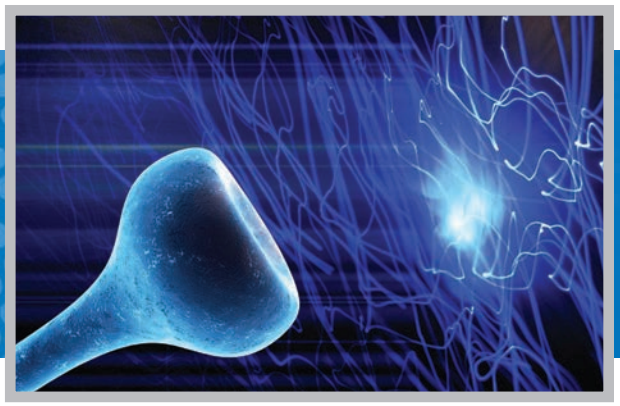
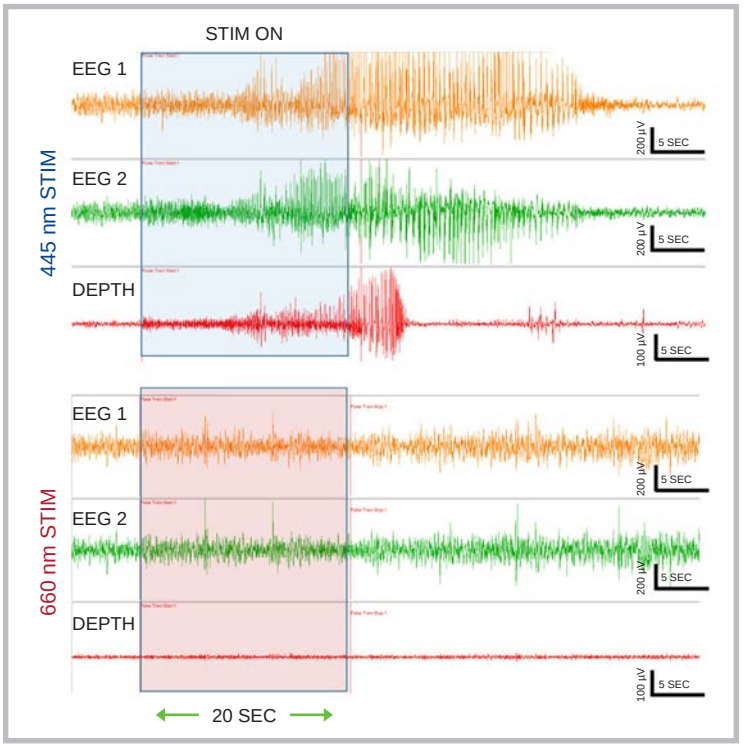
PINNACLE TECHNOLOGY INC

OPTOGENETIC SYSTEMS



STIMULATION INDUCES SEIZURE ACTIVITY



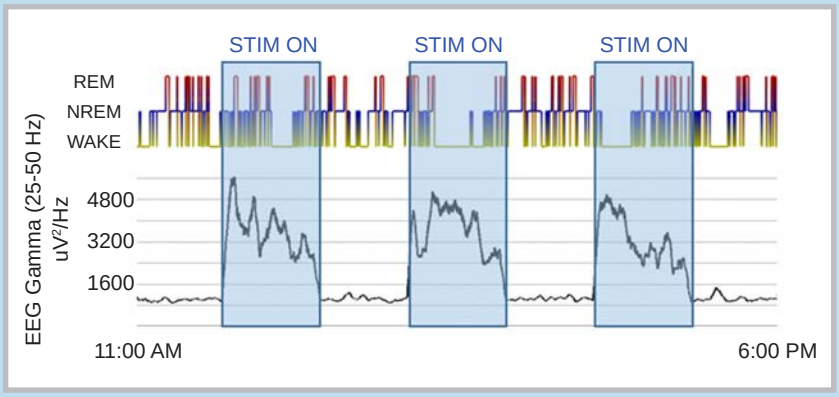
Cortical EEG and hippocampal depth electrode activity during and after stimulation with blue (445 nm) and deep red (660 nm control) light in the same transgenic mice from Jackson Laboratory expressing channelrhodopsin-2. Light pulses (20 Hz, 10% duty cycle, 600 mA) were delivered for 20 seconds. The blue LED power measured prior to implant was 130 mW/mm^2. The deep red LED power measured prior to implantation was 155 mW/mm^2.

Pinnacle's turn-key optogenetic system offers a simple, straightforward solution for a wide range of optogenetic experiments in rats and mice. Researchers from multiple fields can seamlessly integrate optogenetic control with simultaneous biopotential, neurotransmitter and behavioral recordings to accommodate a number of different experimental models. In early optogenetics experiments, broad areas of the brain were often illuminated, and in such cases, too much light is safer than not enough. As the optogenetic toolset improves and the experiments target more specific circuits and structures, precise light delivery is key.

PINNACLE SYSTEM ADVANTAGES

- Integrated biopotential/neurotransmitter recordings with optogenetics.
- Multiple wavelengths of LED probes with most having power >100 mW/mm^2 at the probe tip.
- No optical commutator needed.
- Precision timing and a variety of inputs/outputs.

OPTOGENETIC ALTERATIONS OF SLEEP AND EEG



Sleep activity (top) and EEG Gamma power (bottom) plotted during three periods of 1 hour optogenetic stimulation. Mice expressing channelrhodopsin-2 were implanted with optical fibers bilaterally into the thalamus. During 1 hour stim ON periods, blue light (445 nm) was applied via LED at 40 Hz and 10% duty cycle alternating in 10 second on/off cycles. During these periods, EEG activity demonstrated clear increases in high frequency power and significantly greater increases in waking activity. Corresponding clock time is shown on the X-axis. All stimulations were done during the lights-on period.

SYSTEM OVERVIEW

Our Optogenetics Interface Module controls precise timing and illumination of stimulation events. As the system's key component, it drives the headstage-mounted LED probes through an electrical commutator (no optical commutator required). Pinnacle's LED fiber probes are compatible with standard cannulas and surgical techniques. Multiple headstage configurations are available.

DATA CONDITIONING AND ACQUISITION SYSTEM

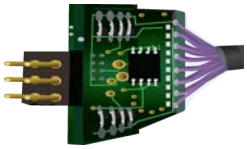
The Optogenetics Interface Module is compatible with our 3- and 4-channel Data Conditioning and Acquisition Systems (DCAS), our Sirenia® Acquisition software suite and most third-party systems.

TECHNICAL SPECIFICATIONS		
PARAMETER	3-CHANNEL	4-CHANNEL
Sampling Rates	200 Hz - 2 kHz	200 Hz - 20 kHz
Software Configurable Low-Pass Filters	10 Hz - 1 kHz	20 Hz - 15 kHz
ADC Resolution	14	16
Input/Output Feature	1 TTL I/O, 3 Analog Outputs	4 TTL I/O, 4 Analog Outputs

COMMUTATOR

A low-torque commutator (< 2 x 10^{-4} N-m) allows unencumbered movement for mice. For rats, we offer a Plastics One commutator with a two-plug setup for smooth, even rotation. Since Pinnacle systems place the light source at the headmount, no optical commutator is needed.

HEADSTAGES



Our headstages amplify and filter the signal at the headmount to deliver clean, artifact-free data. A secure friction fit connects the mouse headstage to the headmount. For rats, a Plastics One threaded coupler ensures a secure connection.

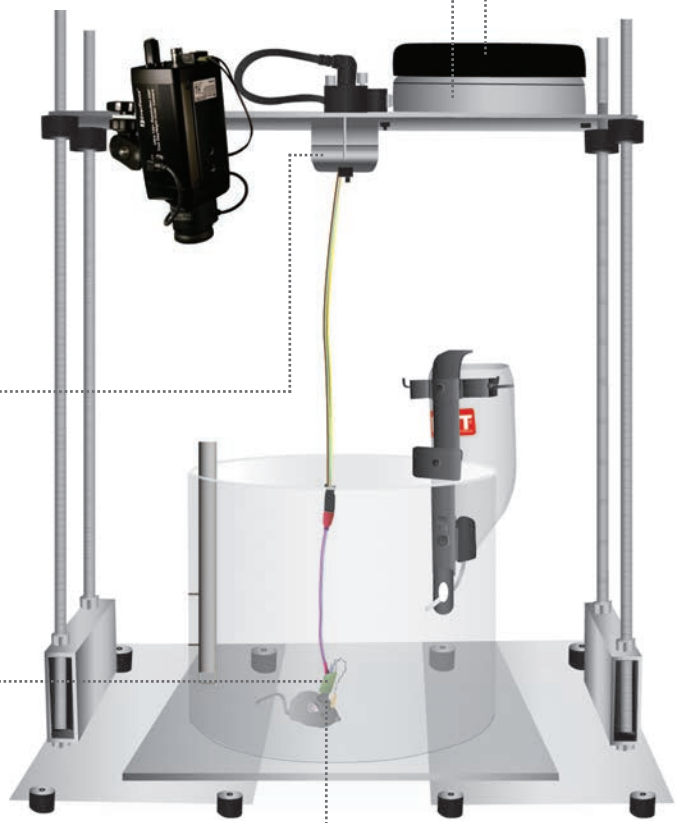
High-Pass Filters: 0.5 Hz EEG, 10 Hz EMG for sleep; 1.0 Hz EEG, 10 Hz EMG for seizure
Gain: X100 (X10 for rat seizure)

HEADSTAGE CONFIGURATIONS	
1 Opto	1 Opto, 4 EEG
2 Opto	1 Opto, 3 EEG, 1 Biosensor
1 Opto, 1 Biosensor	1 Opto 2 EEG, 1 EMG
1 Opto, 3 EEG	2 Opto, 2 EEG, 1 EMG
2 Opto, 3 EEG	1 Opto, 2 EEG, 1 EMG, 1 Biosensor

OPTOGENETICS INTERFACE MODULE

The Optogenetics Interface Module is the heart of the system. It offers precise control of the duration and intensity of optical stimulation for both continuous and discrete events.

- Highly accurate clock ensures < 1 ppm precision timing
- 3 digital inputs, 1 digital output
- 2 isolated inputs for external stimuli



HEADMOUNTS

Prefabricated headmounts reduce surgery time and allow precise, reproducible electrode placement, as well as ready-to-insert EMG leads for targeted electrode placement. Multiple configurations are available, with EMG placement at 90° or 270° rotation. Ask about custom headmount configurations as well.

LED FIBER PROBES

Pinnacle's optogenetics stimulation employs powerful LEDs, with most wavelengths delivering 100 mW/mm^2, coupled to a 200 µm fiber optic cable with 50 µm cladding. Pinnacle's LED fiber probes can be easily implanted using standard stereotaxic techniques. A list of available probes is shown below.

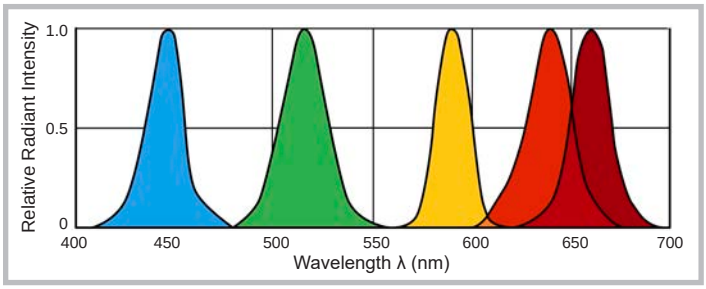
The LED fiber probe wavelength selection is dependent upon the opsin that is being used. By overlaying the opsin absorption spectrum with the LED emission spectrum the appropriate LED probe can be selected.

LED FIBER PROBES

	POWER (mW/mm^2)	
PEAK WAVELENGTH (nm)	@300 mA	@600 mA
445 Deep Blue	69	114
465 Blue	66	112
515 Green	62	NR*
590 Yellow	78	NR*
620 Amber	87	162
640 Red	82	104
660 Deep Red	97	163

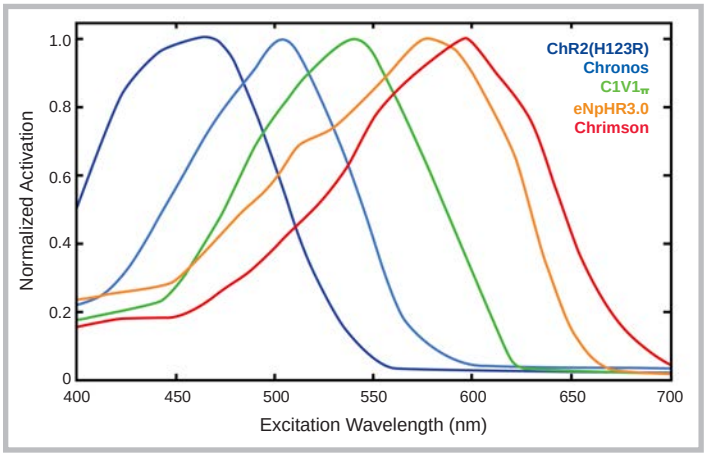
Average power over lots measured at the fiber probe tip (1 kHz, 10% duty cycle).
*Not recommended

LED EMISSION SPECTRUM



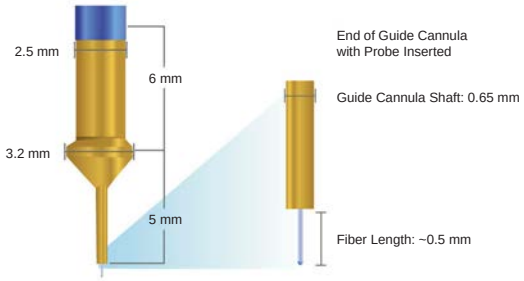
Emission spectra for some of Pinnacle's LEDs.

OPSIN ABSORPTION SPECTRUM

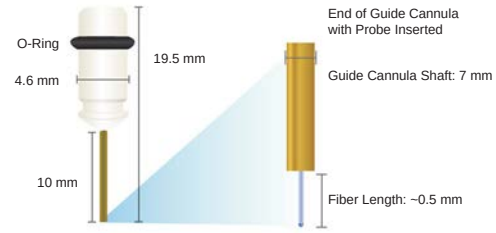


Activation wavelengths for several opsins. Data are summarized from published articles.

OPTOGENETIC PROBES FOR MICE



OPTOGENETIC PROBES FOR RATS



DEPTH ELECTRODES

Histology slice showing the brain region near the implanted cannula and depth electrode. The blue shading shows the estimated LED light penetration from the 200 µm tip. Green coloring indicates channelrhodopsin expression in tissue.

Stereotaxically placed guide cannulas allow for the insertion of LED fiber probes during surgery. Probe cannulas and prefabricated headmounts are affixed to the skull with dental acrylic and the headstage acts as a connection port for up to two probes. Pinnacle offers a wide variety of prefabricated headmounts and connectors to support multiple experimentation requirements.

For researchers interested in deep brain recordings, depth electrodes are available to measure neuronal activity in a localized area before, during and after optical stimulation. These specialized depth electrodes are ideal for EEG measurements that require a high level of recording precision and insight into subcortical neurological functions.

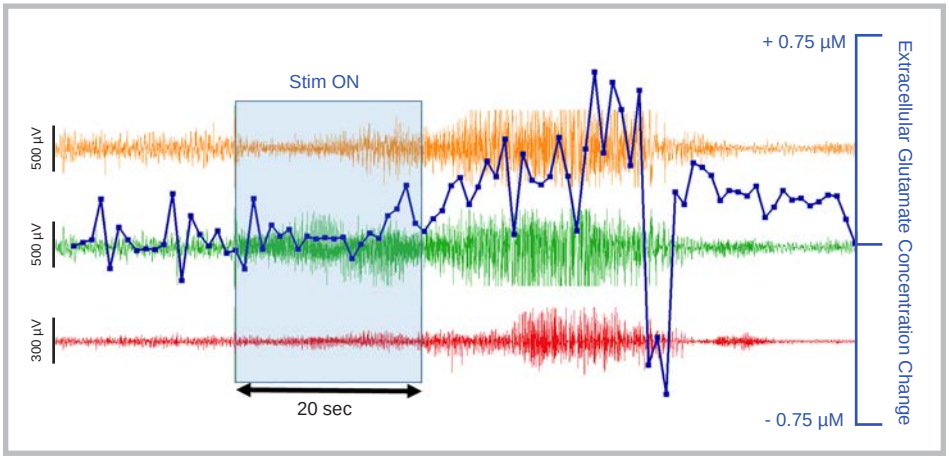
CUSTOM CONFIGURATIONS AVAILABLE

Contact a Pinnacle representative at 785-832-8866

OPTO AND SENSORS

Optogenetics, when coupled with biosensors, allows a better understanding of how networks function and behave in the brain. The graph to the right demonstrates the power of optogenetic techniques combined with simultaneous EEG and biosensor measurements. The ability to precisely measure neurotransmitter activity with optogenetic induced events provides valuable information to researchers in understanding how the neuronal network functions. The real-time second-by-second data from the biosensors is a perfect match for the precision timing of the optogenetic stimulation.

OPTOGENETICALLY INDUCED SEIZURE AND GLUTAMATE ACTIVITY

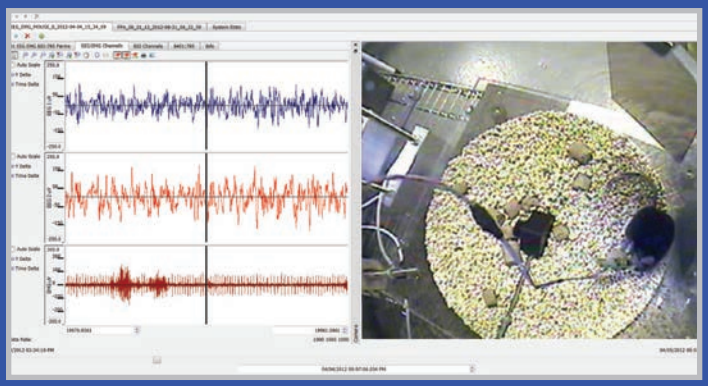


A transgenic mouse expressing channelrhodopsin-2 was surgically implanted with cortical EEG recording screws (EEG1, EEG2), an LED fiber probe and depth electrode in the right hippocampus, and a glutamate biosensor in the right frontal cortex. One week following surgery, blue (445 nm) light (>100 mW/mm^2) was delivered (20 Hz, 10% duty cycle) for 20 seconds. Seizure activity was evident on all three electrode channels during and immediately following application of the stimulation. Extracellular glutamate activity sampled every second demonstrated a seizure-related increase followed by a sharp decline at the end of the seizure event.

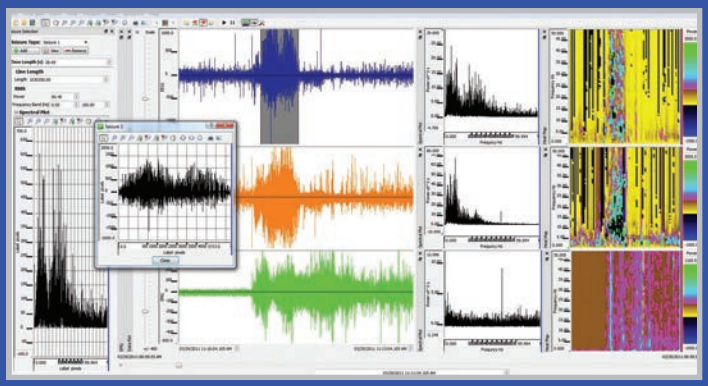
COMMON RESEARCH APPLICATIONS

SLEEP · BEHAVIOR · SEIZURE · LEARNING & MEMORY

SUPPORTING PRODUCTS



EEG and EMG data are recorded simultaneously with full-color video.



Seizure in a C57 mouse selected for automated analysis using Sirenia® Seizure Pro.

SYNCHRONIZED VIDEO

Integrated video recording provides a platform for synchronizing EEG and EMG changes with observable behavioral states. Video can be added to any new or existing hardware system. Captured video is displayed live on screen as it is streamed from the animal and is synchronized with other recorded data in playback mode.

SIRENIA® X-Y TRACKING

Track real-time or pre-recorded locomotor behavior in wireless or tethered animals. Includes quadrant and user-defined zone analysis, speed plots, movement trajectories, heat maps and overlay capabilities.

SIRENIA® SEIZURE PRO

Quickly identify, analyze and log user-defined seizure events over time. Analysis tools include spectral power and peak frequency computation, heat maps, line length, Racine's scale rating, seizure duration and time between seizure events. Generate custom reports and graphs based on aggregate seizure data.

SIRENIA® FEEDBACK PRO

Create rule sets using baseline data, thresholds and power analysis to initiate stimuli in real-time for a variety of sleep, seizure, optogenetics and other studies.