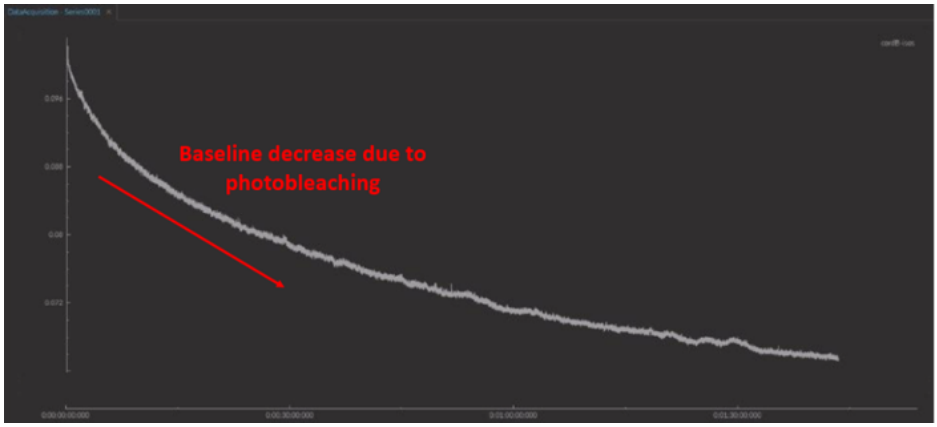

Figure 1.1: *Example of photometry signal with strong photobleaching effect*

To minimize this interference and reduce auto-fluorescence, it is important to photobleach the patch cords using strong blue and UV light prior to recordings. While yellow light can also be used, it has minimal effect on auto-fluorescence and is not recommended as a primary method. As light passes through the patch cord, it naturally reduces auto-fluorescence over time, helping to stabilize the baseline signal in photometry recordings.

Notes

- All LEDs (blue, UV, etc.) can be **ON simultaneously** for bleaching, there is no need to bleach each wavelength separately.
- Always place the **output end of the patch cord in a black box** to safely dissipate the light. High-intensity blue light can be harmful.

As mentioned above, for effective photobleaching, strong blue and UV light should be used. The light can be delivered in continuous wave (CW), sine wave, or TTL pulse mode. However, CW mode is generally more effective due to its higher light output compared to sine wave or TTL pulse mode.

The time required for photobleaching depends significantly on the light power used, as well as the patch cord's core diameter and numerical aperture (NA). Since CW mode light can produce varying maximum power levels depending

on the system, and both system configurations and patch cord specifications may differ between clients, we strongly encourage users to measure autofluorescence and determine the appropriate bleaching duration for their specific setup.

Common approaches include bleaching for approximately 2 hours using high blue light power (in the *mW* range), which typically reduces autofluorescence by ∼75% (see Fig. 1.2), or overnight using lower power (in the *µW* range).

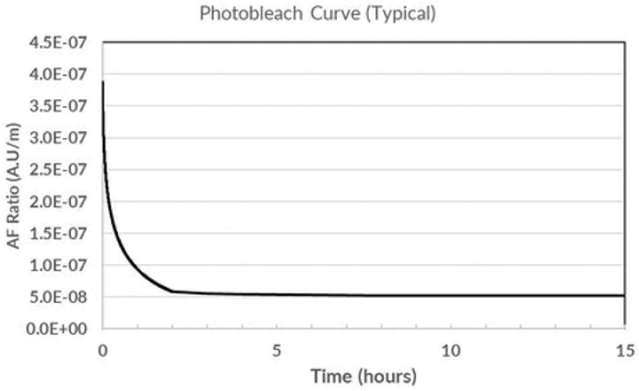

Figure 1.2: *Bleaching Curve*

Please note that the photobleaching effect is not permanent, auto-fluorescence will gradually return over time. Our in-house testing shows that a fully bleached patch cord can regain approximately 25% (for a *400-0.57* patch cord, see Fig. 1.3, top) to 50% (for a *200-0.57* patch cord, see Fig. 1.3, bottom) of its initial auto-fluorescence level after 30 days.

Therefore, we recommend that customers regularly monitor auto-fluorescence levels and define a threshold at which photobleaching should be repeated. Many clients choose to bleach their patch cords **before each recording session** or on a **weekly basis** to minimize autofluorescence effects.

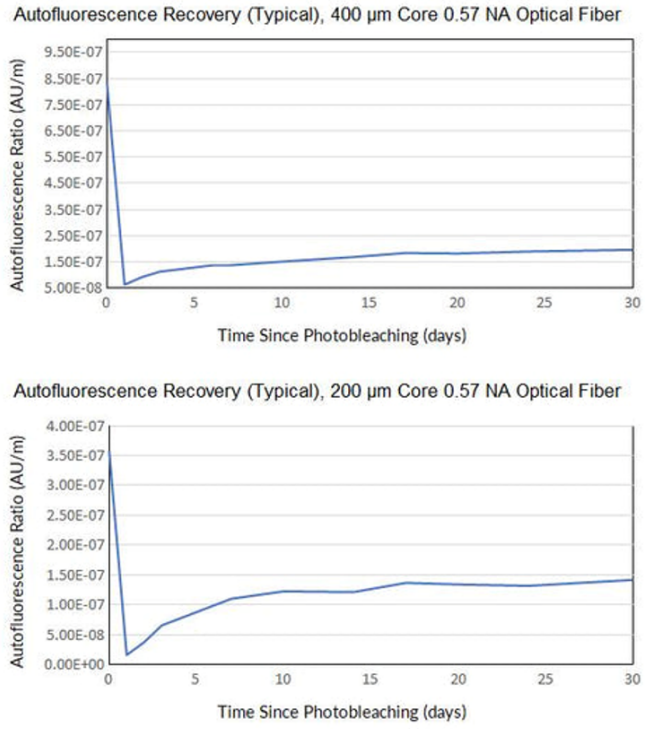

Figure 1.3: *Bleaching Recovery*

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Setting-up a configuration

Below are instructions to create a configuration file specifically for photobleaching optical patch cords using light in CW mode, tailored to each type of photometry system. If you're unsure which photometry system you're using, visit this link to view the comparison table of different photometry systems.

If you need assistance or want ready-made configuration files, feel free to contact us at: sales@doriclenses.com

2.1 FMC Gen3 and Bundle photometry systems

If your photometry system is a **FMC Gen3** with an internal LED driver, or you are using a **Bundle photometry system (BFMC, BFTO, and RBFMC)** with the **BBC300/NC500 console**, use the instructions below to configure the console (see figure 2.1):

1. Click the Configuration tab.
2. Click Add Channel.
3. Select Analog Out.
4. Select the channel number corresponding to the blue LED, and set it to Continuous Wave mode.
5. Set the voltage to the maximum: 4.75 V.
6. Click Add Channel again.
7. Repeat the same steps for the UV LED channel.
8. Click Save Configuration.

2.2 FMC Gen1 and Gen2 systems

If your photometry system is a **FMC Gen1 or Gen 2**, or a **Rotary photometry system (RFMC)**, and the LED driver is external, use the instructions below to make a configuration for the LED driver (note that you do not need to make a configuration in the console module) (see figure 2.2):

1. Click the Configuration tab.
2. Click Add Channel.
3. Select LED.
4. Select the channel number corresponding to the blue LED, and set it to "Continuous Wave" mode.
5. Click Add.
6. Repeat the same steps for the UV LED channel and save the configuration.

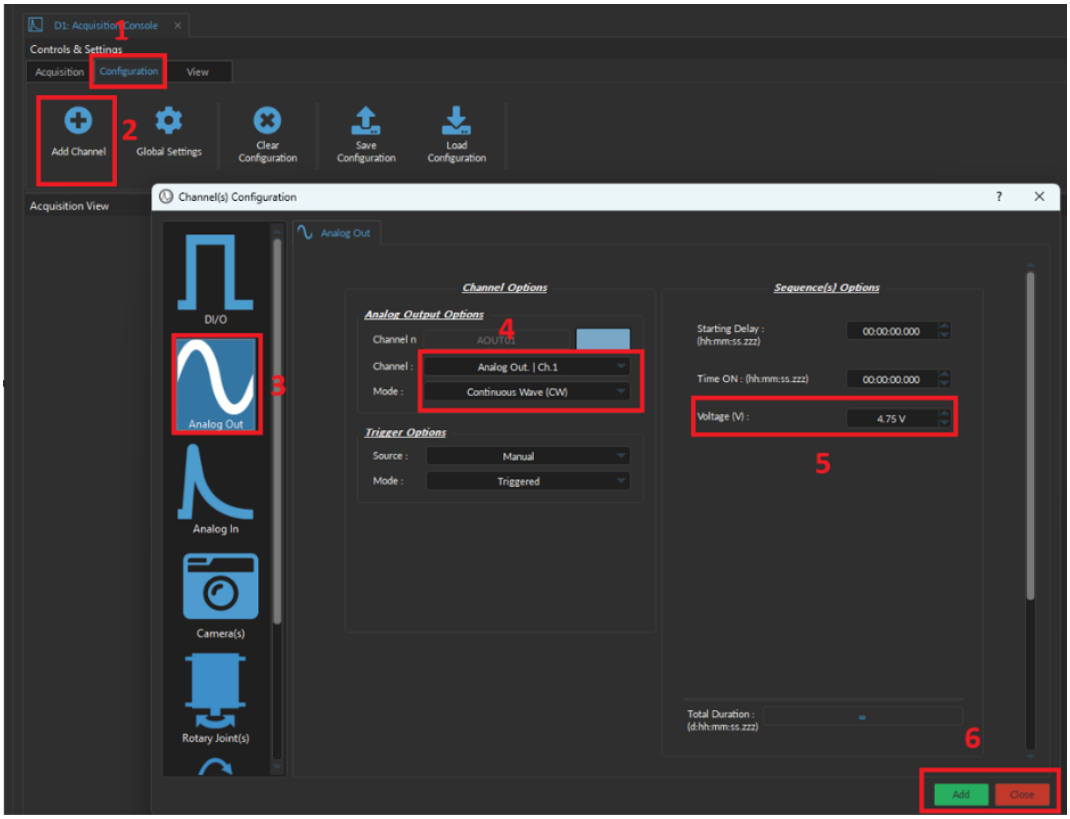

Figure 2.1: *Console Configuration*

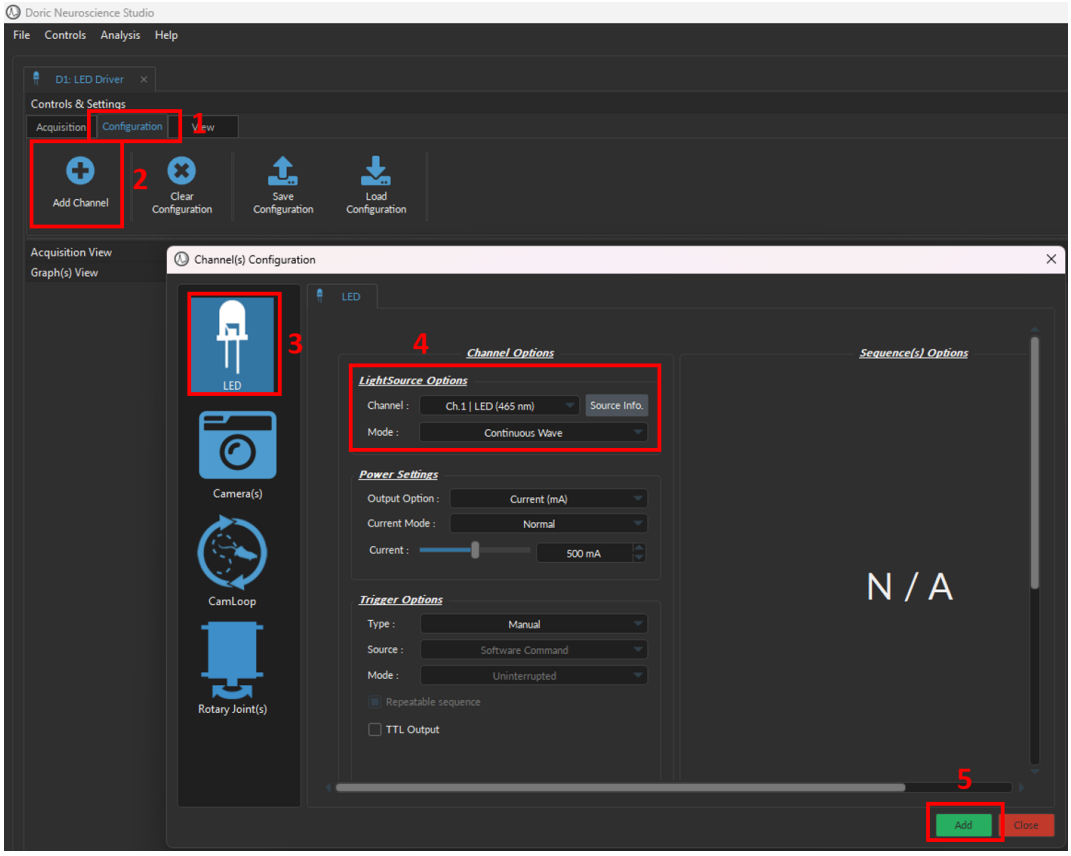

Figure 2.2: *LED Driver configuration*

*** If the LED is external, the patch cord and rotary joint can be connected directly to the LED for improved efficiency (see figure 2.3).

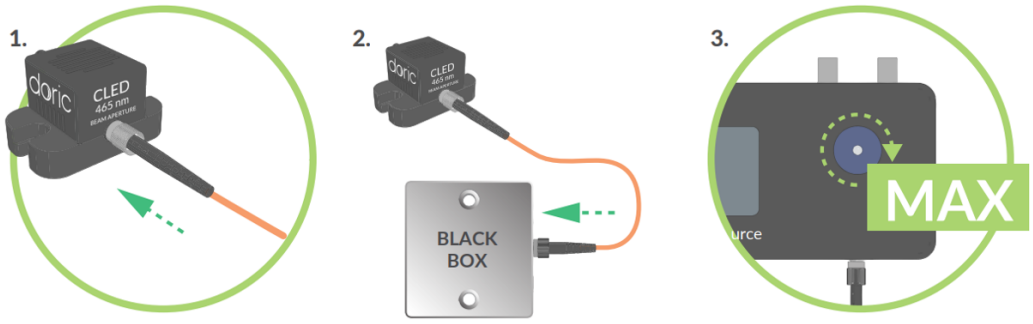

Figure 2.3: *Optical patch cords can be connected directly to the LED for a more efficient photobleaching*