

PlexBright[®] Optogenetic Stimulation System Guide



PlexBright® Optogenetic Stimulation System Guide

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Documentation History

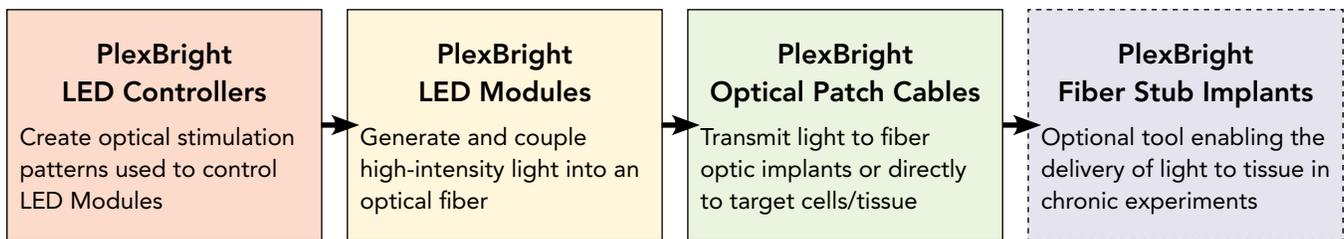
Date	Version	Notes
December 2021	OPTTN0001k	- Added HELIOS® system LED Module, Appendix F minor changes - measured outputs updated
October 2020	OPTTN0001j	- Added Appendix D - Sample Electrophysiology and Optogenetics Experiment Set-up Using NeuroLight Probe
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February 2019	OPTTN0001h	-Added Spectrogram, Appendix C - Sample Electrophysiology and Optogenetics Experiment Set-up, jacket weights
March 2017	OPTTN0001f	-Minor edits
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November 2013	OPTTN0001b	-Added new 16 channel LED commutator and FC Ferrule product information to Steps 4, 5 and Appendix B. - Removed PlexBright Equipment Worksheet. - Minor edits throughout.
June 2013	OPTTN0001a	-Minor changes and reformatted document according to current branding guidelines.

Introduction

Thank you for considering the Plexon PlexBright® Optogenetic Stimulation System for your optogenetics experiments. This guide will help direct you through the process of choosing which PlexBright products are right for your specific applications. Further information about each product is also available on the Plexon website (www.plexon.com). If you come across questions that are not addressed in this guide or on the website, please feel free to contact your Plexon Sales Engineer or email info@plexon.com for more information.

PlexBright System Overview

The PlexBright family of products covers the major aspects of common optogenetics experiments, including the creation of optical stimulation patterns, generation of fiber-coupled light, and delivery of this light into brain tissue. The diagram below covers some of the hardware involved in a basic optogenetic experiment.



PlexBright products also enable more complex experiments. For example, Compact LED Modules may be mounted on a PlexBright Dual LED Commutator to spin with freely behaving animals, preventing the tangling of optical cables. PlexBright products are also compatible with other Plexon hardware systems to enable closed-loop experiments in which optical/electrical stimulation patterns are updated based on feedback from the OmniPlex® Neural Data Acquisition System and/or the CinePlex® Behavioral Research System (see **Appendix D** for more information). Further, some PlexBright products – such as the PlexBright 4 Channel Controller with Radiant™ software (Radiant) – are compatible with some third-party hardware and capable of operating laser equipment or TTL-triggered devices.

It is important to note that special components and custom high performance optical fiber are used throughout the PlexBright family of products. Substitution of any part of the integrated LED system between the controller/driver through to the fiber stub implant will diminish the system's power output.

Selecting the Right Components

Steps 1-7 below will aid in selecting the right components for a given optogenetic experiment.

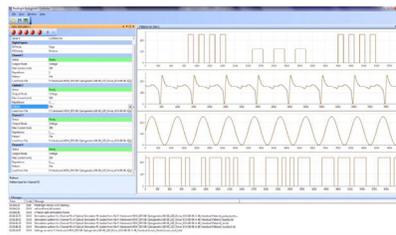
Step 1: Selecting a Controller or Driver

There are two options for controlling the light output from PlexBright LED Modules:

- the PlexBright 4 Channel Controller, and
- the PlexBright LD-1 Single Channel LED Driver.

PlexBright 4 Channel Optogenetic Controller

The PlexBright 4 Channel Controller running Radiant generates output signals to control four LEDs and/or lasers independently. Radiant can be used to easily define simple or complex output patterns through an intuitive software GUI. Digital input and output signals allow precise synchronization and interaction with other devices. Each output channel can control one light source independently or two light sources with the same output pattern, using a PlexBright Series-Y BNC cable.



PlexBright LD-1 Single Channel LED Driver

The PlexBright LD-1 Single Channel LED Driver is an economical solution for controlling one LED (or two LEDs with the same output pattern). The device is equipped with a manual dial and LCD display for setting the light output intensity, and it can accept a digital (TTL) input signal encoding a pulsed output pattern. The LD-1 LED Driver also accepts a 0-5V analog input signal encoding arbitrary output patterns. The LD-1 Single Channel LED Driver can function as a stand-alone device to deliver constant control signals to LED Modules. It is important to note, however, this driver does not generate pulsed or arbitrary patterns. Rather, it receives these patterns as inputs from a separate device (in the form of TTL or analog signal) and generates the corresponding outputs necessary to drive the LED modules. Please see the table below for further details and technical specifications.



Feature	4 Channel Optogenetic Controller	LD-1 Single Channel LED Driver
Output channels	4	1
Software control via GUI	Yes	No
Light source compatibility	LEDs and lasers	LEDs
Outputs	Up to 1.1A (for LEDs) / 0-5V (for lasers)	Up to 1.2A for LED control
Accepts digital inputs (i.e. TTL) to encode pulsed output patterns	Yes	Yes
Generation of arbitrary output pattern	Easily definable via included Radiant™ software GUI	Signal must be generated externally and input as a 0-5V analog input
Accepts digital inputs to trigger pre-defined output patterns	Yes	No
Digital outputs	Yes	No

Step 2: Assessing the Need for a Commutator and Which One

In experiments requiring optical stimulation of freely behaving animals, optical patch cables attached to the animal may become twisted as the animal moves around. In some cases, during short stimulation sessions, this cable twisting may be tolerated. In other cases where extended free movement of the animal is important, a solution to prevent tangling is required. The PlexBright Commutators below offer a unique solution for preventing the tangling of cables.

PlexBright Dual LED Commutator

Up to two PlexBright Compact LED Modules may be mounted on the rotating base of the PlexBright Dual LED Commutator. Because the light sources themselves rotate along with the animal, cable tangling is prevented, without the need for a complex and problematic optical rotary joint.



PlexBright Dual LED + 16 Channel Commutator

The PlexBright Dual LED + 16 channel Commutator, like the PlexBright Dual LED Commutator, will mount up to two PlexBright Compact LED Modules on its rotating, magnetic base (pictured). It also provides commutation of up to 16 channels of neural data and can be combined with any one of our Plexon 16 channel analog headstages.



PlexBright Carousel Commutator

The PlexBright Carousel Commutator has a low-torque, passive design that supports optogenetic experiments requiring one or two LEDs with animals as small as mice. It is also ideal for experiments in which optogenetic stimulation is performed simultaneously with neural recording using up to two digital headstages.



PlexBright Dual LED + Fluid Swivel Commutator

The PlexBright Dual LED + Fluid Swivel is also ideal for experiments in which optogenetic stimulation is performed simultaneously with drug perfusion. It is designed to be used with the PlexBright Head-Mounted LEDs. It also has a removable magnetic torque arm that can be used to help transfer torque from the animal to the commutator.

PlexBright Motorized Carousel Commutator

The PlexBright Motorized Carousel Commutator is ideal for experiments in which optogenetic stimulation is performed simultaneously with neural recording using digital headstages. It can be used with up to four digitizing headstages and two Plexon compact LED modules. There is also a hole down the center of the commutator which can be used for fluid delivery or an optical swivel.



Step 3: Selecting LED Modules

Plexon's PlexBright fiber-coupled LED Modules provide stable, wavelength-specific light at industry-leading output intensities. LED Modules in a spectrum of wavelengths are available in four styles, Table-top, Compact, Head-Mounted and Wireless. The Compact and Head-Mounted are designed for use with our PlexBright Commutators.

Table-top LED Modules

Table-top LED Modules are typically used with:

- ♦ *in vitro* preparations,
- ♦ *in vivo* experiments with anesthetized animals, or
- ♦ *in vivo* experiments with behaving animals when a commutator is not required to prevent tangling of cables.
- ♦ See Appendix F for available wavelengths



Compact LED Modules

PlexBright Compact LED Modules provide the same light output as the PlexBright Table-top LED Modules, only in a smaller form factor. They are mounted directly to the PlexBright Commutators to rotate along with freely behaving animals, preventing cable tangling.

- ♦ See Appendix F for available wavelengths



Head-Mounted LED Modules

PlexBright Head-Mounted LED Modules are powerful sources of fiber-coupled light that are miniaturized for placement directly on the animal's head. They produce high power output and reduce restraints on the experimental environment by eliminating the fiber optic patch cable.

- ♦ See Appendix F for available wavelengths



Head-Mounted Extra Light Infrared Optogenetic Stimulation

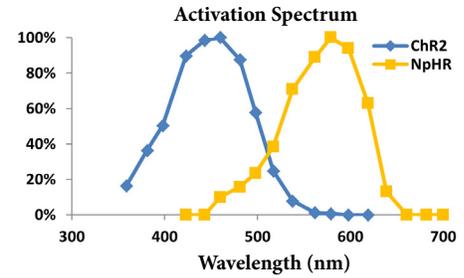
The HELIOS® system is Plexon's newest innovation in optogenetic technology which provides a wireless, lightweight, and reusable solution for high power pulsed optogenetic stimulation in freely moving small animals.

- ♦ See Appendix F for available wavelengths

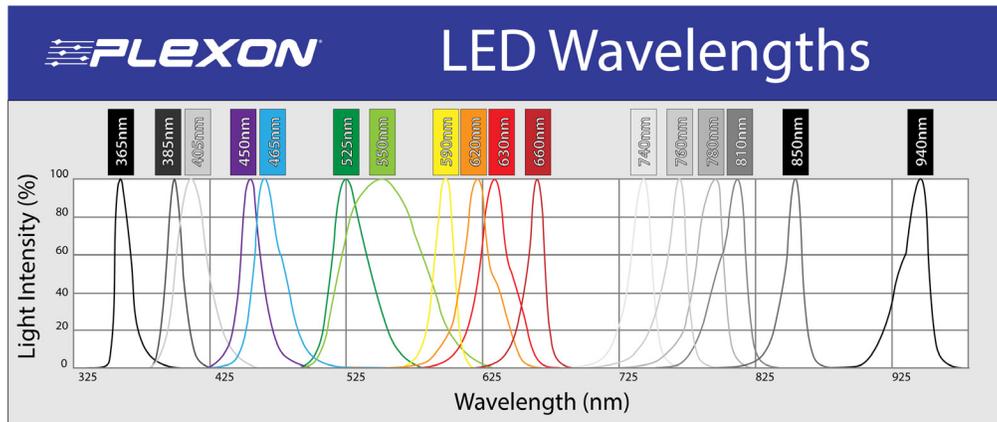


Selecting Wavelengths

Typical optogenetic experiments involve cells transfected with one or more opsins, each of which is sensitive to a particular range of wavelengths. Channelrhodopsin-2 (ChR2), for example, is activated by a range of blue/green wavelengths, though it is most sensitive to light around the 460nm¹. Halorhodopsin (NpHR) is maximally activated by 580nm range yellow light, but it is also significantly activated by wavelengths from green to orange/red¹.



For maximum NpHR stimulation, PlexBright Orange LED Modules are recommended over the lower-intensity Yellow LED modules. Similarly, 550nm Lime LED Modules are recommended for Arch stimulation² (See Appendix D for more information).



Excitatory Opsins

Opsin	Peak Activation	Suggested Wavelength
Channelrhodopsin-2 (ChR2)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (H134R)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (T159C)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (L132C)	474 nm	Blue (465 nm)
Channelrhodopsin-2 (E123A)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (E123T)	490 nm	Blue (465 nm)
Channelrhodopsin-2 (E123T/T159C)	490 nm	Blue (465 nm)
ChIEF	450 nm	Royal (450 nm)
ChRGR	505 nm	Green (525 nm)
VChR1	545 nm	Lime (550 nm)
C1V1	540 nm	Lime (550 nm)
C1V1 CheETA (E162T)	530 nm	Green (525 nm)
C1V1 CheETA (E122T/E162T)	535 nm	Green (525 nm)
C-VChR1	570 nm	Lime (550 nm)
VCOMET	590 nm	Orange (620 nm)
Red activated Channelrhodopsin (ReaChR)	590 nm	Orange (620 nm)

Inhibitory Opsins

Opsin	Peak Activation	Suggested Wavelength
eNpHR 3.0	590 nm	Yellow (590 nm)
Arch/ArchT	566 nm	Lime (550 nm)
eBR	540 nm	Lime (550 nm)
Jaws	632 nm	Red (630nm)

Step 4: Selecting Optical Patch Cables

There are several considerations when choosing a PlexBright Optical Patch Cable:

1. Optical fiber type
2. Termination end – there are three options designed for specific research applications,
3. Reinforcement – certain cables may be reinforced (armored) for strength,
4. LED Module specificity – cables have LED Module-specific connectors, and
5. Length – three standard lengths are available.



Optical Fiber

Plexon's PlexBright Optical Patch Cables are available in two options tailored to meet the needs of optogenetics research: High Performance or High Durability.

♦ High Performance Fiber:

The High-Performance optical fiber has a numerical aperture of .66 and all glass construction (including the cladding) making it heat, chemical, and scratch resistant. This patch cable provides the highest possible output intensities from PlexBright LED Modules.

♦ High Durability Fiber:

The High Durability fiber has a lower numerical aperture (.5NA), but may be more optimal in experiments with highly active animals. If the power output from the High Durability patch cable is satisfactory, Plexon recommends using this fiber type for experiments with freely-moving animals. An optional black rubberized coating can be added to High Durability cables.

Please review Appendix D for information on the power output for each type of fiber

Termination End

PlexBright Optical Patch Cables are available with three application-specific tip options:

- ♦ Bare Fiber Tip: Patch cables terminate in a bare optical fiber that can be inserted directly into tissue. These cables have a reinforced tubing that can be clamped into a micromanipulator for *in vitro* or head-fixed experiments.
- ♦ LC Ferrule Tip: LC Ferrule Tip cables have a smaller ferrule tip for use in experiments with smaller animals such as mice. The LC Ferrule Tip mates with LC-sized Fiber Stub Implants for light delivery in chronic experiments, where researchers need to periodically attach the patch cable to the animal to deliver light. Attachment between the LC Ferrule Tip Patch Cable and LC-sized Fiber Stub Implant is made with ceramic mating sleeves included with each patch cable.
- ♦ Magnetic LC Ferrule Tip: An added feature to the LC ferrule which enables the patch cable to mate with a magnetic fiber stub for magnetic optical fiber connector. The magnetic optical fiber connector allows rotational movement such as spinning and twirling at the connection in freely behaving animal experiments reducing torque on the implant.
- ♦ FC Ferrule Tip: Patch cables are similar to the LC Ferrule Tip cables, but have a larger ferrule tip that offers additional strength useful with larger animals such as rats. The larger FC Ferrule Tip mates with FC-sized Fiber Stub Implants.



Reinforcement Options

This feature is available for PlexBright Optical Patch Cables with LC or FC Ferrule Tips; Bare Fiber Tip cables already have reinforced tubing.

As an added protection from over-bending or chewing (e.g. from freely behaving animals that are bored or agitated), reinforcement is recommended for experiments with rats. For smaller animals such as mice the lighter monocoil wrap may be appropriate. Reinforcement options should be discussed with a Plexon Sales Engineer to ensure intended cable flexibility.

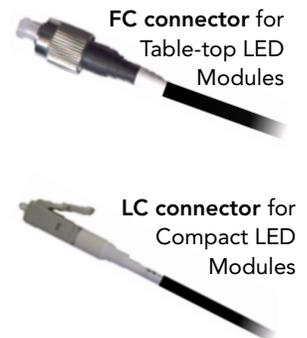
- ♦ **Stainless Steel:** This jacketing offers the maximum amount of protection.
- ♦ **Mono Coil:** The stainless steel, mono-coil wrap offers added protection while still maintaining a large degree of flexibility.



Cable (0.5 meters)	Weight (grams)
Monocoil	2.6 grams
Stainless Steel	4.7 grams

LED Module-Specific Options

Additionally, PlexBright Optical Patch Cables are specific to either PlexBright Table-top LED Modules, or for use with the PlexBright Compact (commutator) LED Modules. The difference between the designs is the method of connection with the LED Module. A PlexBright Optical Patch Cable with the more robust metal bodied FC connector is designed to interface with the PlexBright Table-top LED Module, while a PlexBright Optical Patch Cable with the more lightweight LC connector interfaces with the PlexBright Compact LED Modules, designed for commutator use.



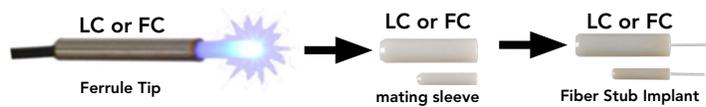
Lengths

Patch cables come in standard lengths of 0.5, 1.0, and 1.5m. For highest possible output intensities, choose the shortest length of patch cable that can be comfortably accommodated by the experiment. For example, experiments with cells, tissue, or head-fixed animals can often accommodate a shorter 0.5m patch cable. Experiments with freely behaving animals often necessitate 1.0m cables. Commutator-mounted Compact LED Modules are typically positioned directly above an animal, and a 0.5m length is common.

Patch cables can be custom made with lengths to the nearest tenth of a meter. Maximum length for High Performance Patch Cables is 2.0m. High Durability Patch Cables can be made in any length to the nearest tenth of a meter.

Step 5: Assessing the Need for Fiber Stub Implants

A PlexBright Fiber Stub Implant is a hollow, ceramic ferrule (or cannula) with an optical fiber at the center that extends beyond one side of the ferrule a specified length. The fiber stub is permanently implanted into the cranium of the subject with the exposed fiber placed



into the tissue. It is then reversibly connected to an Optical Patch Cable using a ceramic sleeve during experimentation to deliver light in chronic stimulation experiments to a specified target depth based upon the fiber length. PlexBright Fiber Stub Implants are available with a choice of body size, optical fiber diameter, and optical fiber length.

Note: The appropriate ceramic mating sleeves are included with the purchase of PlexBright Optical Patch Cables with LC or FC Ferrule Tips.

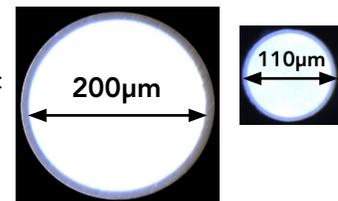
Body Size Options

PlexBright Fiber Stub Implants are available in two body sizes options: LC or FC ferrules. The LC ferrule size is smaller (1.25mm OD x 6.45mm length) and typically used with mice or other small animals, but may also be used with larger animals such as rats. The FC ferrule size is larger (2.5mm OD x 10.5mm length) and typically used with rats and other larger animals. Preference is up to the researcher and often depends on the experimental conditions.

The PlexBright Fiber Stub Implant ferrule size must match the size of the PlexBright Optical Patch Cable ferrule tip. For example, if the subject is a rat and you choose a PlexBright FC Ferrule Fiber Stub Implant, then be sure to also choose an Optical Patch Cable with a PlexBright FC Ferrule Tip.

Optical Fiber Diameters

The typical fiber core diameter is 200 μ m with a 230 μ m outer diameter including cladding (200/230 μ m), a size used in many published optogenetic studies. Fiber Stub Implants are also available in a smaller 110/125 μ m size, which can be used in experiments where minimal tissue displacement is critical.



Note: The 110/125 μ m size transmits approximately 30% as much light as the 200/230 μ m size. Due, however, to the industry-leading light output of PlexBright LED Modules, the light output through the smaller implants is often still more than adequate.

Lengths

PlexBright Fiber Stub Implant length refers to the length of the optical fiber exposed beyond the end of the ferrule (or cannula). Standard lengths are 2, 4, 6, 8, 10 and 12mm, with custom lengths available upon request. Fibers within all PlexBright Fiber Stub Implants are all glass (including the cladding), making them heat, chemical, and scratch resistant. The tips are also precision-finished for a perfect flat tip, without the chipping imperfections from scoring that are typical in competitor products.



Magnetic Fiber Stubs

LC fiber stubs are also available with a magnetic connector. This can be connected to a magnetic patch cable without the need of a ceramic sleeve. These fiber stubs can also be connected to a standard patch cable using a ceramic sleeve.

Step 6: Optical Fiber Cleaning Kit

Plexon wants to ensure that researchers get the most from their PlexBright equipment for the longest use possible. Accordingly, we have assembled the PlexBright Optical Fiber Cleaning Kit recommended for extending the performance and life of high performance optical connectors and fiber tips within the PlexBright System. For your convenience, it contains a specialized cleaning solution and two types of lint-free wipes. Whether you order the PlexBright Optical Fiber Cleaning Kit or use another solution, it is important that the fibers are properly maintained to get the most out of them.

Step 7: Optional Accessories

Plexon offers the following accessories to complement your PlexBright Optogenetic components.

Series-Y BNC Cable

As briefly described earlier in this document, both the PlexBright 4 Channel Controller and the PlexBright LD-1 Single Channel LED Driver are capable of controlling two PlexBright LED Modules per channel with the same output signal. To accomplish this, Plexon developed the PlexBright Series-Y Cable. If you plan to direct the signal from a single output channel into two LED Modules, it is very important that the PlexBright Series-Y BNC Cable be used and not to substitute a splitter or different cable. The Series-Y BNC Cable is not a signal splitter. Rather, it connects both LED Modules in series, not in parallel, resulting in equivalent current being carried through each module as opposed to halving (splitting) the power between the two LED modules.

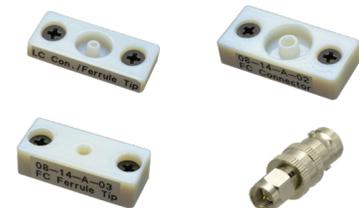
Light Measurement Kit

It is often useful to measure light outputs from the PlexBright System. Light output measurements can validate proper functioning of a PlexBright LED Module and proper connection of any attached PlexBright Optical Patch Cable and PlexBright Fiber Stub Implant. Also, because of variations between PlexBright LED Modules and PlexBright Optical Patch Cables, expected light outputs for a given supply current are only approximate. Light output measurements enable the precise control of optical stimulation that is often required in optogenetics experiments.



To take measurements of light output from the PlexBright System, Plexon highly recommends use of the THORLABS PM100D Optical Power and Energy meter and S140C Photodetector along with specially designed PlexBright Photodetector Adaptor pieces

for the most accurate readings. We have bundled these products together in a convenient kit and highly recommend that any lab purchasing PlexBright equipment also have the PlexBright Light Measurement Kit available for general measurements and troubleshooting.

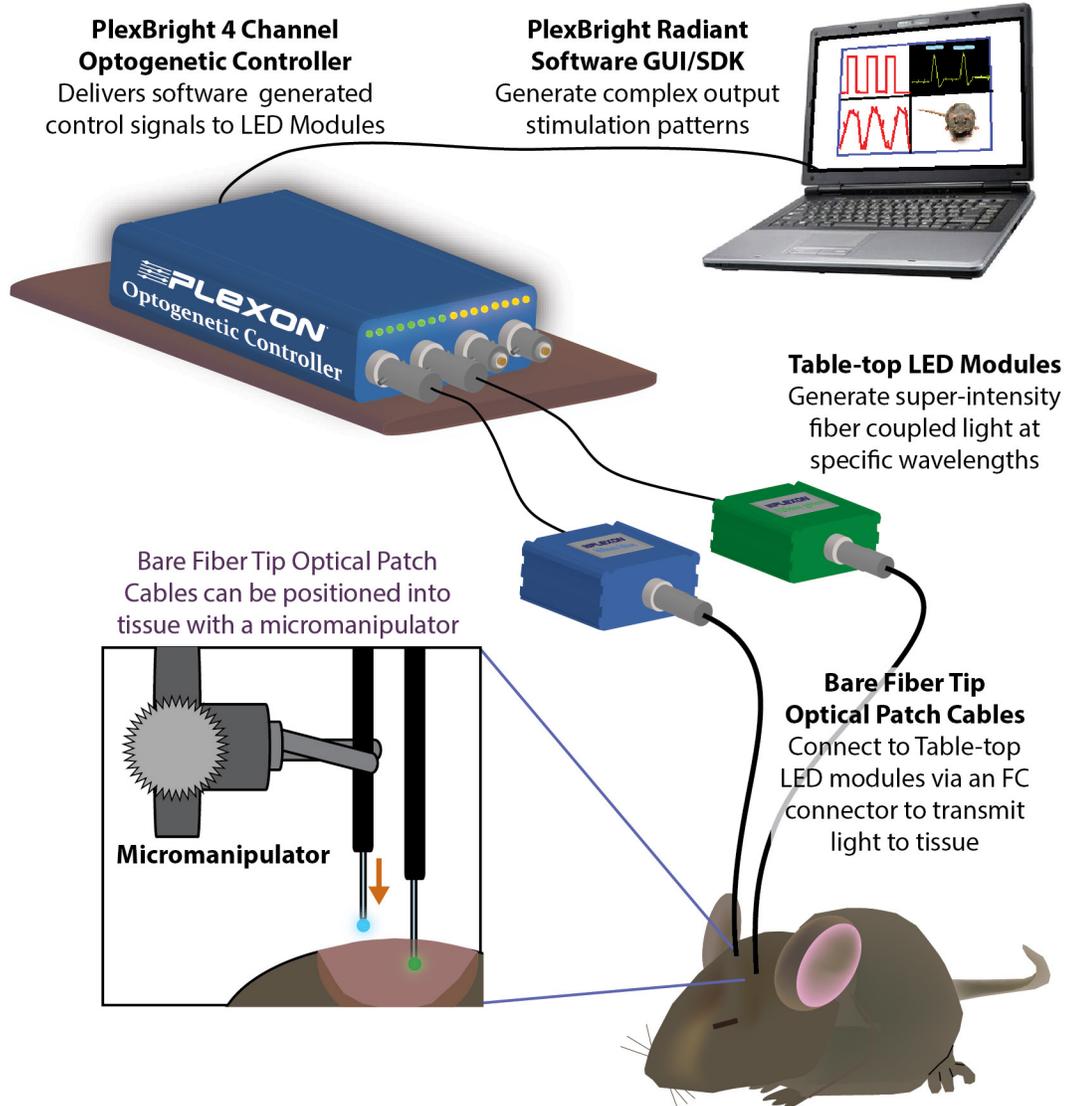


Important Power Output Note

For optogenetic stimulation, a critical metric is the light intensity actually delivered to the tissue. Other companies often provide the light output from the LED module itself, before the drop in light that will result from attaching a patch cable of appropriate size for optogenetic experiments. Appendix D shows power outputs at various points throughout the system light path when using Plexon's high performance or high durability cables.

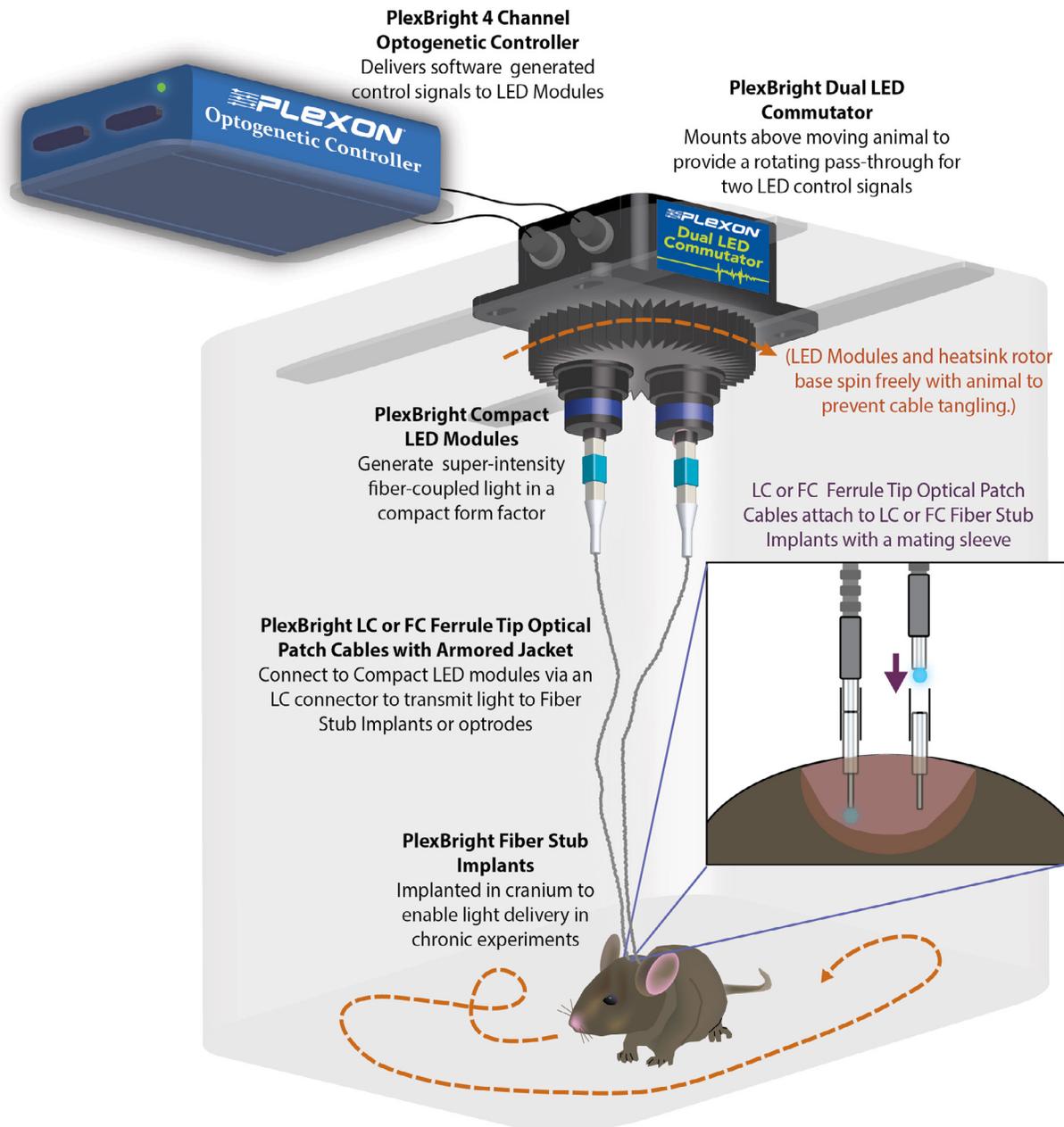
Appendix A - Sample Acute Experiment Set-up

PlexBright Radiant software is used to generate patterns of light stimulation and a 4 Channel Controller is used to deliver these control signals to Table-top LED Modules. FC connector-to-Bare Fiber tip patch cables deliver the light from the LED Modules to an anesthetized, head-fixed animal.



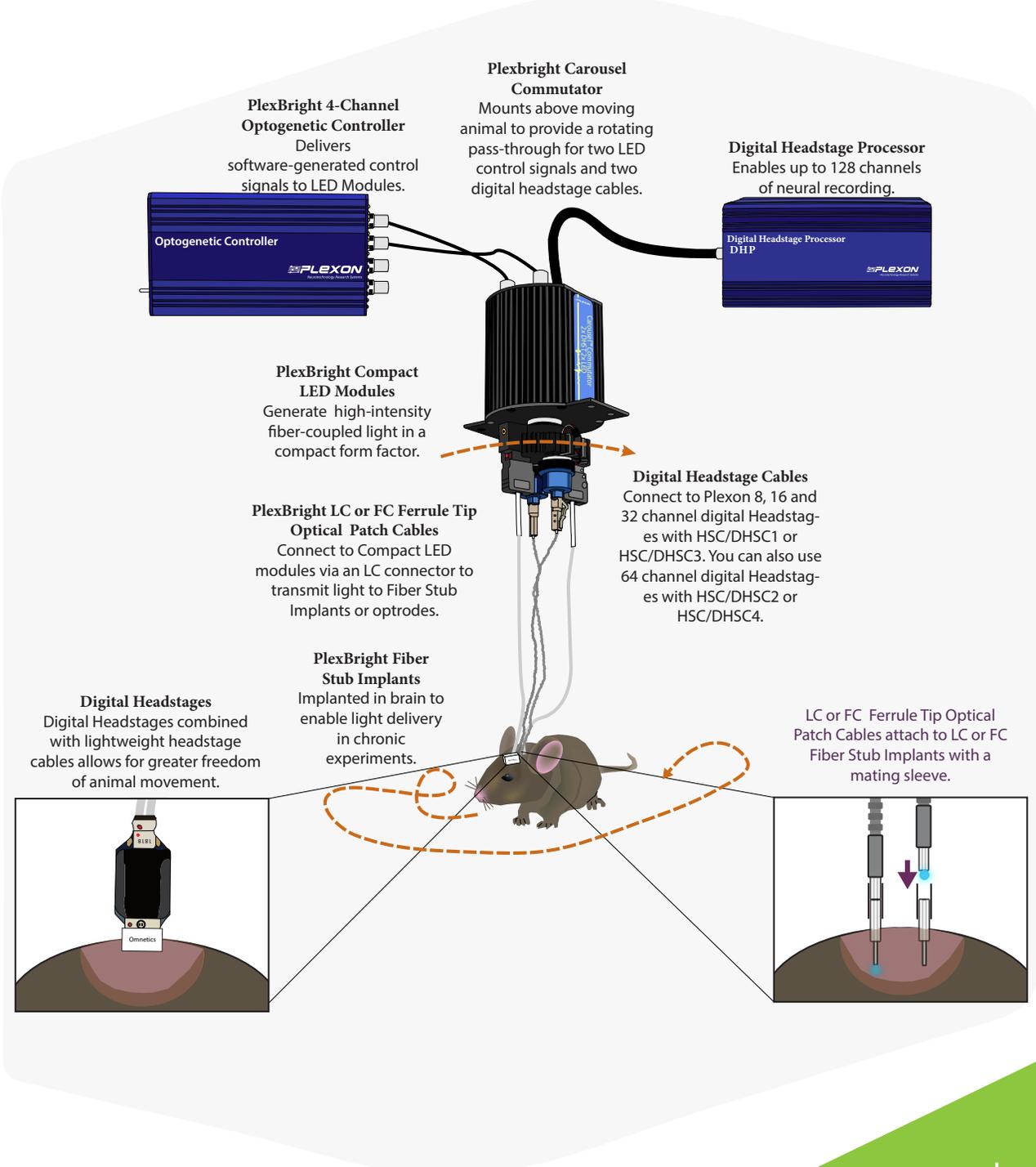
Appendix B - Sample Chronic Experiment Set-up

PlexBright Radiant software is used to generate patterns of light stimulation and a PlexBright 4 Channel Controller is used to deliver these control signals to two PlexBright Compact LED Modules. The control signals first pass through a PlexBright Dual LED Commutator, which allows the mounted compact LEDs to spin freely without tangling. LC connector-to-LC or -FC ferrule tip patch cables deliver the light from the LED modules to PlexBright Fiber Stub Implants on a freely behaving animal. As the animal turns, torque transmitted through the patch cables turns the LED modules and commutator rotor base, preventing tangling.



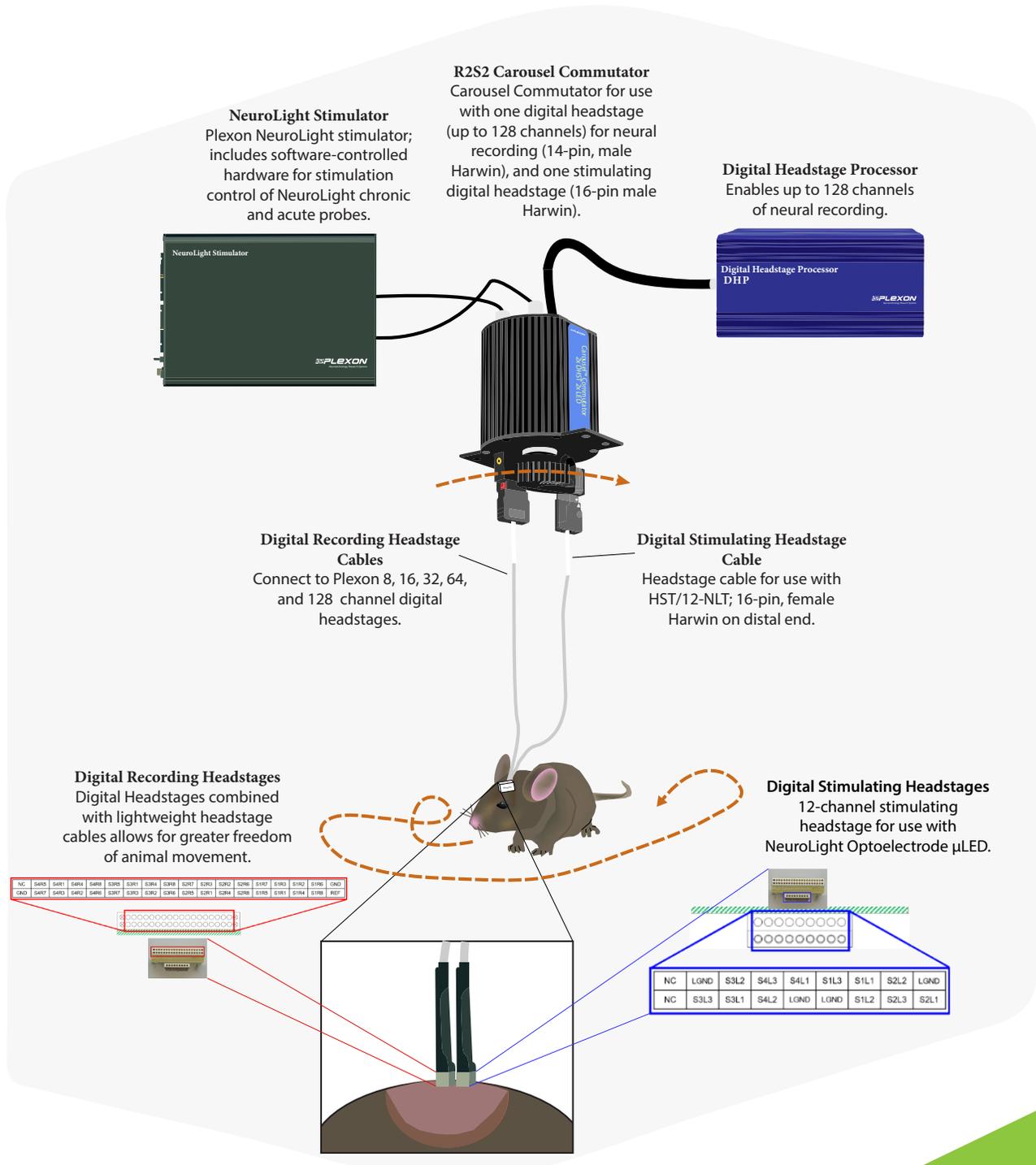
Appendix C - Sample Electrophysiology and Optogenetics Experiment Set-up

PlexBright Radiant software is used to generate patterns of light stimulation (control signals) and a PlexBright 4 Channel Controller is used to deliver these control signals to two PlexBright Compact LED Modules. The control signals first pass through a PlexBright Carousel Commutator, which allows the mounted compact LEDs to spin freely without tangling. The OmniPlex Data Acquisition System allows for simultaneous neural recording. Digital headstage cables are attached to the Carousel commutator and then connected to the digital headstage mounted on the animals head. LC connector-to-LC or -FC ferrule tip patch cables deliver the light from the LED modules to PlexBright Fiber Stub Implants on a freely behaving animal. As the animal turns, torque transmitted through the patch cables and headstage cables



Appendix D - Sample Electrophysiology and Optogenetics Experiment Set-up Using NeuroLight Probe

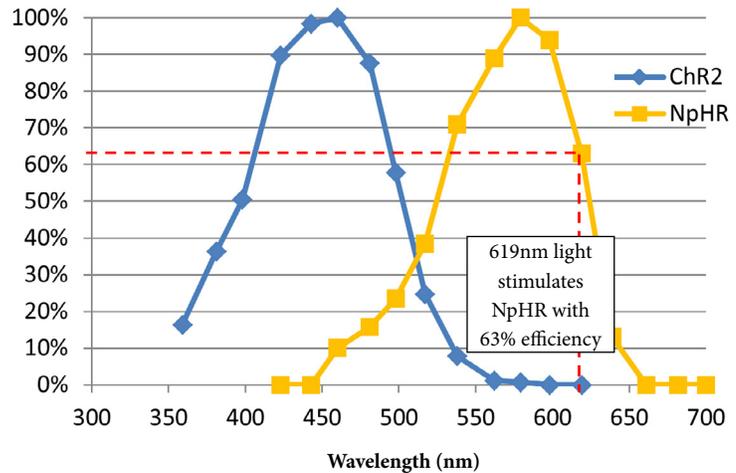
The NeuroLight Stimulator is used to generate patterns of light stimulation (control signals) and the OmniPlex Data Acquisition System allows for simultaneous neural recording. A digital recording headstage cable is attached to the Carousel commutator and then connected to the digital headstage. A stimulating headstage cable is attached to the Carousel commutator and then connected to the stimulating headstage. Both headstages are connected directly to the NeuroLight probe on a freely behaving animal. As the animal turns, torque is transmitted through the headstage cables and prevents the cables from



Appendix E – Selecting LED Module Wavelengths

Typical optogenetic experiments involve one or more opsins, each of which is sensitive to a particular range of wavelengths. Channelrhodopsin-2 (ChR2) is activated by a range of blue/green wavelengths, though it is most sensitive to blue light in the range of 465nm. Halorhodopsin (NpHR) is maximally activated by yellow light in the range of 580nm, but it is also significantly activated by wavelengths from green to orange/red.

Halorhodopsin is maximally activated by yellow range light, but it can be seen from the plot below that it is also significantly activated by other wavelengths.



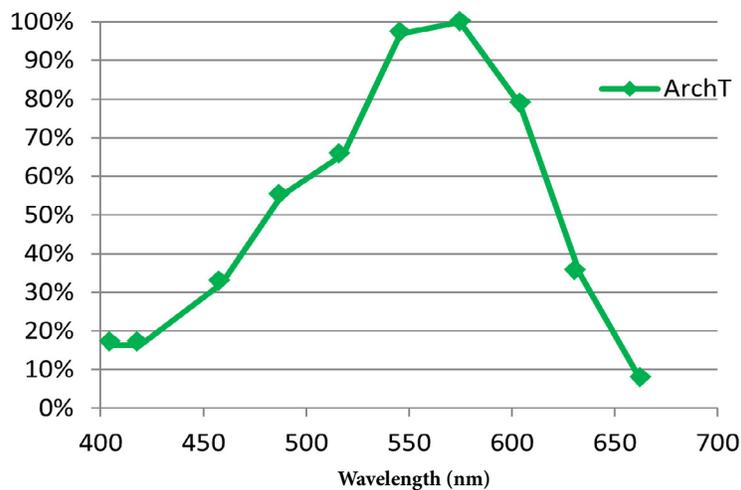
Activation Spectrum for ChR2 and NpHR

¹These plots depict the raw data displayed in Fig. 1 of Zhang F et al., Nature 2007, 446(7136):633-9. (Raw data was graciously provided by Dr. Feng Zhang.)

For maximum stimulation of NpHR, PlexBright Orange LED Modules are recommended.

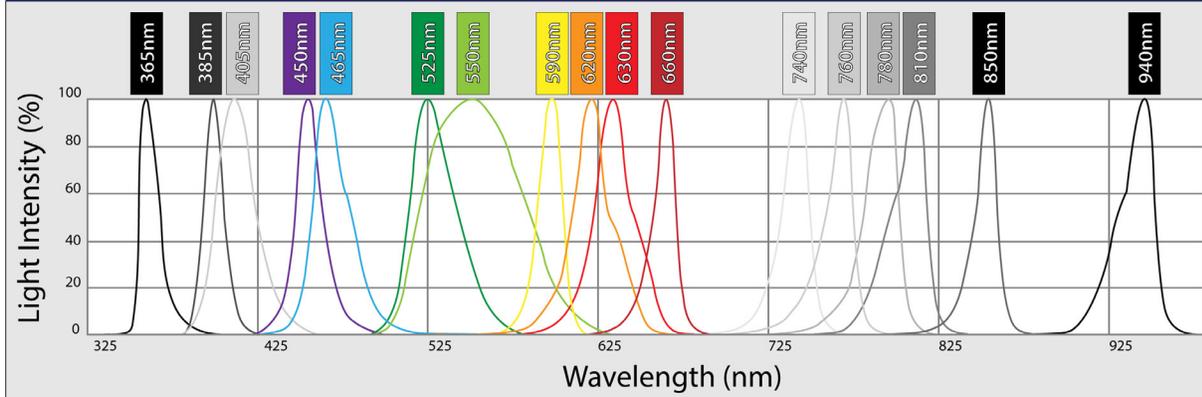
Although the wavelength of PlexBright 590nm Yellow LED Modules stimulates NpHR most efficiently, PlexBright 620nm Orange LED Modules provide more intense light outputs, and thus make up for the lower efficiency of longer wavelength light in activating NpHR. (Additionally, the outputs of Orange LED Modules are more stable at increased temperatures.) Furthermore, the longer wavelength of the Orange LED Modules penetrates farther into tissue and provides further spectral separation from blue and green wavelengths for multi-opsin experiments.

PlexBright 550nm Lime LED Modules are recommended for stimulation of Archaerhodopsin (ArchT).



Activation Spectrum for Archaerhodopsin (ArchT)

²This plot was generated based on Fig. 1b of: Han X, Chow BY, et al., Frontiers in Systems Neuroscience. 2011, 5:18.



Excitatory Opsins

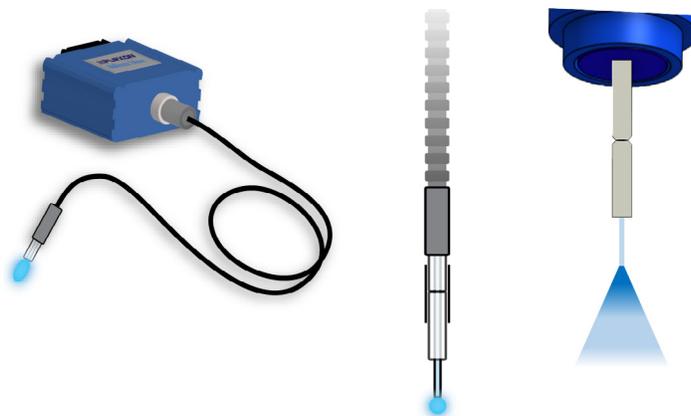
Opsin	Peak Activation	Suggested Wavelength
Channelrhodopsin-2 (ChR2)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (H134R)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (T159C)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (L132C)	474 nm	Blue (465 nm)
Channelrhodopsin-2 (E123A)	470 nm	Blue (465 nm)
Channelrhodopsin-2 (E123T)	490 nm	Blue (465 nm)
Channelrhodopsin-2 (E123T/T159C)	490 nm	Blue (465 nm)
ChIEF	450 nm	Royal (450 nm)
ChRGR	505 nm	Green (525 nm)
VChR1	545 nm	Lime (550 nm)
C1V1	540 nm	Lime (550 nm)
C1V1 CheETA (E162T)	530 nm	Green (525 nm)
C1V1 CheETA (E122T/E162T)	535 nm	Green (525 nm)
C-VChR1	570 nm	Lime (550 nm)
VCOMET	590 nm	Orange (620 nm)
Red activated Channelrhodopsin (ReaChR)	590 nm	Orange (620 nm)

Inhibitory Opsins

Opsin	Peak Activation	Suggested Wavelength
eNpHR 3.0	590 nm	Yellow (590 nm)
Arch/ArchT	566 nm	Lime (550 nm)
eBR	540 nm	Lime (550 nm)
Jaws	632 nm	Red (630nm)

Appendix F - LED Power Output at Various Points Throughout the System Light Path

PlexBright LED Modules Color (Wavelength/Max Current)	Measured Output ¹ (Normalized Output) At the LED Module	Measured Output ¹ (Normalized Output/High Performance .66NA Fiber)			Measured Output ¹ (Normalized Output/High Durability .5NA fiber)		
		At the tip of a 200/230µm Patch Cable*	At the tip of a 200/230µm Fiber Stub**	At the tip of a 110/125µm Fiber Stub***	At the tip of a 200/230µm Patch Cable^	At the tip of a 200/230µm Fiber stub^^	At the tip of a 110/125µm Fiber Stub^^^
Royal (450nm/300mA)	44.9mW	28.1mW (894mW/mm ²)	22.5mW (715mW/mm ²)	6.9mW (724mW/mm ²)	21.8mW (694mW/mm ²)	17.4mW (554mW/mm ²)	5.4mW (569mW/mm ²)
Blue (465nm/300mA)	40.2mW	24.9mW (792mW/mm ²)	19.9mW (634mW/mm ²)	6.1mW (645mW/mm ²)	19.0mW (605mW/mm ²)	15.2mW (484mW/mm ²)	4.7mW (495mW/mm ²)
Green (525nm/300mA)	11.8mW	7.8mW (249mW/mm ²)	6.2mW (199mW/mm ²)	1.9mW (201mW/mm ²)	5.8mW (185mW/mm ²)	4.6mW (146mW/mm ²)	2.4mW (253mW/mm ²)
Lime (550nm/500mA)	19.8mW	11.5mW (336mW/mm ²)	9.2mW (293mW/mm ²)	2.8mW (295mW/mm ²)	10.1mW (321mW/mm ²)	8.1mW (258mW/mm ²)	2.5mW (263mW/mm ²)
Yellow (590nm/250mA)	5.8mW	3.2mW (102mW/mm ²)	2.6mW (82mW/mm ²)	0.8mW (83mW/mm ²)	3.0mW (95mW/mm ²)	2.4mW (76mW/mm ²)	0.8mW (84mW/mm ²)
Orange (620nm/250mA)	17.5mW	11.0mW (349mW/mm ²)	8.8mW (279mW/mm ²)	2.7mW (283mW/mm ²)	9.2mW (293mW/mm ²)	7.4mW (236mW/mm ²)	2.3mW (242mW/mm ²)
Red (630nm/1000mA)	19.5mW	11.8mW (375mW/mm ²)	9.4mW (300mW/mm ²)	2.9mW (304mW/mm ²)	9.7mW (309mW/mm ²)	7.7mW (245mW/mm ²)	2.4mW (253mW/mm ²)
Crimson (660nm/1000mA)	22.8mW	15.1mW (476mW/mm ²)	12.0mW (381mW/mm ²)	3.7mW (386mW/mm ²)	11.5mW (366mW/mm ²)	9.1mW (290mW/mm ²)	2.8mW (295mW/mm ²)
Infrared 1 (850nm/1000mA)	21.3mW	13.2mW (422mW/mm ²)	10.6mW (337mW/mm ²)	3.2mW (342mW/mm ²)	10.7mW (341mW/mm ²)	8.6mW (274mW/mm ²)	2.6mW (274mW/mm ²)
InfraRed 2 (940nm/1000mA)	21.5mW	14.0mW (445mW/mm ²)	11.2mW (356mW/mm ²)	3.4mW (360mW/mm ²)	10.4mW (331mW/mm ²)	8.3mW (264mW/mm ²)	2.5mW (263mW/mm ²)
NIR 1 (740nm/1000mA)	12.3mW	8.5mW (270mW/mm ²)	6.8mW (216mW/mm ²)	2.1mW (219mW/mm ²)	6.5mW (206mW/mm ²)	5.2mW (165mW/mm ²)	1.6mW (163mW/mm ²)
NIR 2 (760nm/1000mA)	14.7mW	11.2mW (356mW/mm ²)	8.9mW (285mW/mm ²)	2.7mW (288mW/mm ²)	7.8mW (250mW/mm ²)	6.3mW (200mW/mm ²)	1.9mW (198mW/mm ²)
NIR 3 (780nm/1000mA)	11.0mW	7.2mW (228mW/mm ²)	5.7mW (182mW/mm ²)	1.8mW (184mW/mm ²)	5.7mW (180mW/mm ²)	4.5mW (144mW/mm ²)	1.4mW (143mW/mm ²)
NIR 4 (810nm/1000mA)	11.7mW	8.4mW (268mW/mm ²)	6.7mW (215mW/mm ²)	2.1mW (217mW/mm ²)	6.1mW (195mW/mm ²)	4.9mW (325mW/mm ²)	1.5mW (154mW/mm ²)
UV 1 (365nm/1000mA)	28.1mW	13.5mW (430mW/mm ²)	10.8mW (344mW/mm ²)	3.3mW (348mW/mm ²)	15.4mW (489mW/mm ²)	12.3mW (391mW/mm ²)	3.7mW (388mW/mm ²)
UV 2 (385nm/1000mA)	42.0mW	26.0mW (829mW/mm ²)	20.8mW (663mW/mm ²)	6.4mW (672mW/mm ²)	23.0mW (732mW/mm ²)	18.4mW (586mW/mm ²)	5.5mW (581mW/mm ²)
UV 3 (405nm/1000mA)	41.8mW	27.2mW (865mW/mm ²)	21.7mW (692mW/mm ²)	6.7mW (701mW/mm ²)	22.5mW (717mW/mm ²)	18.0mW (573mW/mm ²)	5.4mW (569mW/mm ²)



*Measured at the tip of a PlexBright High Performance (.66NA) Optical Patch Cable with a 200/230µm fiber 1.0m long.

**Measured at the tip of a PlexBright 200/230µm Fiber Stub Implant connected to a PlexBright High Performance (.66NA) Optical Patch Cable with a 200/230µm fiber 1.0m long.

***Measured at the tip of a PlexBright 110/125µm Fiber Stub Implant connected to a PlexBright High Performance (.66NA) Optical Patch Cable with a 200/230µm fiber 1.0m long.

^Measured at the tip of a PlexBright High Durability (.5NA) Optical Patch Cable with a 200/230µm fiber 1.0m long.

^^Measured at the tip of a PlexBright 200/230µm Fiber Stub Implant connected to a PlexBright High Durability (.5NA) Optical Patch Cable with a 200/230µm fiber 1.0m long.

^^^Measured at the tip of a PlexBright 110/125µm Fiber Stub Implant connected to a PlexBright High Durability (.5NA) Optical Patch Cable with a 200/230µm fiber 1.0m long.

¹ Power values are measured using pulsed stimulation (1 msec pulses)

Appendix F - LED Power Output at Various Points Throughout the System Light Path Cont.

PlexBright Head-Mounted LED Modules	Measured Output ¹ (Normalized Output)	Measured Output ¹ (Normalized Output/.66NA Fiber)
Color (Wavelength/Max Current)	At the LED Module	At the tip of a 200/230µm Fiber Stub (no patch cable required)
Head-Mounted Blue (465nm/300mA)	55mW	19.2mW (611mW/mm ²)
Head-Mounted Green (525nm/300mA)	15mW	5.2mW (166mW/mm ²)
Head-Mounted Lime (550nm/500mA)	22mW	7.7mW (245mW/mm ²)
Head-Mounted Orange (620nm/250mA)	24mW	8.4mW (267mW/mm ²)

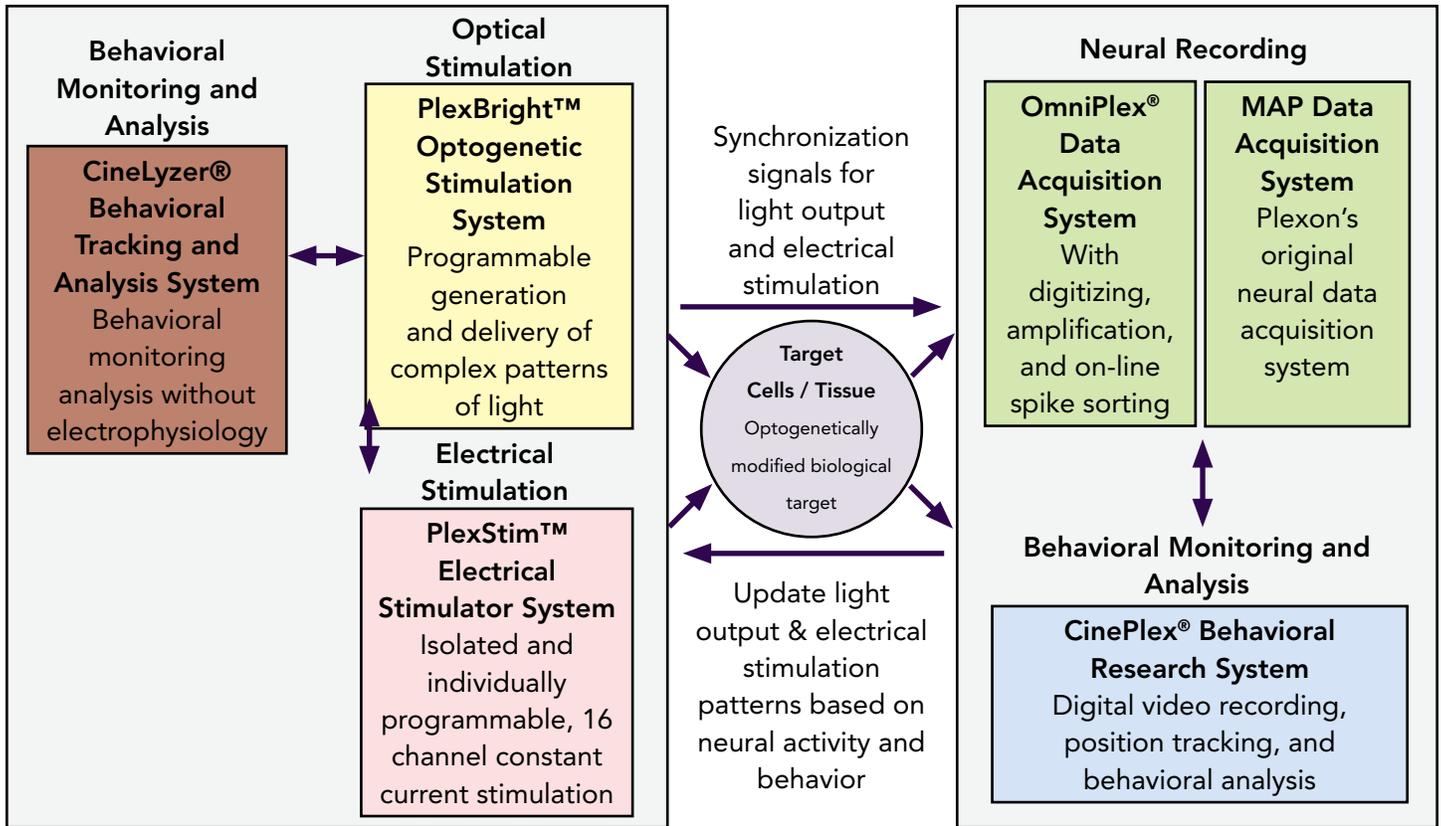
PlexBright Magnetic Head-Mounted LED Modules	At the LED Module	Measured Output ¹
Color (Wavelength/Max Current)	Measured Output ¹	At the tip of a 225/245µm Fiber Stub (no patch cable required)
Magnetic Head-Mounted Blue (465nm/300mA)	35.3mW	28.3mW (712mW/mm ²)
Magnetic Head-Mounted Green (525nm/300mA)	12mW	9.6mW (241mW/mm ²)
Magnetic Head-Mounted Lime (550nm/500mA)	14mW	11.2mW (322mW/mm ²)
Magnetic Head-Mounted Orange (620nm/250mA)	13mW	10.4mW (362mW/mm ²)

HELIOS LED Modules	Measured Output ¹	Available Light Output
Color (Wavelength/Max Current)	At the LED Module using 1ms pulses with 1% duty cycle	Per charge; Based on minimum power outputs
Blue (465nm/300mA)	55mW	2.3mWh
Green (525nm/300mA)	15mW	0.63mWh
Lime (550nm/500mA)	22mW	1.0mWh
Orange (620nm/250mA)	24mW	0.92mWh

¹ Power values are measured using pulsed stimulation (1 msec pulses)
 *Magnetic Head-Mounted LED modules have lower values for Measured Output at the the LED module because they are made with a smaller fiber (200/230)

Appendix G – Designing Closed-loop Experiments

PlexBright products are designed to pair with other hardware systems to offer powerful, closed-loop experiments where optical/electrical stimulation patterns are programmatically updated based on real-time feedback from neural acquisition and/or behavioral analysis systems. For more information about these systems, please visit the Plexon website or contact your Plexon Sales Engineer at info@plexon.com.



About Plexon Inc

Plexon is a pioneer and leading innovator of custom, high-performance data acquisition, behavior and analysis solutions specifically designed for scientific research. We collaborate with and supply thousands of customers including the most prestigious neuroscience laboratories around the globe driving new frontiers in areas including basic science, brain-machine interfaces (BMI), neurodegenerative diseases, addictive behaviors and neuroprosthetics. Plexon offers integrated solutions for *in vivo* neurophysiology, optogenetics, and behavioral research – backed by its industry-leading commitment to quality and customer support. For more information, please visit www.plexon.com.

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