Optogenetics

INTRODUCTION

Neuroscience has made great strides in the last twenty years. Technological innovations have enabled scientific discoveries in this field at a time where an aging population has placed great emphasis on the understanding of neurodegenerative conditions such as Parkinso n's and Huntington's disease.

To help understand the basis of neurodegenerative disorders researchers have turned to a new tool- optogenetics. By using both genetics and light to control the response of specific cells within living mammalian tissue, optogenetics is viewed as a potent new method to understand the basic of mechanisms of a host of diseases.

APPLICATIONS

In 2005 Karl Deisseroth reported the first use of transfected microbial opsins to control mammalian neurons through action potentials created by pulses of visible light.[1] The technique has since expanded to include additional proteins that can be stimulated by precise light frequencies. For example, phytochromes, cryptochromes and light-oxygen-voltage (LOV) domains are all now used to study applications not just in the neurosciences, but other fields as well.[2]

The use of optogenetics to investigate cellular processes is widely published. In one area of study, researchers used optogenetic techniques to understand cell proliferation and tumorigenesis. Specifically, the plant protein Phytochrome B is transfected into numerous types of mammalian cells to study proliferation and cell signaling- and optogenetic methods provide very precise light control to activate the opsins in question and pathways under study.[3]

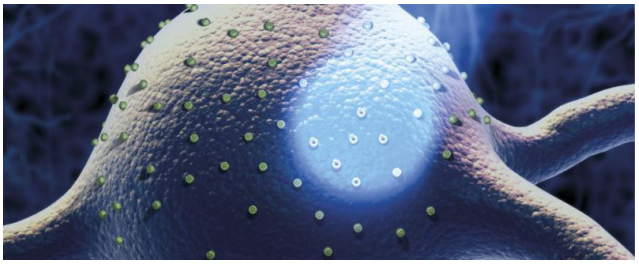


Image courtesy of Ed Boyden, McGovern Institute for Brain Research at MIT, Sputnik Animation.

At a molecular level, opsins such as bacteriorhodopsins, channelrhodopsins and halorhodopsins are utilized in the study of areas as diverse as opening and closing of ion channels, driving protein-protein interactions, observing conformational changes and controlling gene expression. The ability to employ these types of molecules permits the investigator to create experiments that will trigger the required physiological effect, provide the required kinetic properties of the light-modulated property under study as well as modulate the necessary power and spatial extent of the light signal that is employed.[4]

EXPERIMENTAL CONSIDERATIONS

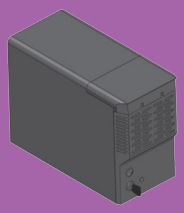
Optogenetic-based experiments broadly fit into two categories: whole animal *in vivo* experiments and microscopy-based *in vitro* experiments. This article will focus on the microscopy-based *in vitro* experiments. Regardless of application, the primary consideration in effective opsin regulation is the ability to activate or deactivate the cells or tissue under investigation. Several factors must be considered, including the type of cell or tissue under study, application of light to whole cells or smaller subregions and the wavelength of light required to activate or suppress the opsin in use and pathway under study.

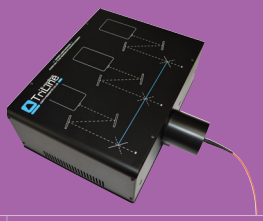
Opsins respond to very specific wavelength ranges so careful selection of your activation wavelength as well as any fluorescence probes being used is critical. Opsins respond

Continued on back →

Optogenetics Solutions

Choose Light Source





	LED	LDI (multimode lasers)	Laser Bank (single mode lasers)
Full field illumination	✓	✓*	×
Sub array only	×	×	✓
Full field or sub array	✓	✓	×

**With 1.5mm fiber*

TYPICAL WAVELENGTHS USED IN OPTOGENETICS

Wavelength	405	470	535	590	630
Rhodopsin / probe	Photoactivation	ChR2	CIVI	NpHR	Crimson

Many other wavelengths available

LED and Laser Controller Options

	OptoLED Lite	OptoLED	OptoFlash	LDI	Laser Bank
Channels	2	2	1	Up to 7	Up to 6
Feedback	×	✓	✓	✓	×
Over drive	×	✓	✓	✓	×
Digital modulation	50µsec	<1µsec	<0.1µsec	10µsec	<1µsec*

**Varies depending on laser*

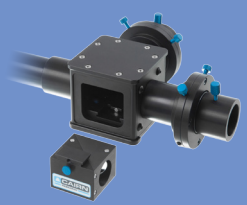
Multiport Couplers

Choose up to 4

1

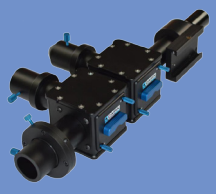
Single port coupler

2



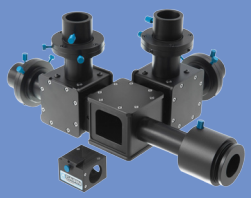
Duel port coupling

3



Triple coupling

4



Quad coupling

Optional fiber coupling for any LED

Any of the above light sources can be mounted on any port. Can also bring in LLG-coupled light source for standard imaging.

Microscope Interface

EPI-ILLUMINATION OR TRANSMITTED LIGHT PORT

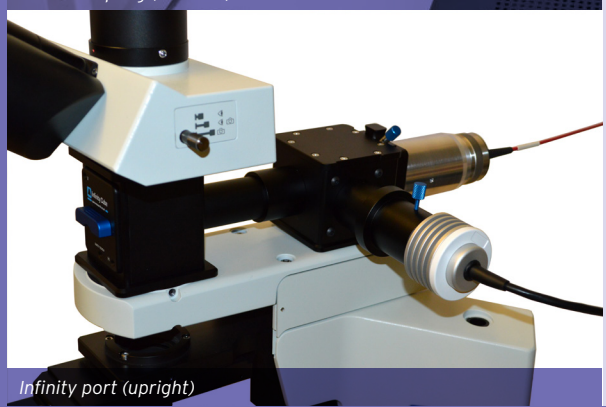
Epi illuminator (upright/inverted)

- *Port up to 4 light sources into epi or transmitted light path*
- *Enables full field illumination (sub field via microscope field stop)*

Transmitted port often used in conjunction with E-phys reading to activate ChR

DIRECT COUPLING TO UPRIGHT OR INVERTED STANDS

Direct coupling (inverted)



Infinity port (upright)

- *Port up to 4 light sources into epi or transmitted light path*
- *Increased power at sample (compared to epi port)*
- *Laser can be used to create a diffraction limited spot*
- *Full field or sub field imaging via optional adjustable field stop*
- *Optional safety interlocks on infinity port*

Easily configure one port for full field illumination and second for simultaneous sub region activation.

Continued from front

to light from 450 nm through 700 nm, depending on the specific opsin with 470 nm, 550 nm and 590 nm being the most common. For that reason, fine spectral tuning is a necessity, especially if activation/deactivation is accomplished through closely separated wavelengths.

HARDWARE REQUIRED

The hardware used for *in vitro* optogenetic-based experiments must be able to control light intensity, field of view (FOV) and multiple light inputs. Activation of opsins may require low to moderate levels of light over prolonged periods, or may require high intensity light in short bursts, depending on the system under study. As the intensity and kinetic requirements for opsins vary, the ability to modulate illumination intensity and pulse frequency is essential. LED's are often looked to as a reliable light-emitting source for optogenetic applications. LEDs are inexpensive, can be rapidly modulated and are easily adapted to most microscopes. To account for rapid pulsing of light required to excite or inhibit opsin activity, reliable and repeatable triggering methods are necessary. Millisecond-level temporal regulation necessitates electronic shuttering found in simple transistor-to-transistor logic (TTL) circuits. These circuits are well known and used extensively throughout a variety of light-based techniques and work equally well with and LED based optogenetic applications.

FOV must also be considered. *In vitro* applications may require activating a small field of view while imaging the entire field. And in the case of microscopy-based optogenetic applications it may well be necessary to combine an imaging light source with specific activation light sources.

THE 89 NORTH SOLUTION

There are two critical aspects of any optogenetics systems; the light sources themselves and how they are coupled into the microscope. The OptoLED is the preferred choices for microscope-based optogenetics.

The OptoLED is a 2-channel LED controller with closed-loop feedback control to ensure high stability and the ability to transiently over-drive the LEDs for additional power in short pulses. LEDs are available from 365 nm through 940 nm including, 470 nm, 565 nm and 590 nm.

The Multiport illuminator from Cairn Research Ltd. combines up to four separate illumination sources into a single easy to configure hardware solution. The Multi-Port Illuminator can be mounted to both the transmitted and reflected light pathways of upright and inverted microscopes and can selectively crop the FOV of illumination source while allowing full field illumination of the other sources. On upright microscopes, an infinity port adapter is available for many microscopes that couples the light directly into the infinity space between the trinocular head and microscope body, leaving the epi illumination port open. On inverted microscopes, a direct coupling is available that replaces the existing microscope epi illumination path, increasing throughput and power at the sample.

[1] Deisseroth K, Feng G, Majewska AK, Miesenböck G, Ting A, Schnitzer MJ. Next-generation optical technologies for illuminating genetically targeted brain circuits. *J Neurosci*. 2006 Oct 11; 26 (41): 10380-6.

[2] Pathak GP, Vrana JD, Tucker CL. Optogenetic Control of Cell Function Using Engineered Photoreceptors. *Biology of the cell / under the auspices of the European Cell Biology Organization*. 2013;105(2):59-72.

[3] Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. *Cell*. 2013 Dec 5;155(6):1422-34.

[4] Optogenetics in Neural Systems. Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K. *Neuron*. 2011 Jul 14;71(1):9-34.

89 NORTH
1 Mill St., Unit 285, Burlington, VT 05401 USA sales@89north.com www.89north.com
toll free 1.877.417.8313 main +1.802.881.0302 fax +1.802.881.0308