A Low-cost Flexible Multispectral Light Engine and Controller for Visual Stimulation and Optogenetics

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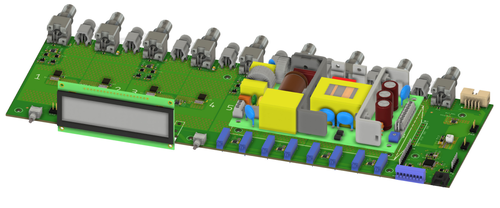
Abstract

We have developed a multispectral light engine and controller for visual stimulations that can also be used for flexible optogenetic activation. The system is designed to precisely control up to eight high power light sources for full field projection, or patterned projection through a digital micromirror device. The open-source controller can independently drive each of the eight LED outputs up to 1.8 A each, with milliamp precision and little ripple, throughout the entire current range, and can be modulated through external analog signals. While many conventional solutions for presenting visual stimulations during *in vivo* imaging experiments rely on optical filters, tedious screening, or both, they generally offer only partial solutions, with artifacts appearing in the recorded movies. The controller is designed to interface with two-photon microscopes to allow for flexible strobing only during line-reversals or flyback to minimize interference in the functional imaging measurements, which is accomplished by a synchronous timing scheme with microsecond accuracy. Critically, the controller contains processing circuits that detect and automatically synchronize to the microscope's scanner timings to simplify incorporation into many imaging workflows. Given its flexible design, combined with our custom stimulus design package, this engine allows visual neuroscientists to span a large range of most organism's color gamut, without sacrificing imaging quality. In addition, the same methodology can be implemented during all optical experiments.

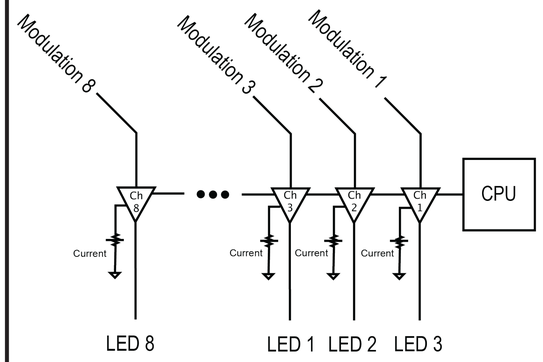
Introduction

Separation of fluorescence responses from optical stimulation during 2-photon imaging is critical to the capture of high fidelity, low-noise neural data. Here, we present an 8-channel LED driver tailored for optogenetics and visual stimulation that can also work as a general-purpose controller. The blanking features of our design minimize crosstalk between the LED illumination and the fluorescence of indicators such as GCaMP. Additionally the fast and high resolution constant current drivers provide accurate, linear LED illuminations.



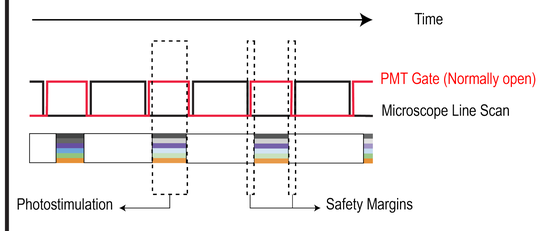
Technical Overview

Each LED has a constant current setting and a separate fast modulation input that sets the current from 0 to the maximum setting for each channel using analog voltages while maintaining frequency responses over 1 MHz. The CPU is a relatively simple STM32 (Cortex-M4F) processor. Yet, it is inexpensive and runs up to the 125MIPS range. The drivers are composed of a simple current comparator circuit, controlling a high power MOSFET. Driver modulation is processed by a 10MHz op-amp that is shut down by a separate FET switch when blanking is required.

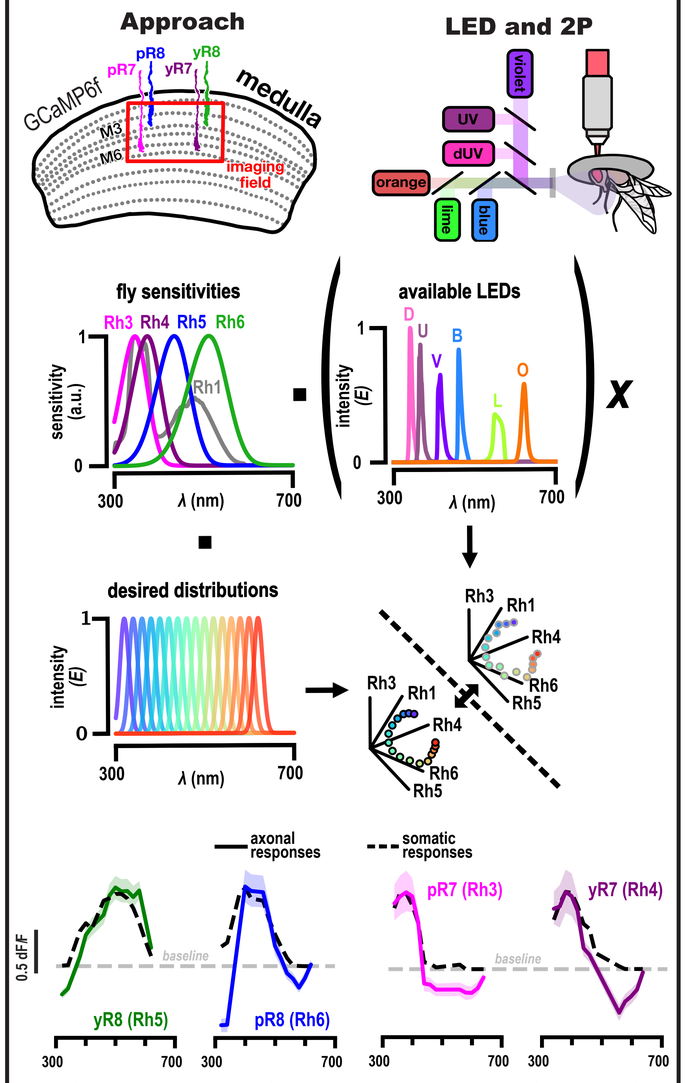


Blanking

When provided with a blanking signal, all channels can be turned off. As an example, a synchronous bus that is processed out of the horizontal galvanometer synchronization can be used to blank the LEDs during the retrace periods of the galvo mirrors. Our design is fast enough to analyze the horizontal scanning waveform and generate a blanking waveform to shut down the LEDs a few uS before the scanning starts and restore illumination a few uS after scanning ends to minimize light bleedthrough, within microsecond timescales that can be defined by the user. Additionally a signal is generated to gate the microscope detector if such functionality is available. Blanking times can be easily modified by switches on the front panel or using parameters within the code.

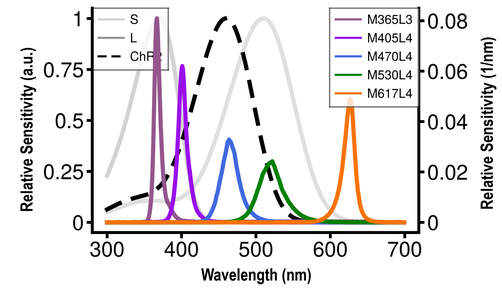


Single wavelength reconstruction for the fruit fly using six LEDs

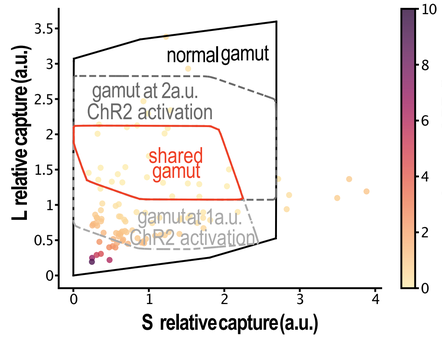


Using the blanking ability of the light engine, we can simultaneously stimulate the fly eye using a combination of LEDs and record the activity of neurons *in vivo* using a two photon microscope. Using our hardware and animal agnostic computational package, dreye[1], we have measured the responses of photoreceptor axons to LED mixtures that span the single wavelength manifold in the fly color space. Thanks to the LED blanking, the signal-to-noise ratio of our imaging movies increased more than 30-fold, and were able to measure clear opponent responses in single axon terminals[2].

Other uses of the stimulator in conjunction with the dreye package: Optogenetic and visual stimulation of the mouse retina



LS color space



Our light engine can also be used to excite photoreceptors, while isolating the contribution of ChR2-expressing neurons. We can find planes in the L and S cone mouse color space that correspond to different activations of the ChR2. In this conceptual example, we use five LEDs to reconstruct a large gamut of mouse colors, while being able to switch between two different magnitudes of ChR2 activation. This technique can also be used with other optogenetic tools whose sensitivities overlap heavily with an animal's photoreceptor sensitivities.

Resources

Dreye App | Color Geometry Preprint | Dreye Docs | LSD Docs

1. Christenson, et al. 2022, BioRxiv
2. Heath, Christenson, et al. 2020, Current Biology