University of Colorado
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Optogenetics and Neural Engineering

A proposed methods section that could help ensure that a researcher is thinking about their optogenetic stimulation parameters in adequate depth:

Optogenetic light activation was achieved with a ***wavelength*** ***laser company (ex: SLOC)*** laser delivering collimated light through a ***core diameter***, ***numerical aperture*** patch cord connected through a sleeve ***ex: Precision Fiber Products SM-CS125S*** with index matching solution ***ex: Thorlabs G608N3*** to a ***flat/tapered***[2] faced implantable fiber, of the same diameter and NA of the patch cord. A ***light power meter (ex: Thorlabs S121C connected to a PM100D)*** was used to capture the entire beam profile at ***number*** mW power. The area of the patch cord, ***number*** mm^2 over the power gives an irradiance of ***number*** mW/mm^2. Stimulation was pulsed at ***parameters (ex: 1 ms 20 Hz pulses)*** using a TTL signal generated by ***generator number and manufacturer***. Rise and fall times of the pulsed laser resulted in a pulse average irradiance of ***number*** mW/mm^2 across a single pulse, as measured with ***fast detector (ex: Thorlabs DET10A)*** connected to an ***oscilloscope (ex: Tektronix TDS 2024C)***.

[1] **ON/OFF Considerations**: No system is instantaneous. The inevitable delay between a signal to change the status of a system (input) and the actual change (output) can be reasonably negated, but should still be acknowledged. Two examples:

1. An LED was pulsed on for 10 ms. It was noted that there is a ramp on time taking 1 ms, and a ramp down time of 0.5 ms. This can be estimated as a loss of 0.5 ms to the overall desired pulse. That represents (0.5/10) or 5%. It would therefore be reasonable to replace the last sentence with:

    a. Pulsing the LED resulted in ON/OFF rise/fall times, as measured with ***fast detector (ex: Thorlabs DET10A)*** connected to an ***oscilloscope (ex: Tektronix TDS 2024C),** of about 5% less than the power as measured continuous on and can therefore be negated.

2. A Laser was pulsed at 5 ms. It was noted that the ramp on time is near instantaneous, but *overshoots* the continuous on values by about 50% and then decays down to the continuous value over about 2.5 ms. When the TTL pulse ends, the laser ramped down in near instantaneous. Therefore, when this laser is pulsed, it delivers more optic power than when continuously on. Assuming linear decay, it can be estimated to be 25% more power over this pulse. You may account for this by either turning down the laser (suggested) or adjusting the TTL pulse duration. Use the last sentence of the paragraph above to describe this system.

[2] **Tapered Fibers**: Tapering the end of a fiber (typically through pulling or etching (warning: hydrofluoric acid is nasty stuff) can allow for greater volume of light distribution/collection. However, the tip of the fiber optic canula is no longer a circle, but rather a cone (if etched) or some curvy volume (if pulled). Measure all the light that is emitted from the tip for the power and report the irradiance as the power per area of the cone (or close estimation to this). The area of a cone or radius r and height h is given as $Area = \pi * r * h$

A well etched fiber with hydrofluoric acid will be at 45° and the equation simplifies to $Area = \pi * r^2$

**<u>Please</u>** acknowledge our facility in your publications. An appropriate wording would be:
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