Light Sources for Optogenetics Experiments

Ver. 02

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Light Sources for Optogenetics Experiments

To truly understand the intricacies of how cells carry out processes requires the ability to precisely alter the activity of specific cells at specific times. This precise control is now possible by combining optics and genetics, also known as optogenetics.

To alter cellular behavior, genes that code for light-responsive proteins known as opsins are inserted into cells. Photo-excitation or photo-inhibition of these proteins causes them to alter cell function in specific ways, allowing scientists to observe the effects that such changes have on cellular activity.

The light source used for photoexcitation or photo-inhibition is a key part of the optical setup for optogenetic studies. Well-defined spectral, temporal, and spatial control is important as well

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as homogenous and constant illumination (Yizhar O. July 2011). In experiments involving multiple opsins, narrow emission spectrum is important to selectivity activate each opsin. In some experiments illumination stability is very important because any fluctuations or "hot spots" may cause inconsistent protein activation in the cells under illumination.

Optogenetics illumination sources include lasers and LEDs, and photoactivation can be done under a microscope or via a fiber for in vivo applications. Using a Prizmatix LED illumination system for optogenetics studies provides many advantages compared to laser-based systems including:

- Illumination Homogeneity and Stability
- Opsin Selectivity
- Light Switching
- Illumination Intensity Control
- Versatility

Laser Illumination

Diode lasers and diode-pumped solid state (DPSS) lasers are commonly used for optogenetics studies. For these studies, the diode lasers are usually used at 405 nm and 488 nm, and diode-pumped solid state (DPSS) lasers at 473 nm, 532 nm, 561 nm and 593.5 nm. The market is flooded by various inexpensive products that are low quality and do not perform well. Here we will discuss only high-end products.

Diode lasers use a semiconductor diode as laser gain medium and have a laser head and controller. In DPSS lasers a solid gain medium such as a Nd:YVO4 crystal is pumped with a high-power near IR laser diode to enable wavelength conversion from the near infra-red to visible or even ultra violet. DPSS lasers have all components of diode lasers as well as many additional components that make it a very expensive illumination source.

The main advantage of lasers is their intrinsically highly collimated beam, which can be focused to very small point. This is important for efficient coupling to very thin optical fibers such as ones with core diameters of 50 microns or less. In addition, the narrow spectral width (typically $\leq$1 nm) is helpful for selectively illuminating multiple opsins. However, lasers do present some significant disadvantages in performance and usability for Optogenetics studies including:

- Mode hopping (diode lasers)
- Back reflections (diode lasers)
- Instable TTL modulation (DPSS lasers)
- Speckle noise
- Safety issues
- Not suitable for wide field fluorescence microscopy

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Mode Hopping Noise

Most diode lasers simultaneously emit very close wavelengths called modes. During operation the laser spontaneously changes the mode distribution. This effect is called mode hopping, and in diode lasers it causes noise that contributes to power and wavelength instability (Carlsten 2005), (Hertsens 2005).

Back reflections

Diode lasers are very susceptible to back reflections from external components such as the optical fiber facet. These back reflections disturb the standing wave oscillations within the laser cavity. This phenomenon increases the effective power noise of the laser. A strong back reflection causes certain lasers to become highly unstable and in some cases can even cause permanent damage to the diode laser. To eliminate this problem additional optical component called optical isolator may be needed.

TTL modulation

Optogenetics studies require well-defined temporal control, in other words the light source must be turned off and on in a very precise manner. Most DPSS laser controllers do not provide direct TTL modulation and adding an optical switch can complicate and increase the cost of the optical set up. DPSS laser controllers that do provide TTL modulation are mostly limited in functionality and speed, they have a slow rise and fall time, the actual delivered power during TTL - ON state is much lower than the power measured at CW conditions. Due to these limitations DPSS lasers often use fast mechanical shutters for switching, though these can be acoustically noisy (especially in experiments with free moving animals) and have relatively short expected life times.

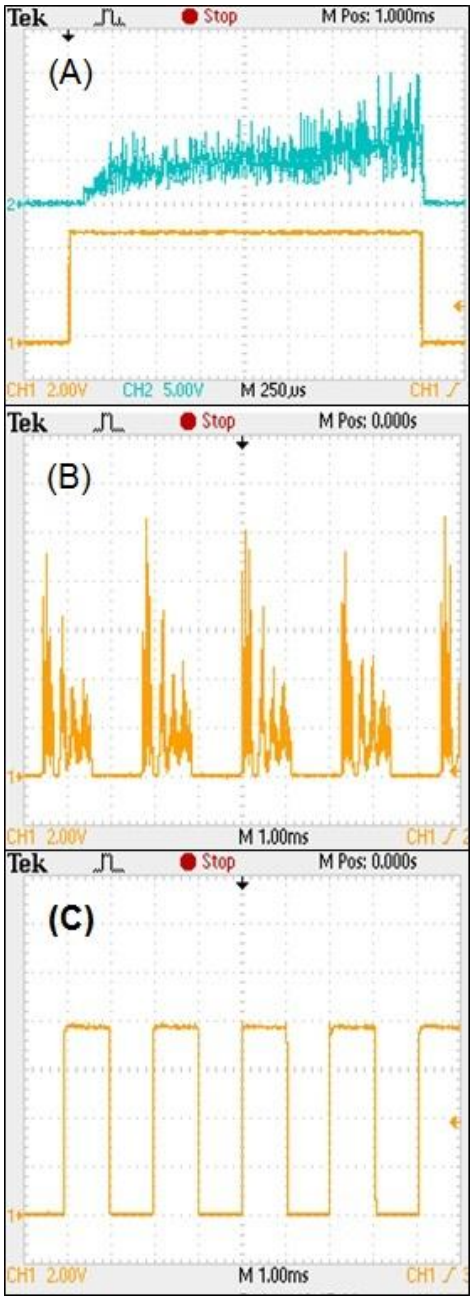

Figure 1: Comparison of DPSS laser and LED performance with TTL modulation (ON/OFF switching):
(**A**) 473 nm DPSS laser, Ch1 the TTL signal, Ch2 – photodiode measurement
(**B**) 561 nm DPSS laser, Ch1 – photodiode measurement
(**C**) Ultra High Power LED, Ch1 – photodiode measurement

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Speckle

Because laser output has a narrow wavelength bandwidth and is highly coherent, self-interference of the laser beam occurs. This causes a random spatial intensity variation at the illuminated site called a speckle pattern. The speckle pattern produces significant spatial intensity fluctuations, and its distribution can change in response to even minor temperature and current changes in the laser or at fiber banding. The resulting random fluctuations in local intensity are called speckle noise. When coupled to a multimode optical fiber, the speckle effect worsens because the output from the fiber has significant variations in local intensity. Additionally, significant changes in the fiber output power can be caused from bends in the fiber, which increase attenuation of higher propagation modes.

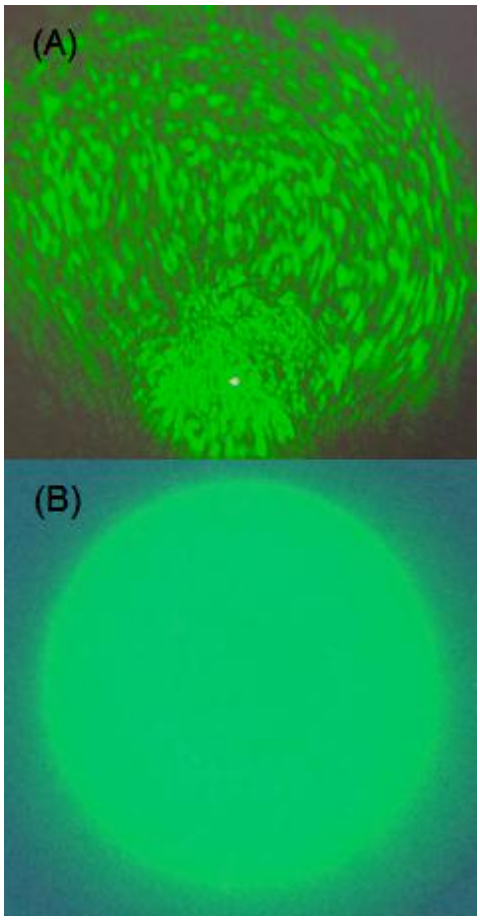

Figure 2: (A) Speckle pattern produced by fiber coupled 561nm DPSS laser. (B) Homogenous beam produced by fiber coupled Ultra High Power LED

Laser safety issues

Due to the brightness, beam collimation and coherence properties of lasers there are risks for eye and skin injury and are therefore subject to government regulations. The ANSI Z136 standard in the US and IEC 60825 international standard define "classes" of lasers depending on their power and wavelength. The laser class defines safety measures, such as labeling, warnings and safety goggles required for laser-equipped labs.

Wide field illumination

The adantage of the laser's thin collimated beam can be a disadvantage in applications such as wide field microscopy. For wide field fluorescence microscopy the laser beam must be correctly expanded and delivered to the microscope's epifluorescence excitation port. The speckle noise from the laser beam causes a highly irregular pattern that is not suitable for wide field imaging. Therefore, laser devices cannot be used for optogenetics fiber-coupled applications and wide field microscopy.

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LED Illumination

Using LED illumination system for optogenetics studies provides many advantages without most of above-mentioned disadvantages of lasers. These include:

- Illumination Homogeneity and Stability
- Opsin Selectivity
- Light Switching
- Illumination Intensity Control
- Versatility

Illumination Homogeneity and Stability

The Prizmatix Microscope LED , and Ultra High Power Microscope LED (UHP-Microscope-LED) systems feature high-end LED drivers that ensure stable output from the LED. All driver circuits are current sources that provide stable current for LED operation. The power is stable over time because the LED thermal management features high-end heat sinks and even fans if necessary.

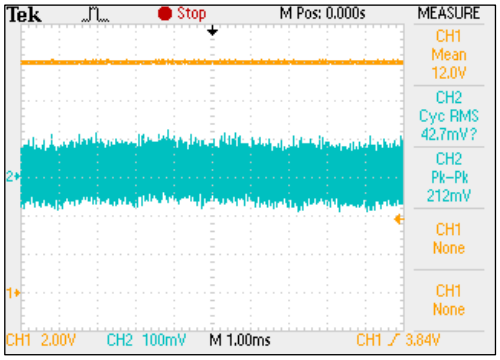

Figure 3: Measurements of power noise for Ultra High Power LED. Channel CH1 measure DC, Channel CH2 measure AC component of the photodiode output voltage. Noise: AC_{rms} / DC = 0.0427 / 12.0 = 0.36%

Because LED systems don't have a resonant cavity like lasers, they don't exhibit the noise related to emission modes or instability from back reflections that can be found with diode lasers.

Opsin Selectivity

In experiments involving multiple opsins a narrow emission spectrum from the illumination source is important to achieve selective photoactivation. The emission spectrum of most LEDs is from 10 to 30 nm, a spectral width that is ideal for selective activation of multiple opsins. Interference filters can be used to achieve a narrower spectrum.

Light Switching

Optogenetics studies require well-defined temporal control, in other words the light source must be turned off and on in a very fast and precise manner. Most DPSS laser systems with TTL modulation input become instable at fast switching rates, so mechanical fast shutters are necessary.

Prizmatix LED current drivers feature a direct TTL input for fast switching with a rise/fall time of microseconds, much faster than millisecond pulses required for optogenetics applications. The Prizmatix UHP-Microscope-LED series feature a standard fast opto-isolator at the TTL input that ensures complete isolation of the sensitive electrophysiology electronics from the LED driver electronics.

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Illumination Intensity Control

The output of LEDs is directly dependent on current, yet most LEDs do not perform well at low currents. To precisely control illumination levels most commercial LED systems use pulse width modulation (PWM). However, PWM is not suitable for most scientific applications such as fluorescence microscopy or the fast switching used for photoactivation in optogenetics. For optogenetics experiments, the LED must be operated with a stable current and the ON/OFF controlled through the TTL input. All Prizmatix LED current controllers feature a constant current operation mode and provide direct TTL modulation. The LED current can manually adjusted using a precise 10-turn potentiometer with a locking dial, or it can be set using a computer through the optional analog modulation input.

Versatility

The Prizmatix Microscope-LED and UHP-Microscope-LED series can be combined in many different configurations to enable multiple or single wavelength outputs for various flexible connection ports, adaptors, and couplers. They can be directly connected to a microscope via epi-fluorescence port adaptors or a Liquid Light Guide (LLG).

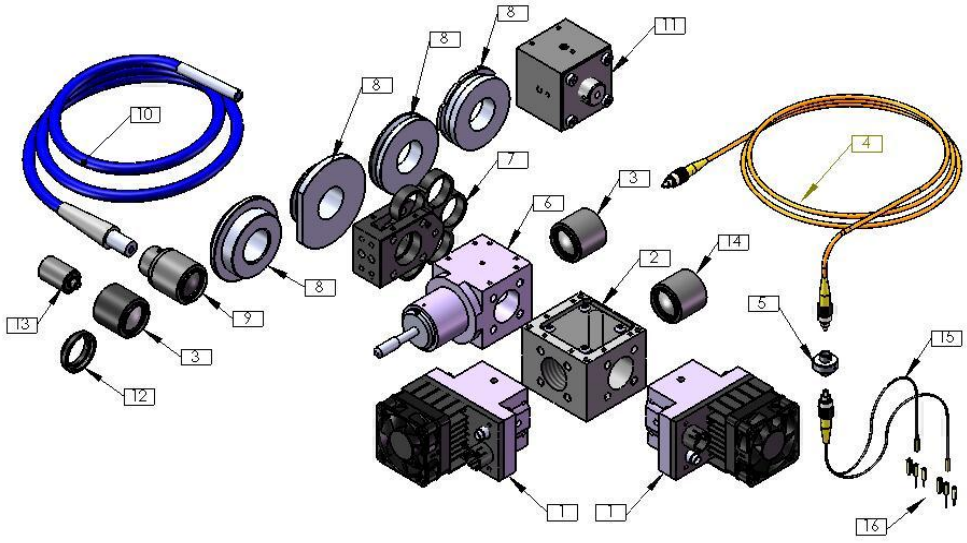

Figure 4: Optogenetics Toolbox assembly diagram shows the system versatility. [1] Ultra High Power LED, [2] Beam Combiner, [3] Fiber Coupling Adaptor, [4] Fiberoptics, [5] Rotary Joint, [6] Beam Switcher, [7] Filter Wheel, [8] Microscope Adaptors, [9] Liquid Light Guide Adaptor, [10] Liquid Light Guide, [11] Liquid Light Guide XYZ Collimator, [12] C-Mount Adaptor, [13] Fiberoptic Collimator, [14] Monitoring Photodiode, [15] Single/Dual Fiber, [16] Optogenetics Implantable Cannulae / Ferrules. For more detailed description see:
http://www.prizmatix.com/optogenetics/Optogenetics-LED-Light-Sources-and-Fiber-Optics.htm

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The UHP-Microscope-LED-White with the optional Filter Wheel is very versatile because it allows one light source to be connected to a fiber coupler for single wavelength illumination or through a single fiber or multiple fibers for photoactivation with multiple wavelengths.

An OptiBlock Beam-Switcher can be added to a LED system attached to microscope for additional versatility. It enables simple manual switching between two illumination modes. For example, it can be used to switch between direct epifluorescence illumination and fiber optic illumination without disconnecting the LED system from the epifluorescence system.

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