Optical control for vision restoration

Samar Mohanty

Biophysics and physiology Group
University of Texas-Arlington

Outline

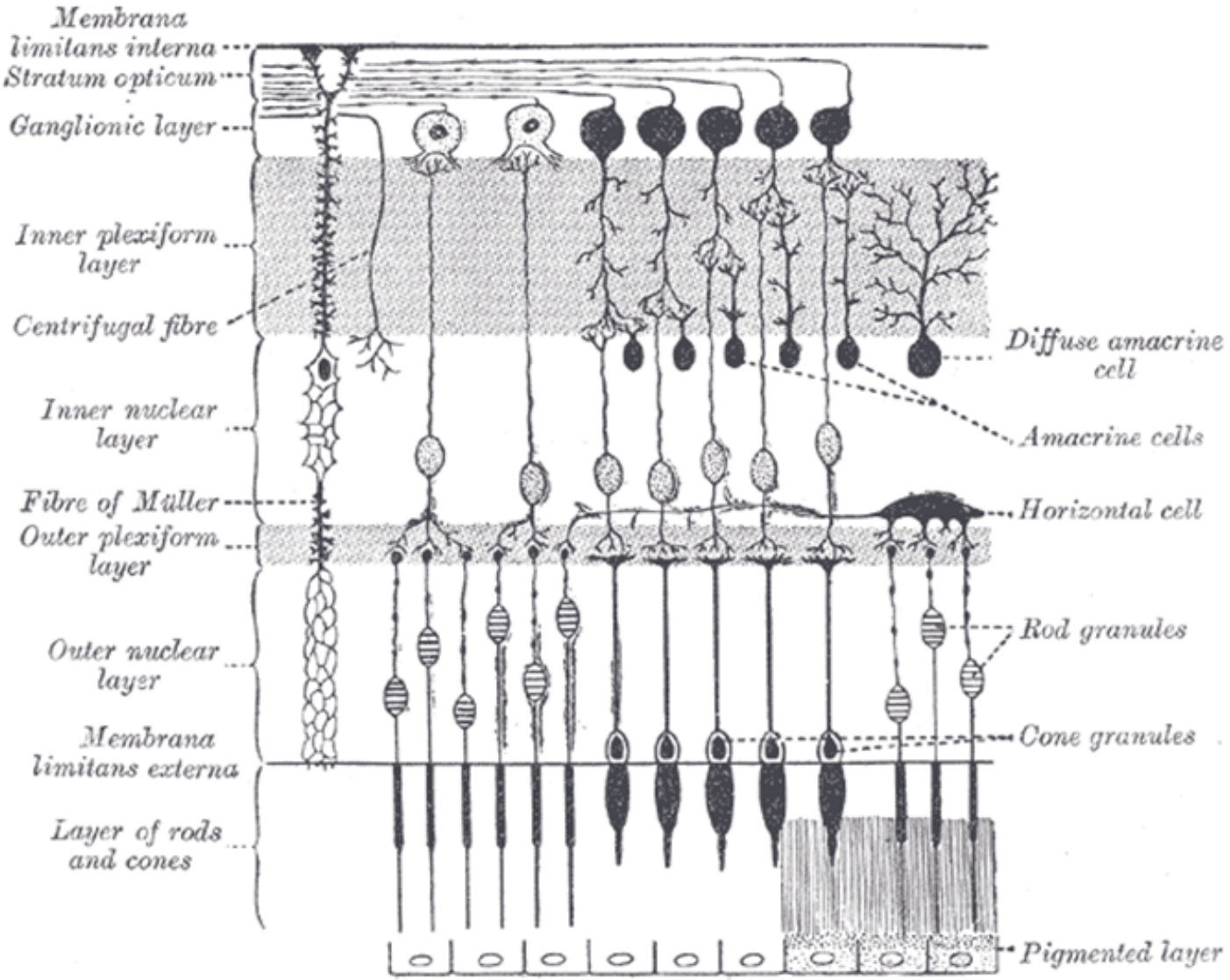
- Introduction
- Optogenetics basics
- Non-viral delivery
- Vision restoration in RP mouse models
- Retinal prosthetic development

==========

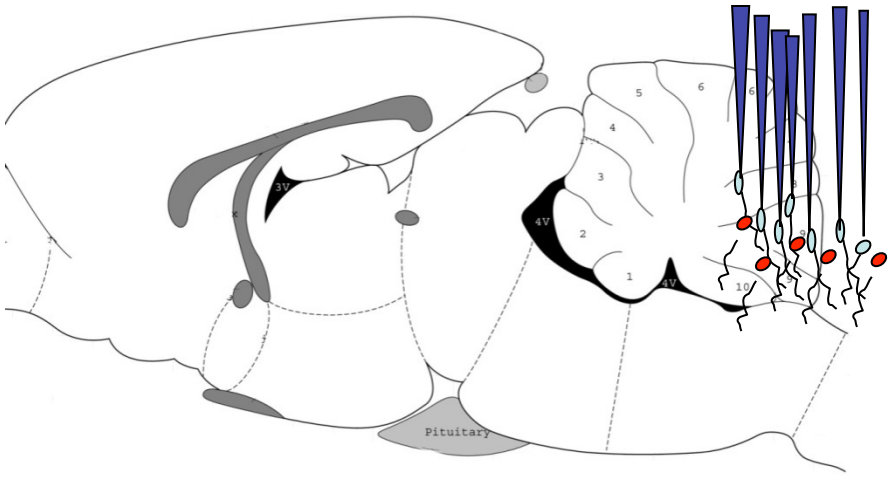
- Enhancing light –sensitivity
- Targeted optical gene delivery
- Optical detection of neural activity
- Probing retinal circuitry by localized stimulation
- Conclusions

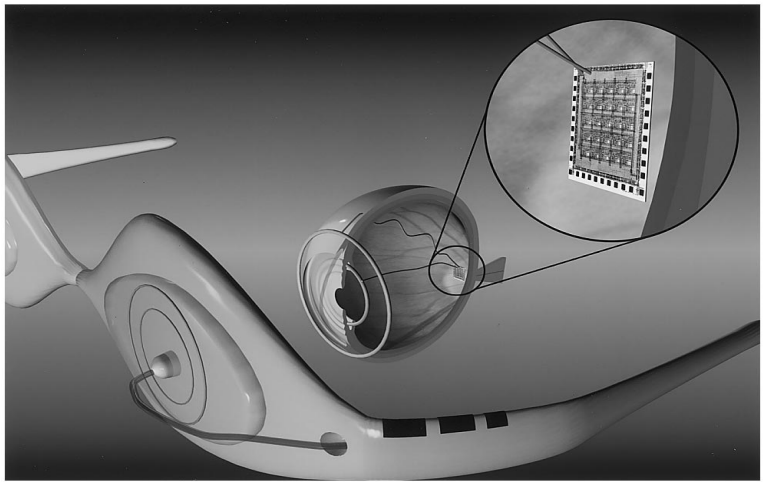
Retinitis Pigmentosa

- RP causes night-blindness and a loss of peripheral vision through the progressive degeneration of the retina.
- Loss of photoreceptors !!!!



Electrical stimulation: The problem with selective activation of chemically defined groups of neurons…



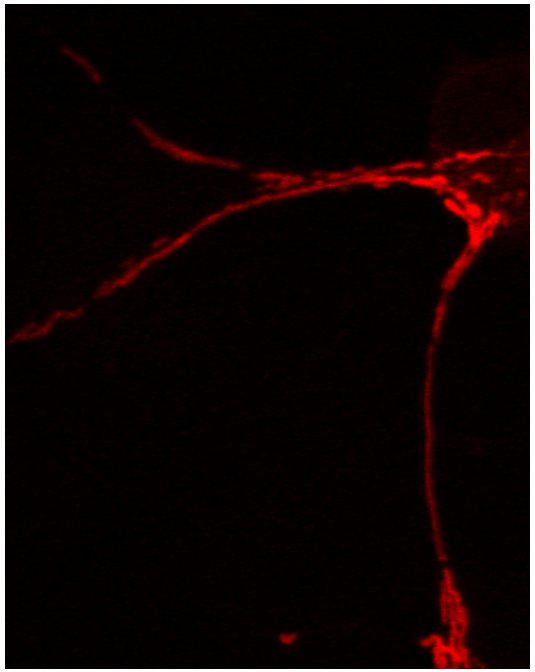


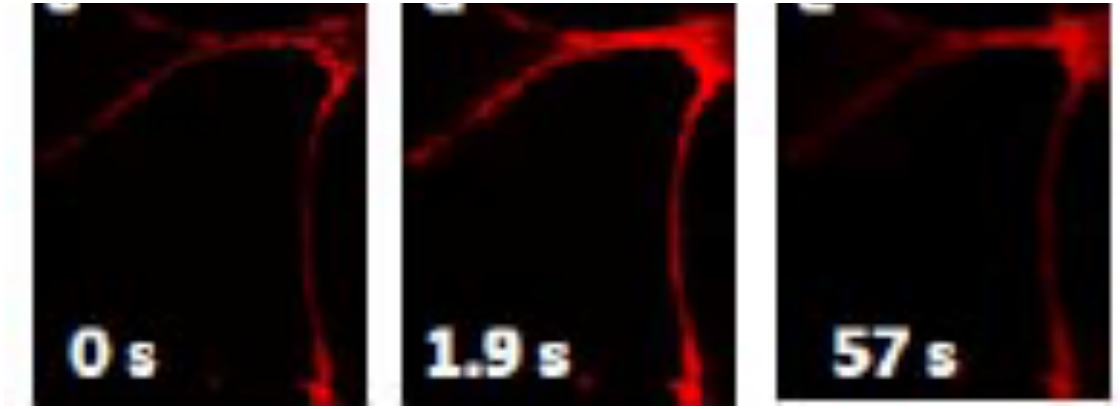
Controlling function of specific neurons (e.g. ON vs OFF) in native circuitry

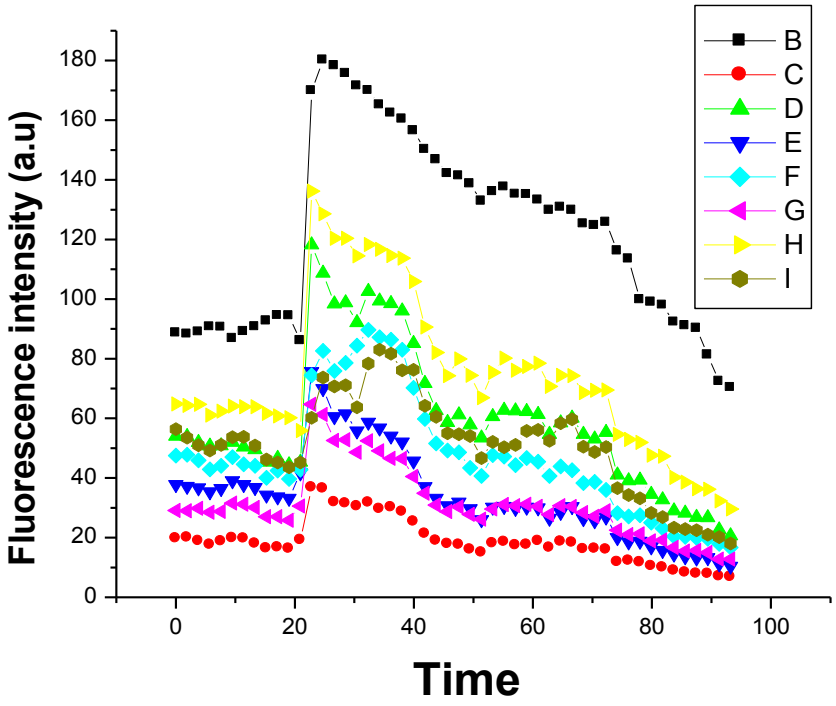
→ Requires dissection to allow accurate electrical stimulation

→ Thus inherently difficult in live animals.

Optical stimulation : The problem with selective activation persists

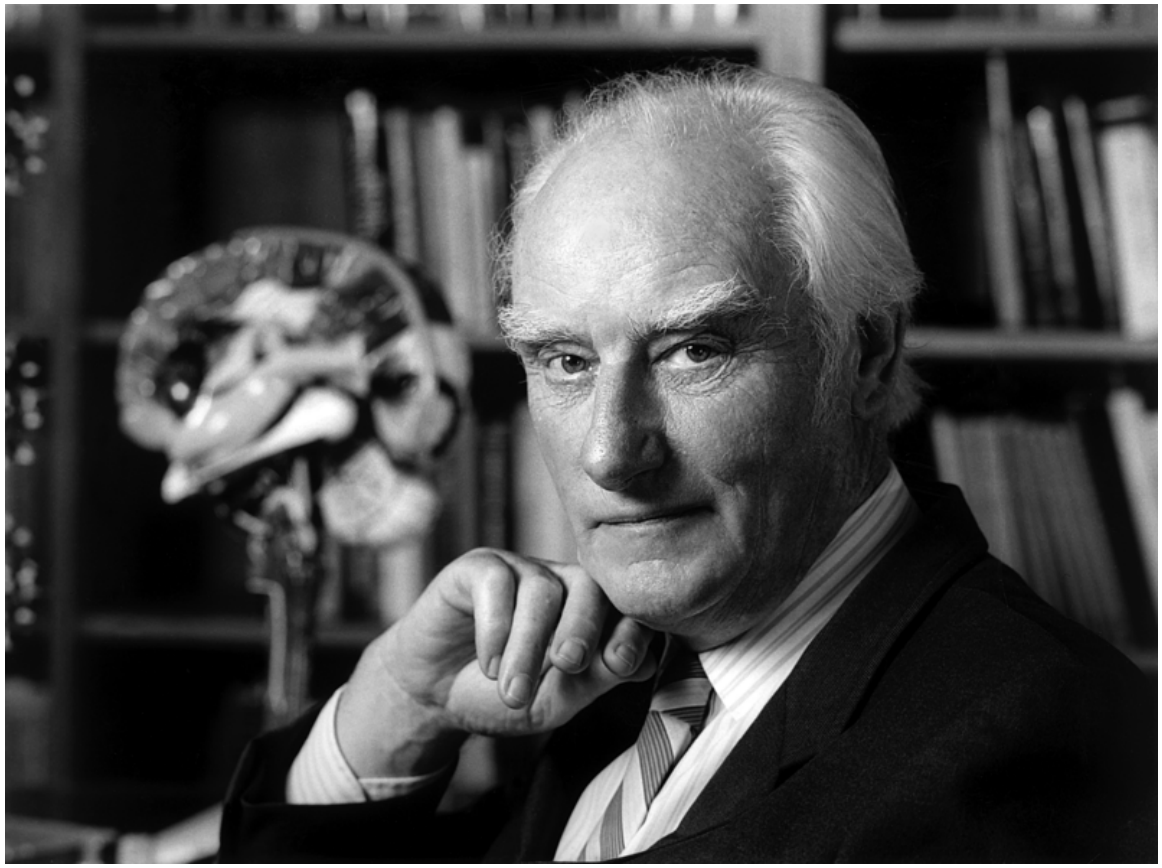






- High power levels (800 nm, 76 MHz, 25 mW)

→Activation (calcium changes) observed



Francis Crick (1916-2004); Nobel Prize : 1962

“a method by which all neurons of just one type could be inactivated, leaving the others more or less unaltered”

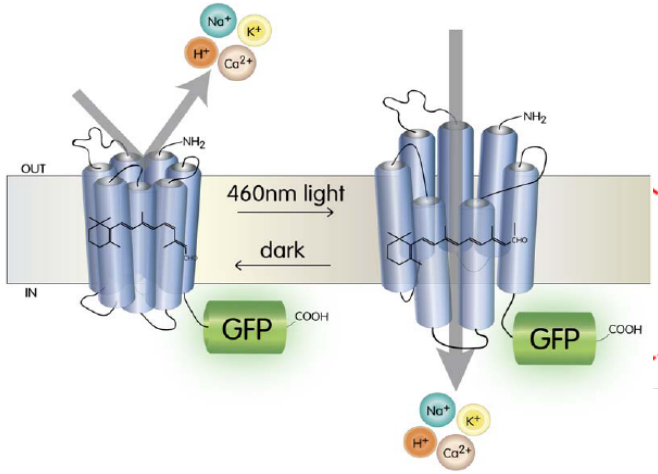
Emerging methods that combine genetics and optics have neuroscientists glowing about the possibilities of

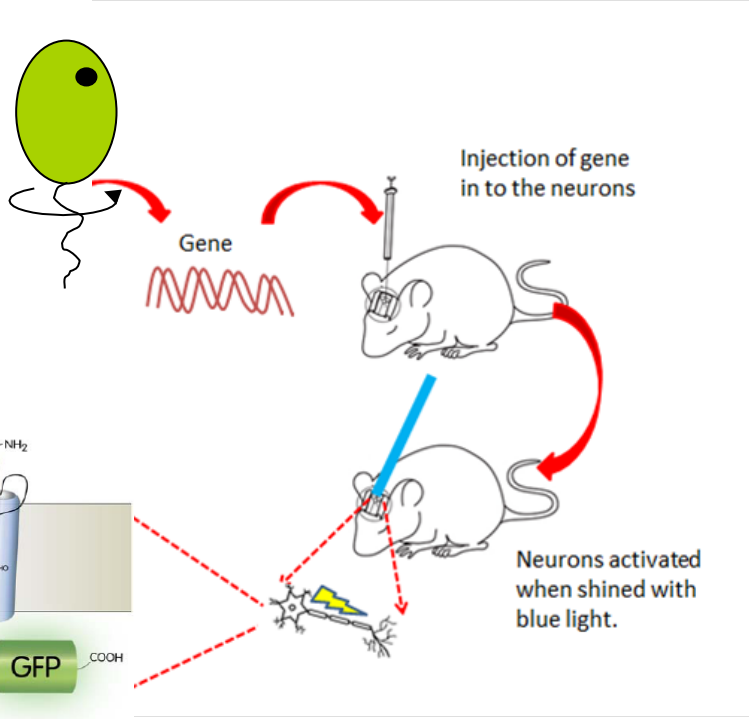
How to get a
remote-controlled switch into the brain/ retina?

Opsin: the perfect photo-chemical switch

Light-gated cation channel from the green alga *Chlamydomonas reinhardtii*

- Eye Spot contains 2 light-sensing proteins:Channelrhodopsin-2 (Cation channel) 7-transmembrane-helix proteins





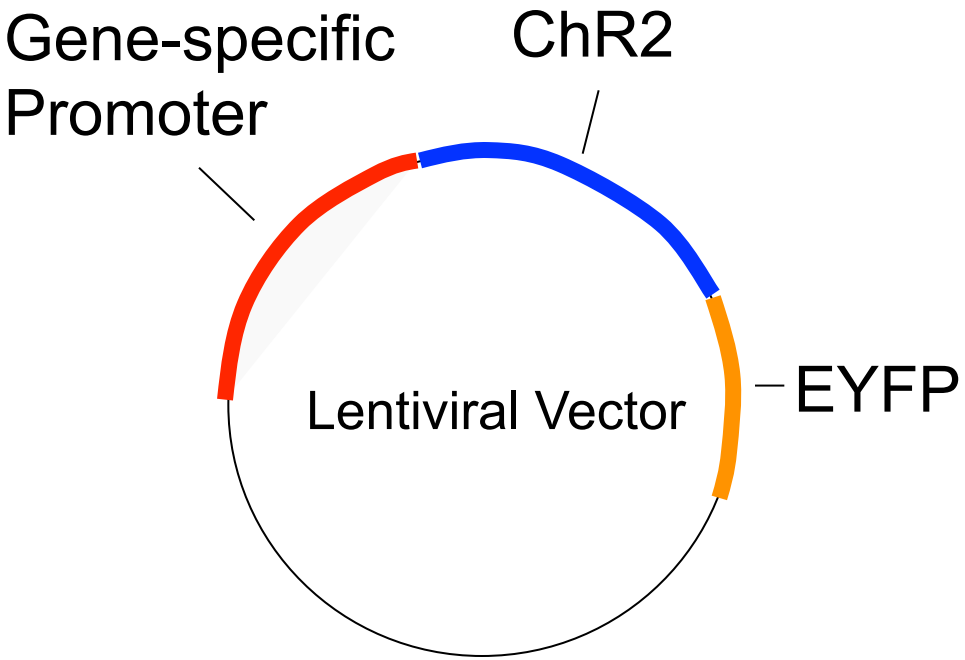
- Activated by a band of blue light (440-500 nm)
- Intensity required:< mW/ mm^2

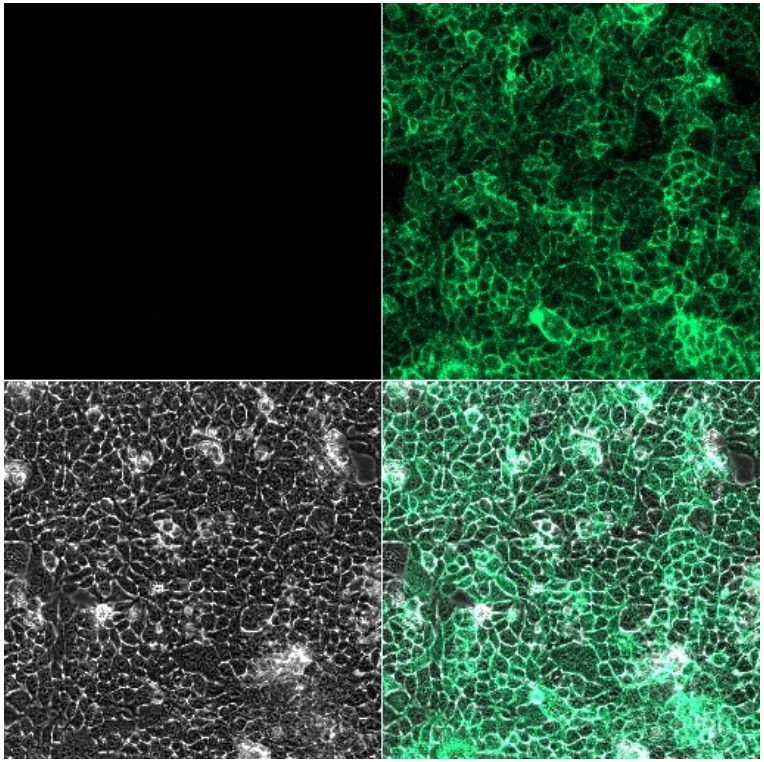
→ in-vivo application possible

- Ca^{2+}/Na^{+} channel with ms-kinetics

- **Genetically targeted**
- **Temporally precise**
- **Minimally-invasive**

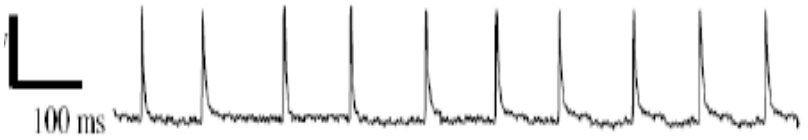
Model: ChR2-EYFP transfected HEK 293 cells





- Specific Promoter: ensures expression in cells that produce the transmitter/gene-of-interest.
- EYFP: allows easy identification for opto/ electrophysiology.
- Electrophysiology/ calcium imaging.

YFP only, Ex: 514 nm, red channel detection minimized



Advantages (over electrical stimulation)

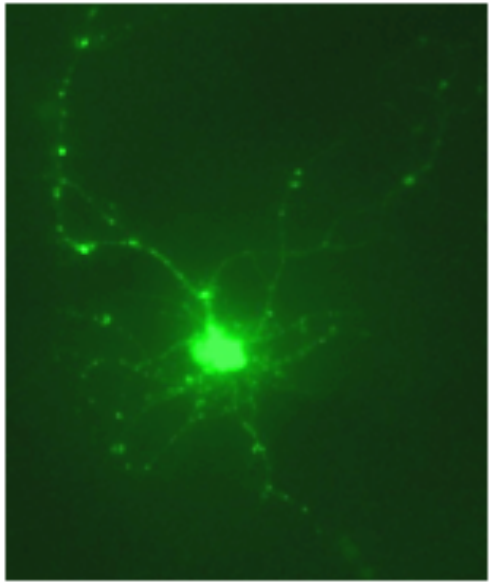
- Optogenetics (minimally invasive) eliminates the highly challenging requirement of placing electrode arrays.
- Higher spatial resolution (single cell or sub-cellular level) and Offers the necessary millisecond-temporal precision.
- Cellular specificity (ON/OFF etc).
- Allows rapid reversibility.
- High throughput (parallel stimulation of multiple cells, wide area)
- Less susceptible to contamination and no requirement of mechanical stability & electrical noise reduction.

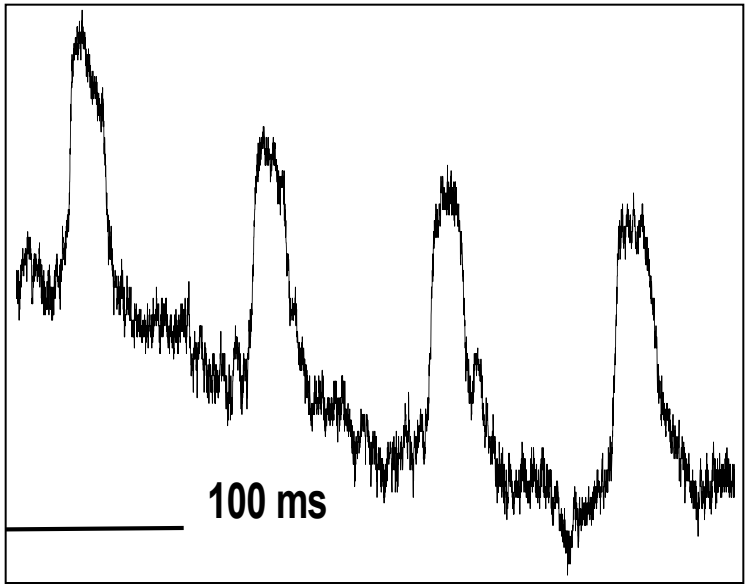
Optogenetic restoration of vision

Retinitis pigmentosa: Loss of photoreceptors !!!!

Thy1-specific expression of ChR2 in retinal ganglion cells or mGluR6-specific expression in ON Bipolar cells.

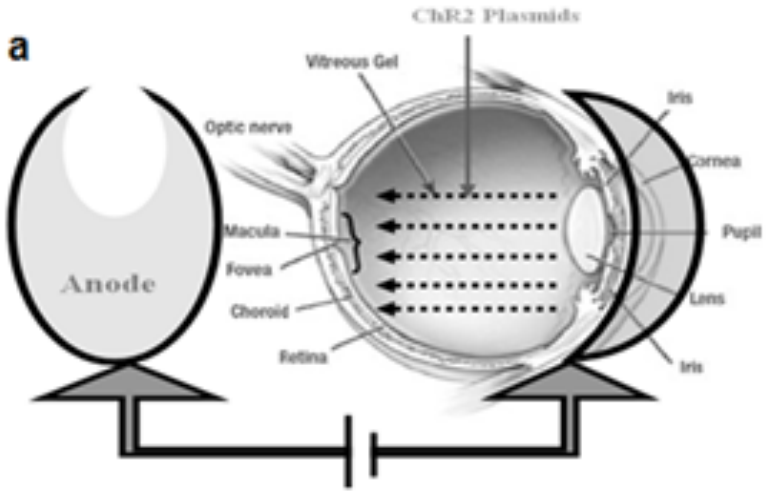
→ Potential strategy for the restoration of vision after rod and cone degeneration.

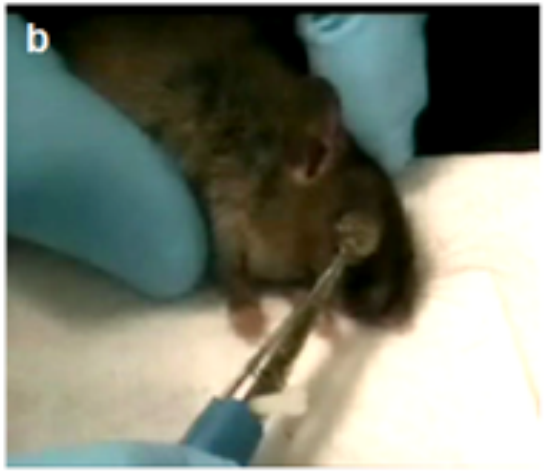


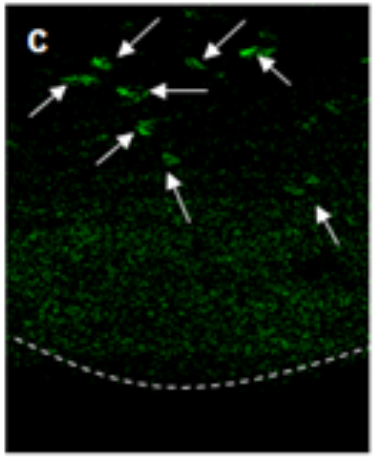


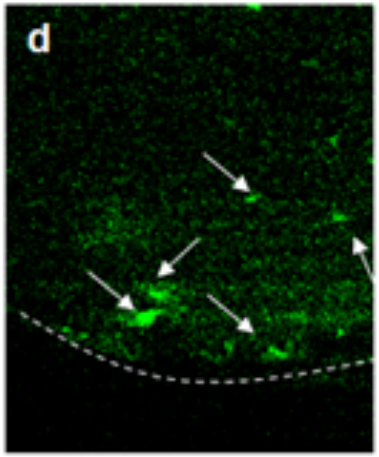
Left: Neuron expressing opsin. Right: Optogenetically-evoked potential in retinal ganglion cells.

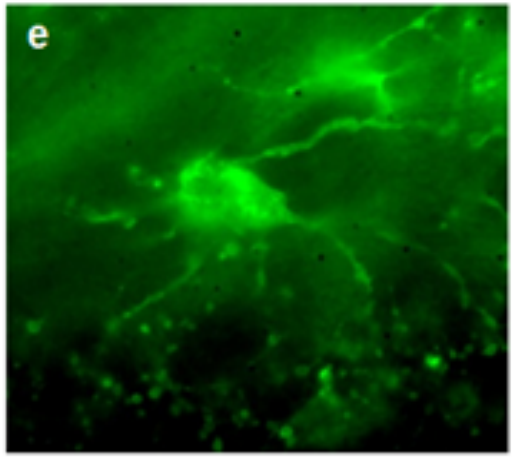
Non-viral delivery of opsin-encoding genes







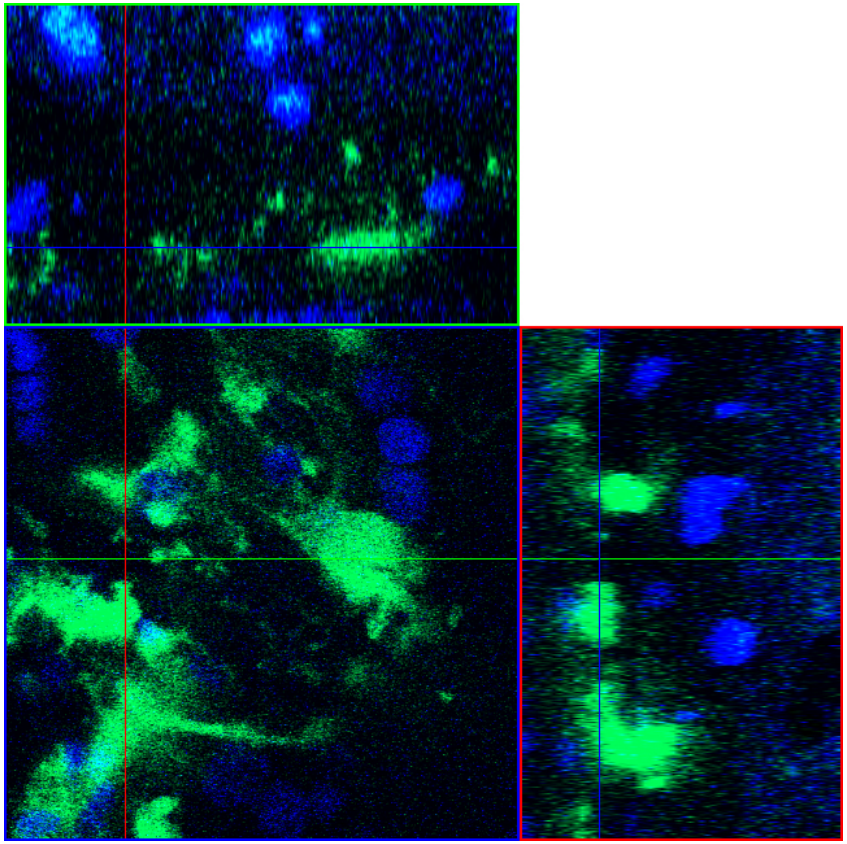


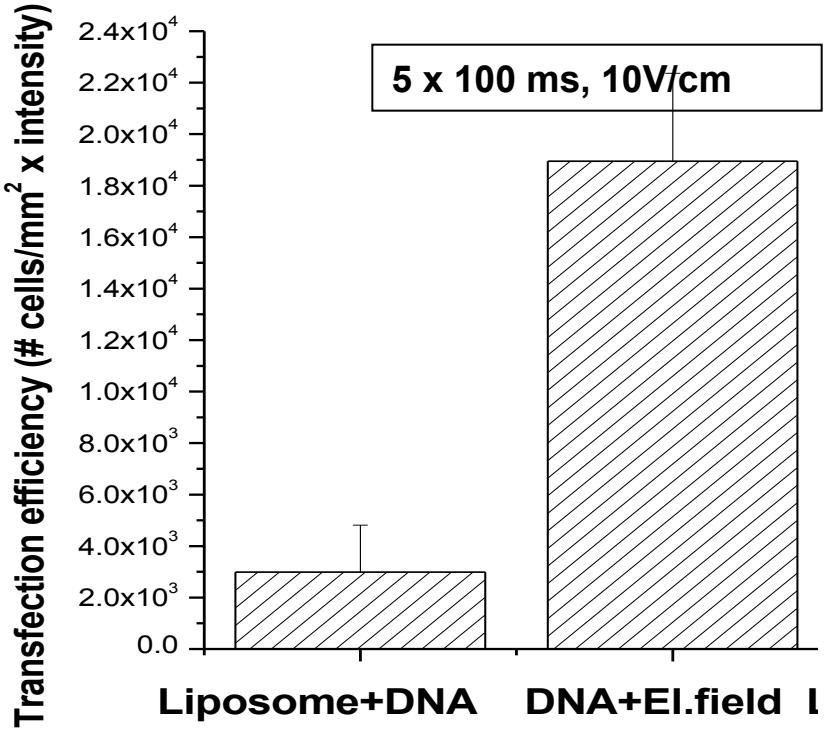


Non-viral delivery of opsin to *rd1* mice retina. (a) Schematic method for electroporation-based delivery of ChR2. The dotted arrows show the direction of movement of plasmids via electroporation. (b) Picture of electrode for *in-vivo* transfection of ChR2 encoding plasmids into the retina by electroporation.

(c-d) *In-vivo* confocal image of *rd1* mice; the panels c & d show the Z-stack images of the same eye separated by 30 microns along the axial direction. Dotted curve lines represent the outline of the pupil. (e) ChR2-YFP expressing retinal ganglion cell in an explant.

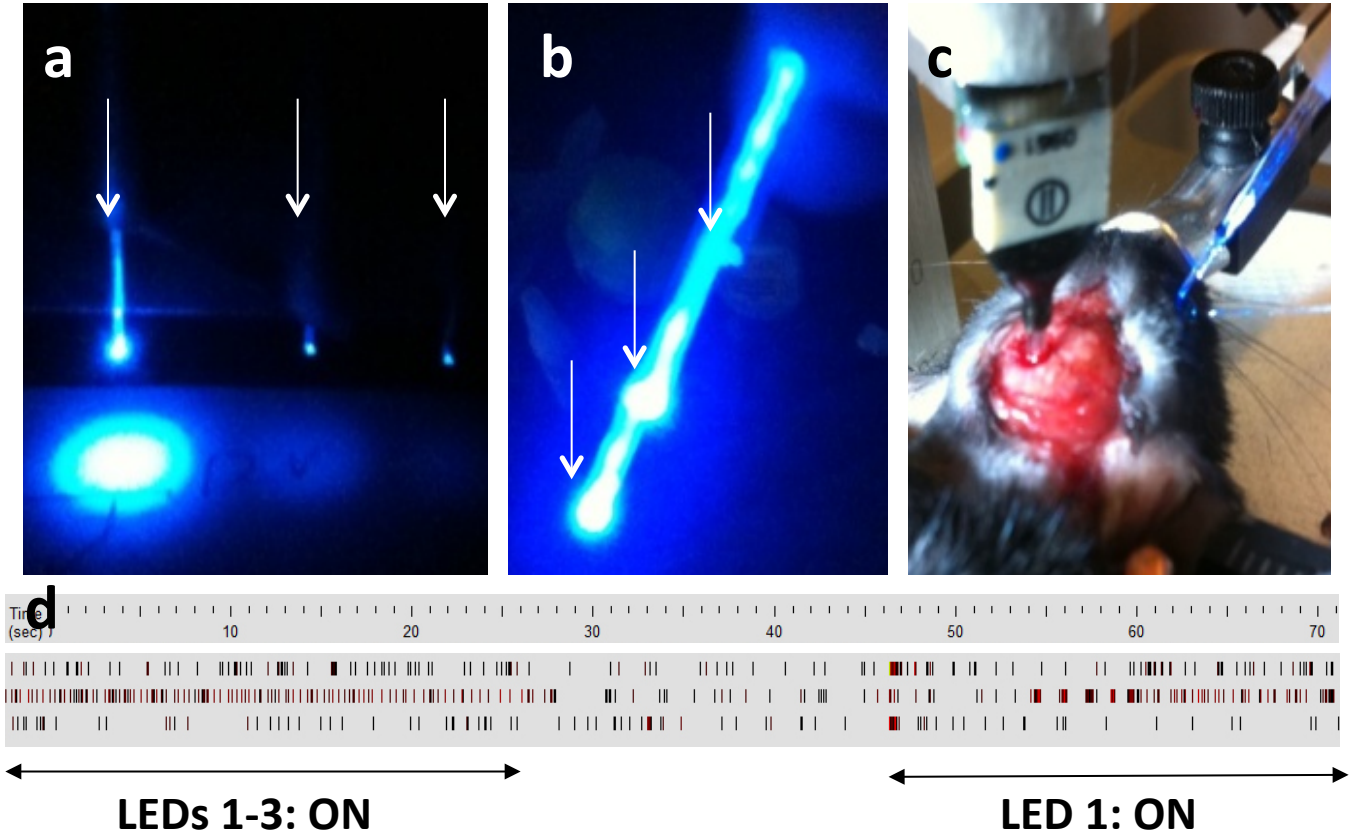
Efficiency of transfection





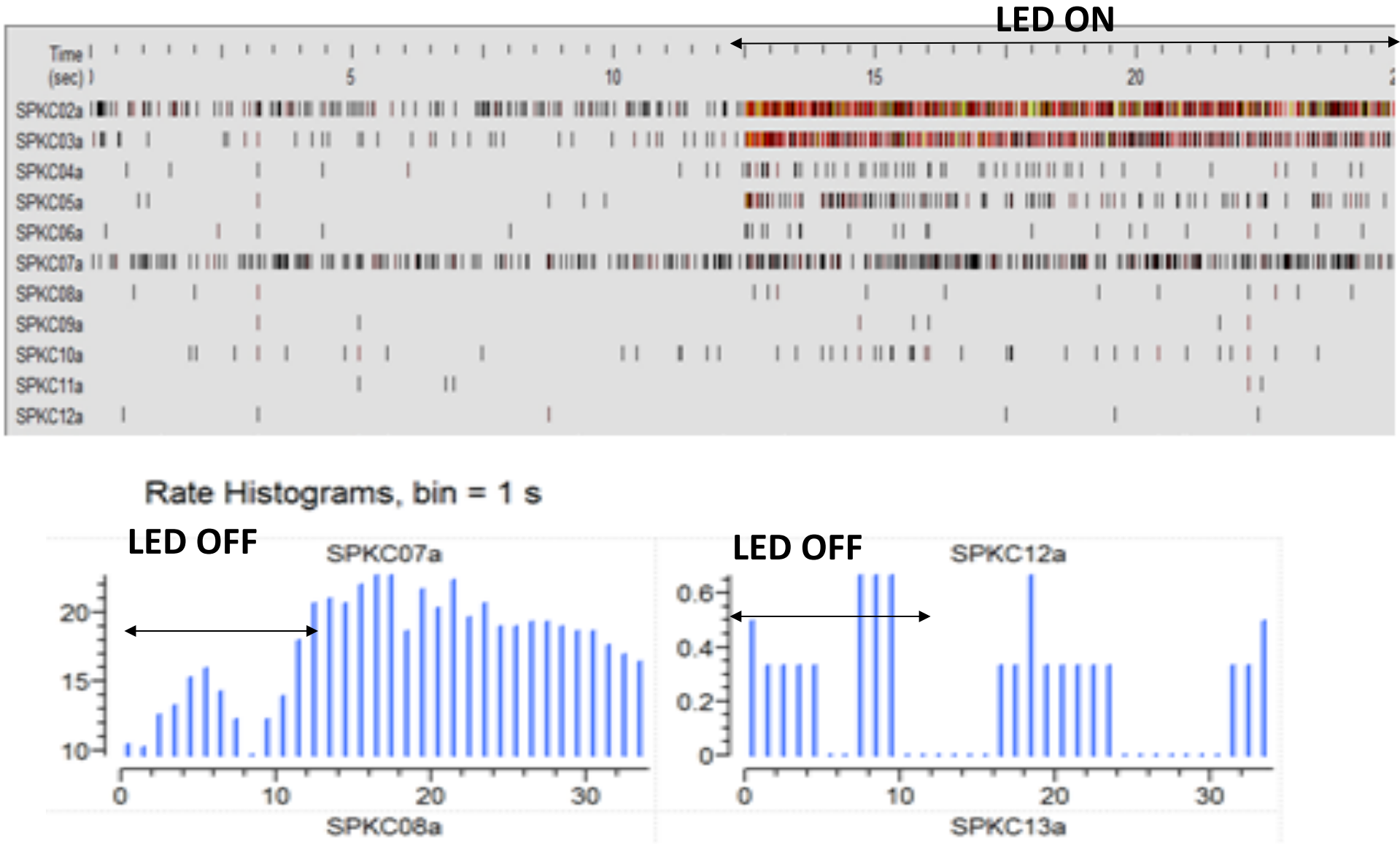
Confocal imaging of YFP expression in the retinal explant from rd1/rd1 mouse eye being treated with Thy1-ChR2-YFP by electroporation.

In vivo recording of VEP from rd1/rd1 cortex

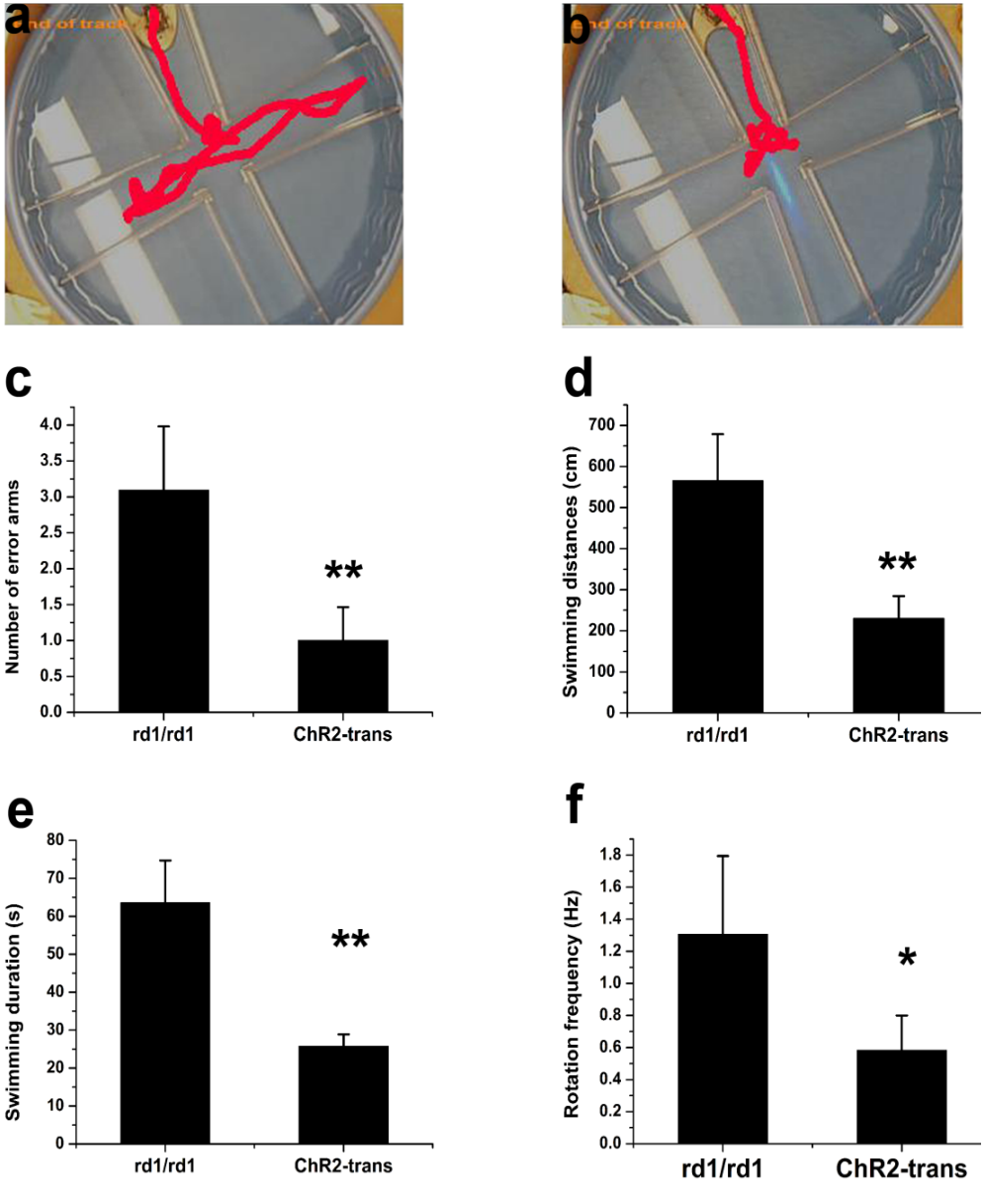


Light-evoked spiking activities in visual cortex of mice having optogenetically-sensitized retinal ganglion cells. A typical spatial pattern of *in-vivo* spiking activities in visual cortex (V1) subsequent to stimulation of the Thy1-ChR2-YFP expressing eye by blue (473 nm) LED.

In vivo recording of VEP from rd1/rd1 cortex

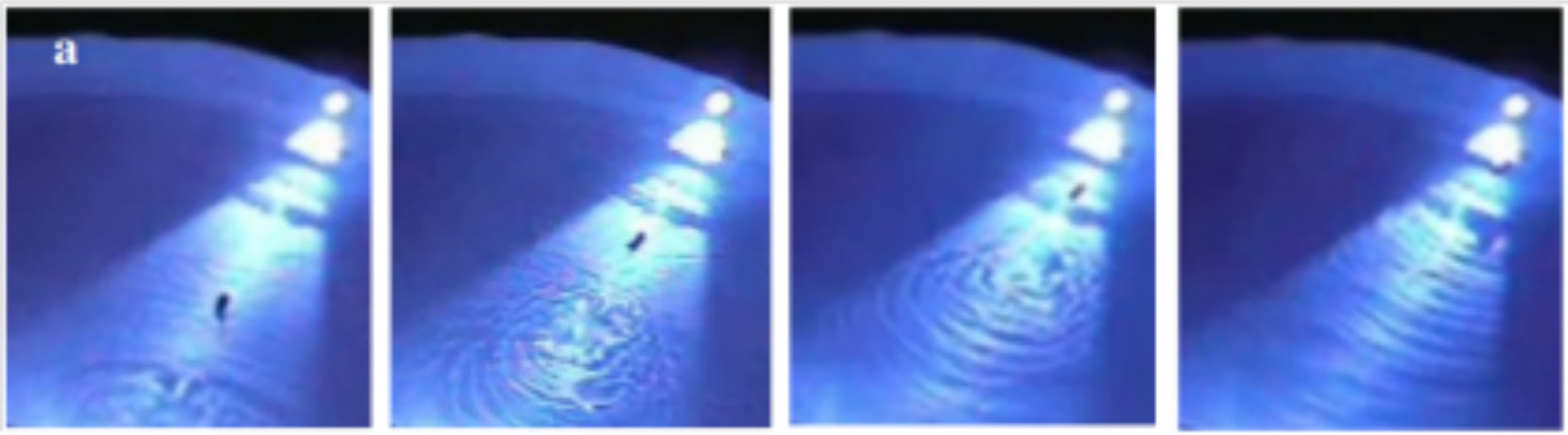


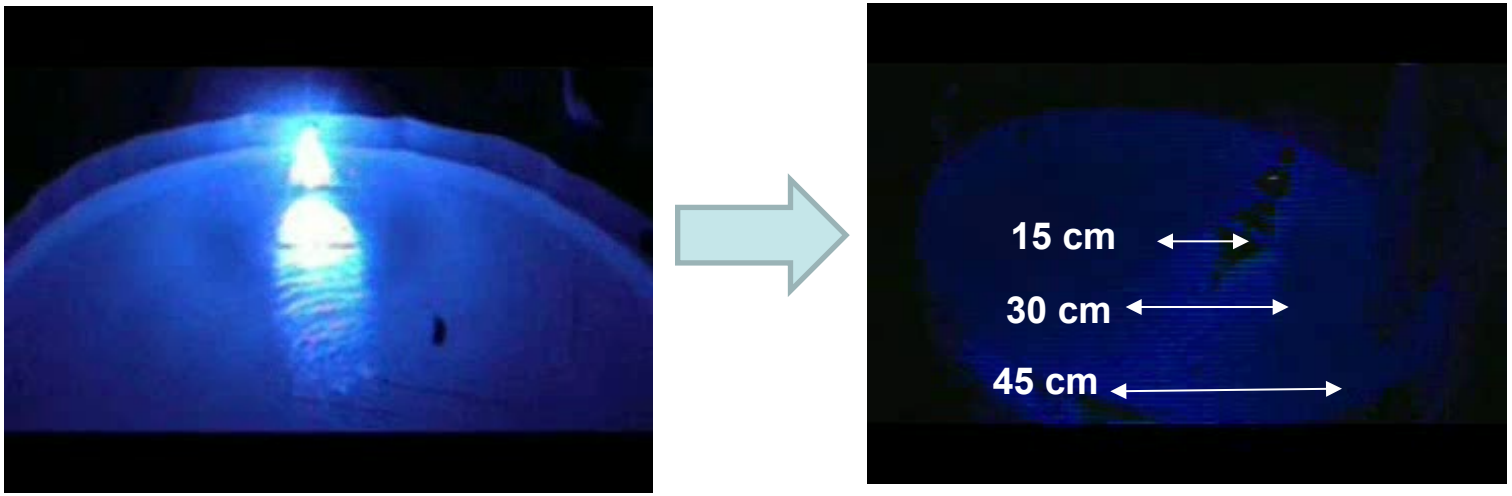
Water-maze test for restoration of vision



Performance of mice transfected with ChR2 in eyes improved as compared to mice without transfection. Representative track of *rd1* mouse (a) without injection of ChR2 gene, and (b) with injection of ChR2 genes in the eyes. (c) Numbers of error arms mice swam before achieving the platform; (d) distances mice swam before reaching the platform; (e) total time of mice swam before finding the platform; (f) rotation frequency of mice head during swimming.

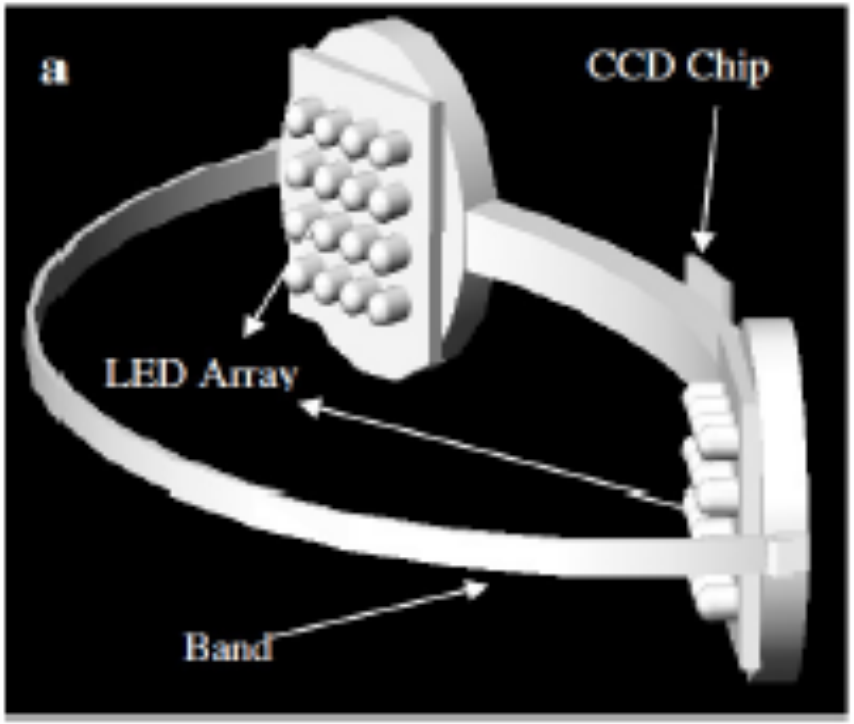
Long-range directional control by light

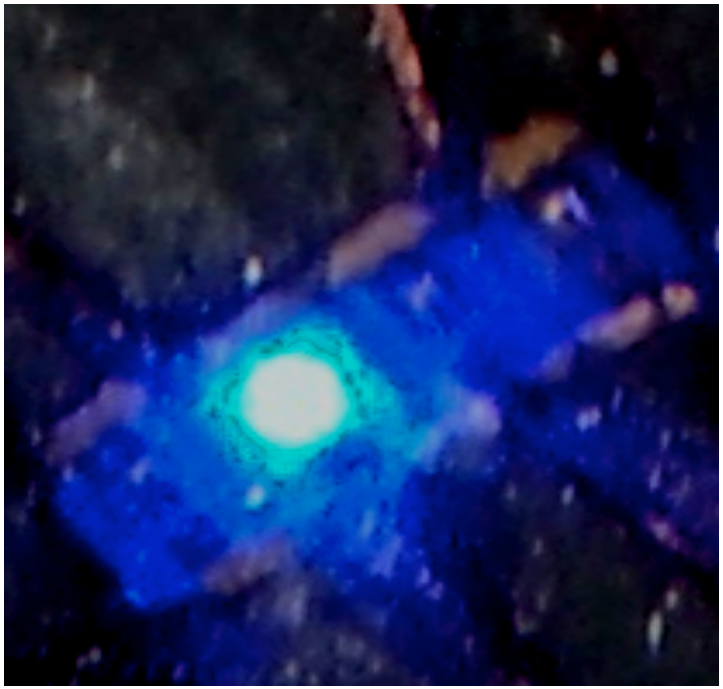


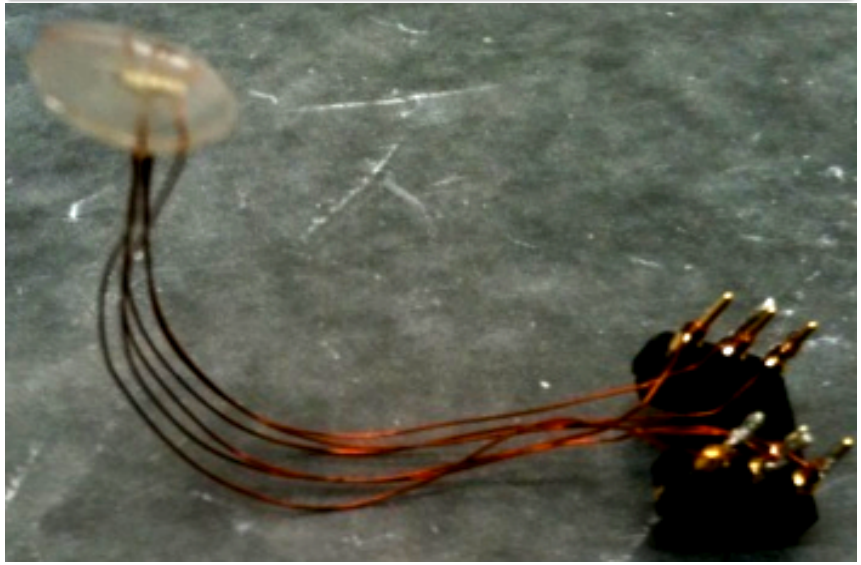


Intensity dependent directional light sensitivity of mice with opsin-sensitized degenerated retina. Movement of Thy1-ChR2-treated *rd1* mouse towards light source from a distance of 150 cm.

Retinal optogenetic prosthetic

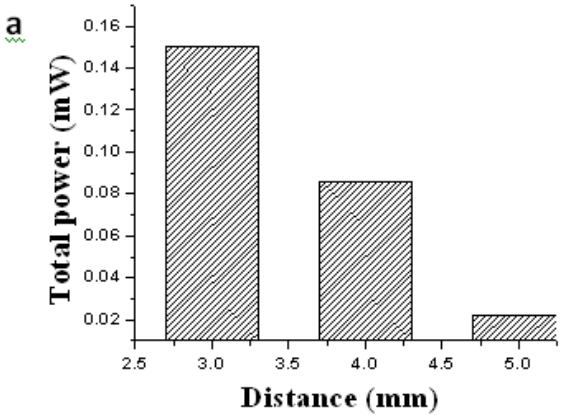


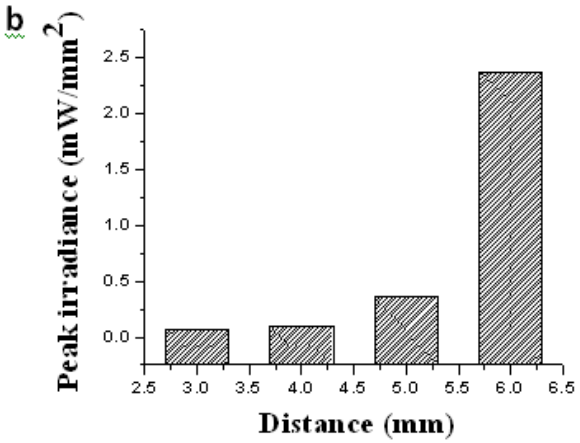


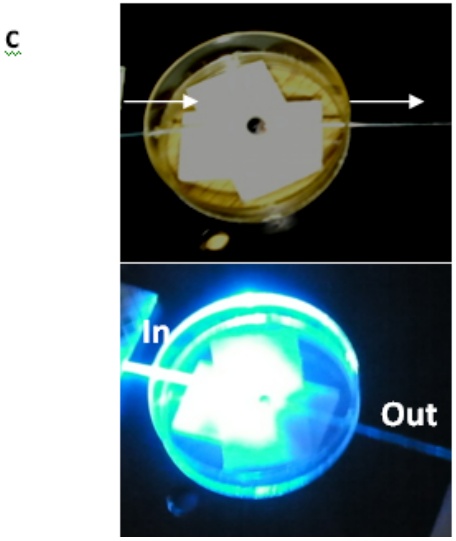


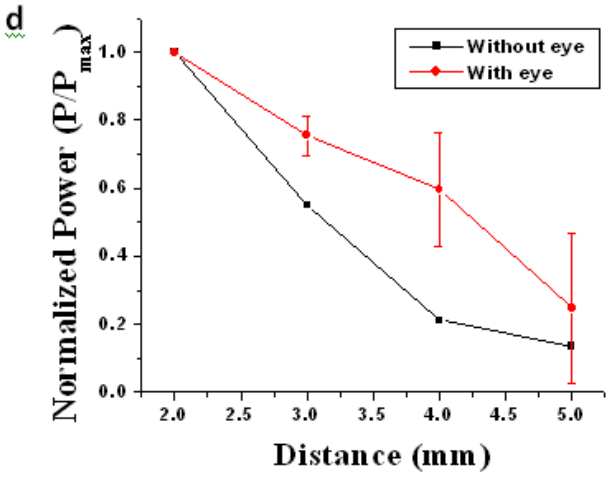
- Requires spatio-temporal modulation of retinal neuron activity based on visual sensory feed-back.
- Optimal light delivery to retina (intensity, resolution etc)

Optimizing light delivery to retina by active LED-array illumination



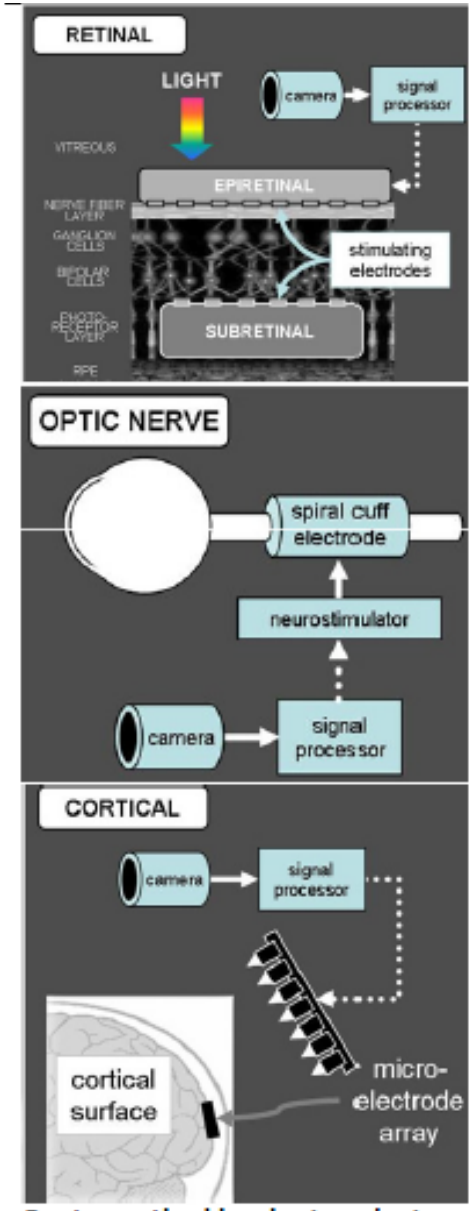






(a) Simulation of total power output at retina. (b) Peak irradiance reaching the retina, of mouse eye being irradiated at varying distances using a diverging optical fiber source. (c) Setup for measurement of light transmitted to retina, (d) Normalized power measured at varying distances from the source fiber. Symbol (-■-) denotes measurements without placing an eye in between the source and receiver fiber, symbol (-●-) denotes the condition where eye is placed in between the two fibers.

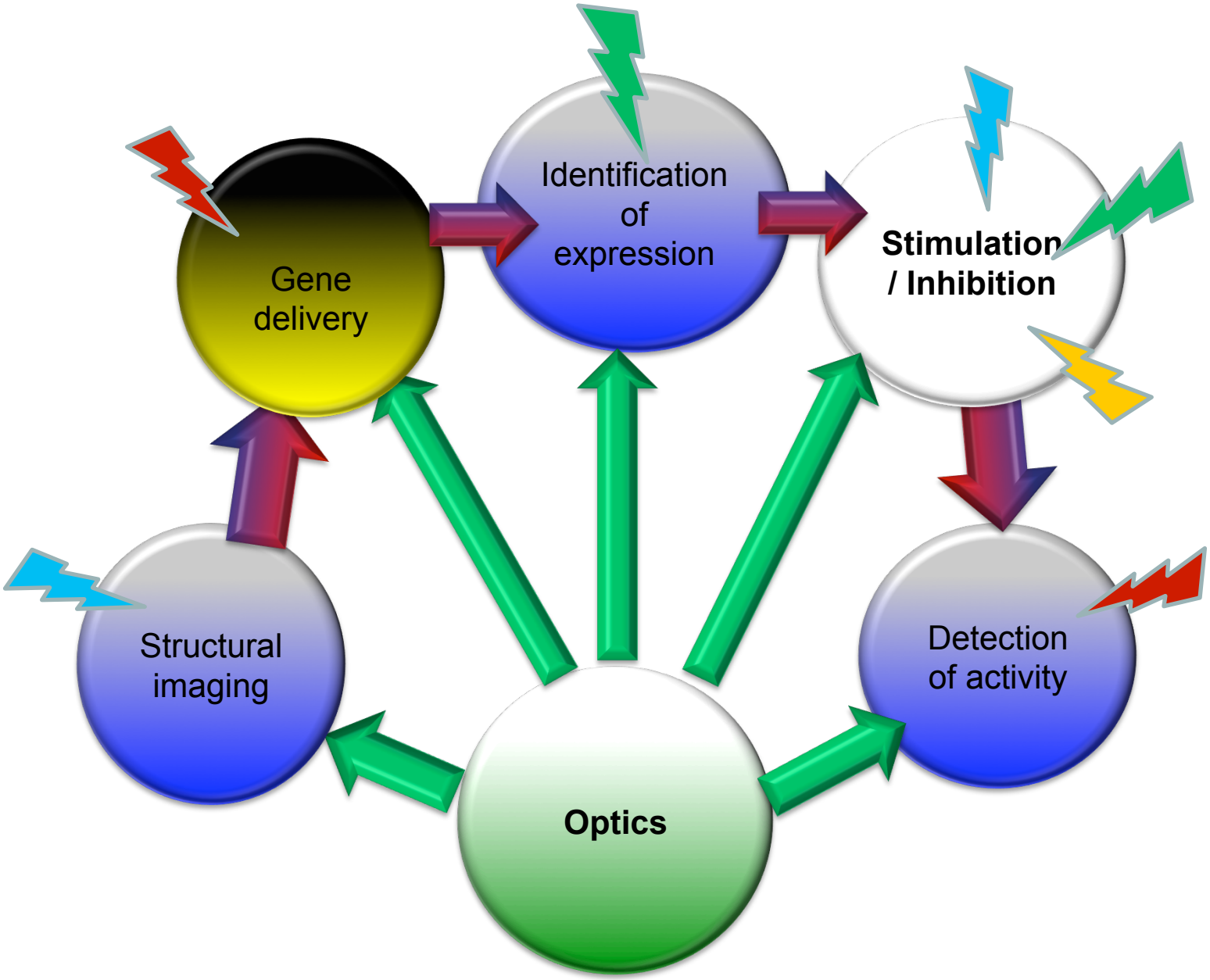
Electrical vs optogenetic approach



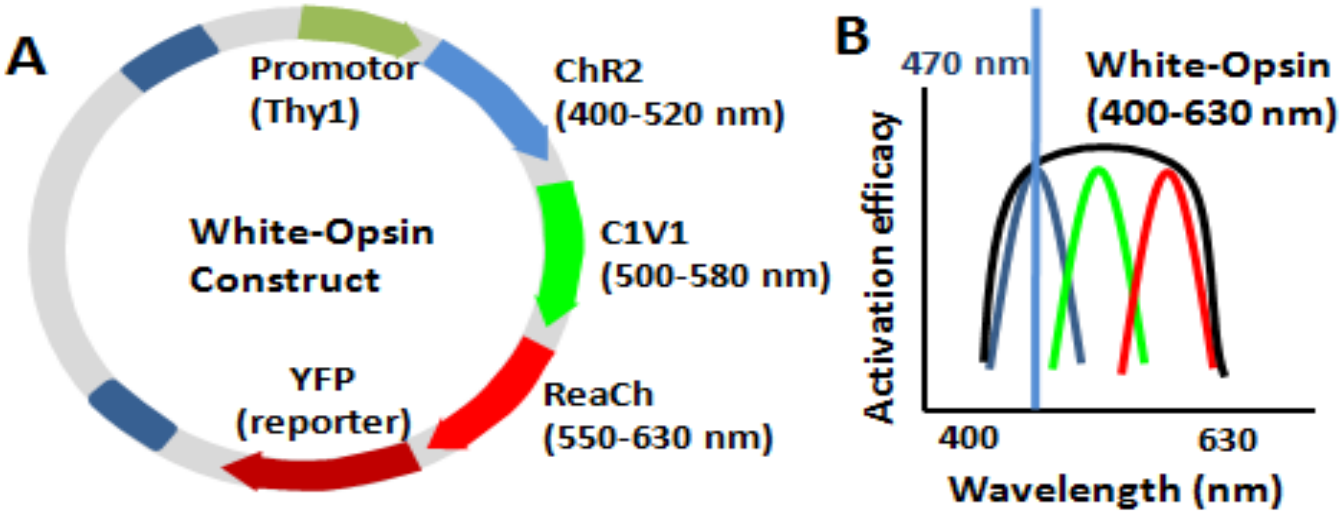
Boston retinal implant project

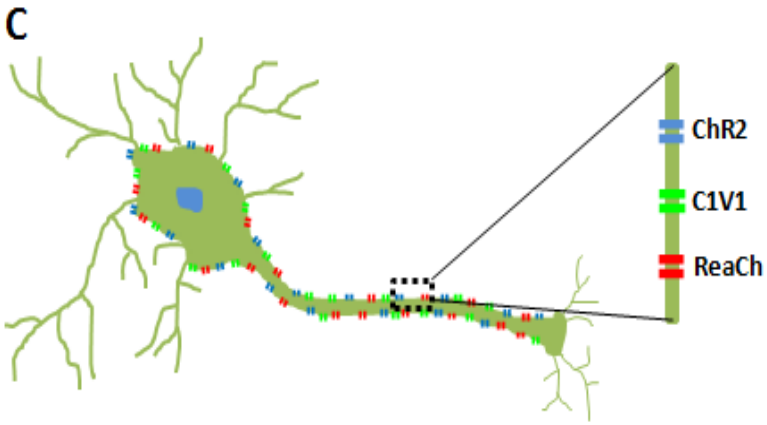
Current approaches: limitations	Optogenetics :Solution
• *Invasive surgical procedure*	• *Minimally-invasive:* -Eliminates challenge of placing electrode arrays
• *Subretinal implants :* -Microphotodiode not producing sufficient current to stimulate neurons under ambient light	• *Genetically targeted* -Cellular specificity (ON/OFF cells)
• *Epiretinal /cortical implant*: - Cellular proliferation - Disordered stimulation pattern due to non-specific electrical stimulation.	• *Less susceptible to contamination* • *Precise spatially-resolved pattern stimulation*
• *Poor long-term stability* -Electrode delamination and dissolution -Limited electrode density	• *Stable expression up to 1 yr* - Long term stability needs evaluation
• *Limited resolution due to* - Size of electrode - Distance between retina and electrodes	• *Higher spatial resolution* - Single cell or sub-cellular level
• *No true restoration of vision* - Only detect motion	• *Millisecond-temporal precision* - Real time vision
• High *threshold current* - Depends on degree of blindness	• *Low light power required for RGC stimulation* - Not dependent on degree of blindness
• *Heating induced damage* -1000 electrodes needed to restore visual function will require *125 mW (125 µW/electrode)*	• *High throughput* - Parallel stimulation of multiple cells, wide area
• *Electrical noise*	• *No requirement of electrical noise reduction*

All-optical technologies for vision restoration



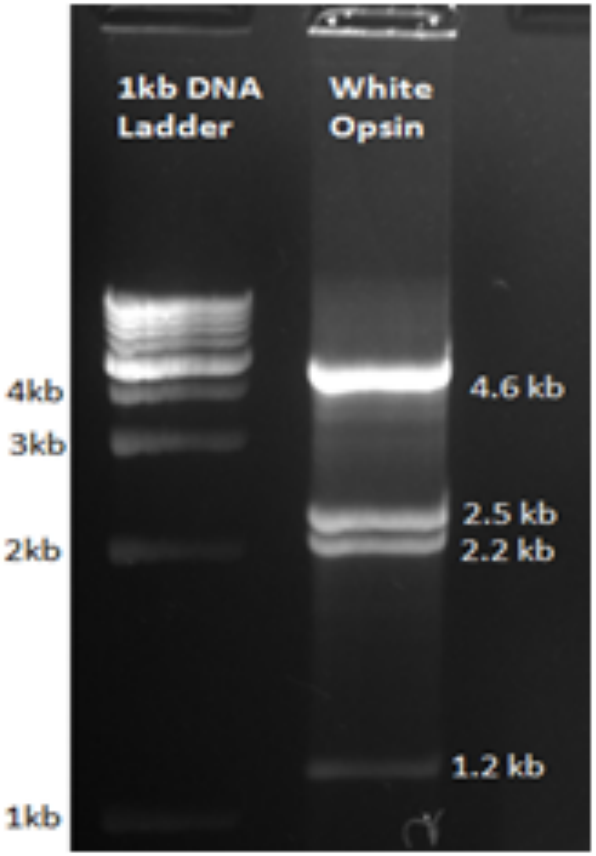
White-opsin: enhancing light-sensitivity of retinal neurons



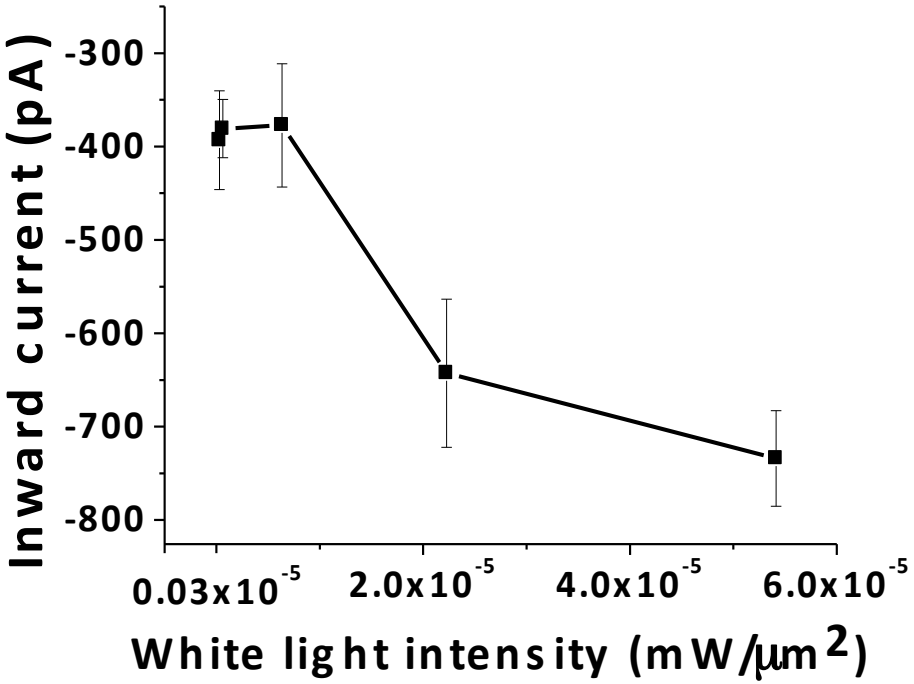


(A) Plasmid map of broad-band activatable, white-opsin made by integration of three opsins ChR2 for blue, C1V1 for green and ReaCh for red sensitivity. (B) Broad-band activation spectrum of white-opsin that can be stimulated by white light in contrast to conventional approach of narrow-band intense blue light (470 nm). (C) Retinal ganglion cell expressing white-opsins. The different spectral bands of white light will stimulate the corresponding opsin-components expressed in retinal cells.

White-opsin: enhancing white-light sensitivity

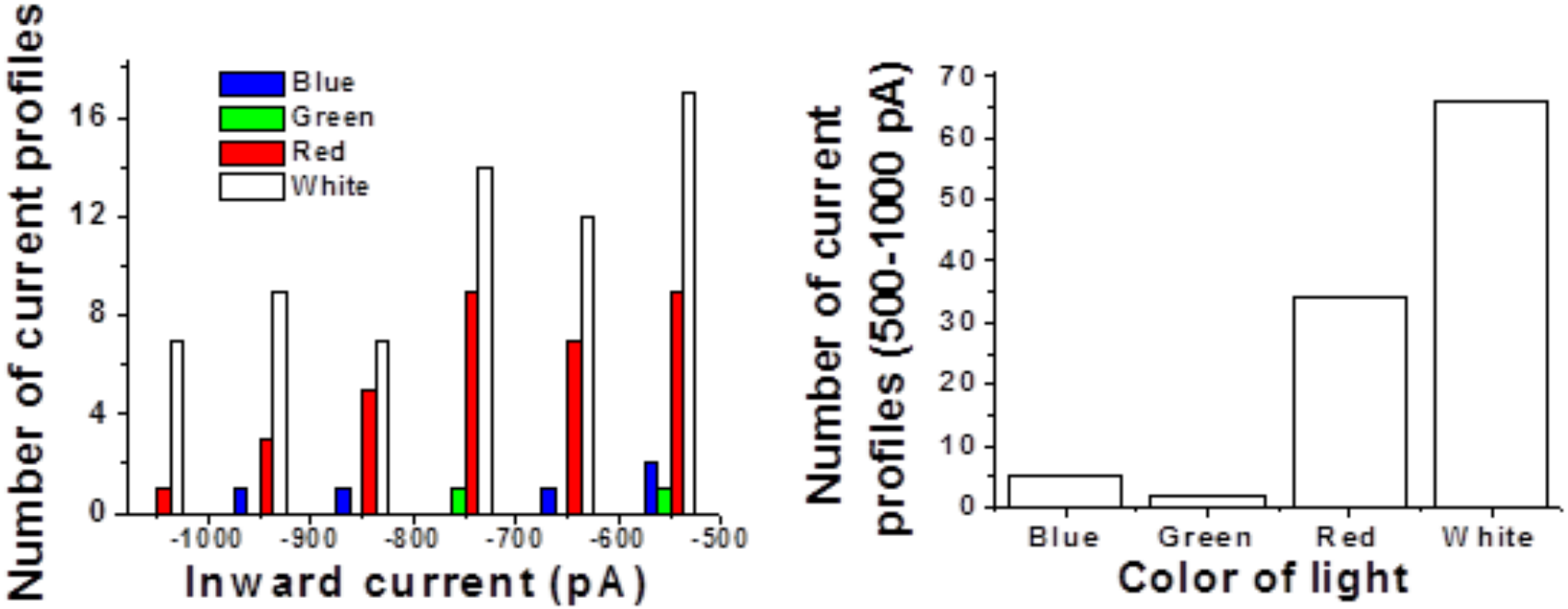


SDS-PAGE of white-opsin construct (digested by restriction enzyme Bgl II with restriction fragments: 4.6, 2.5, 2.2 and 1.2 kb).



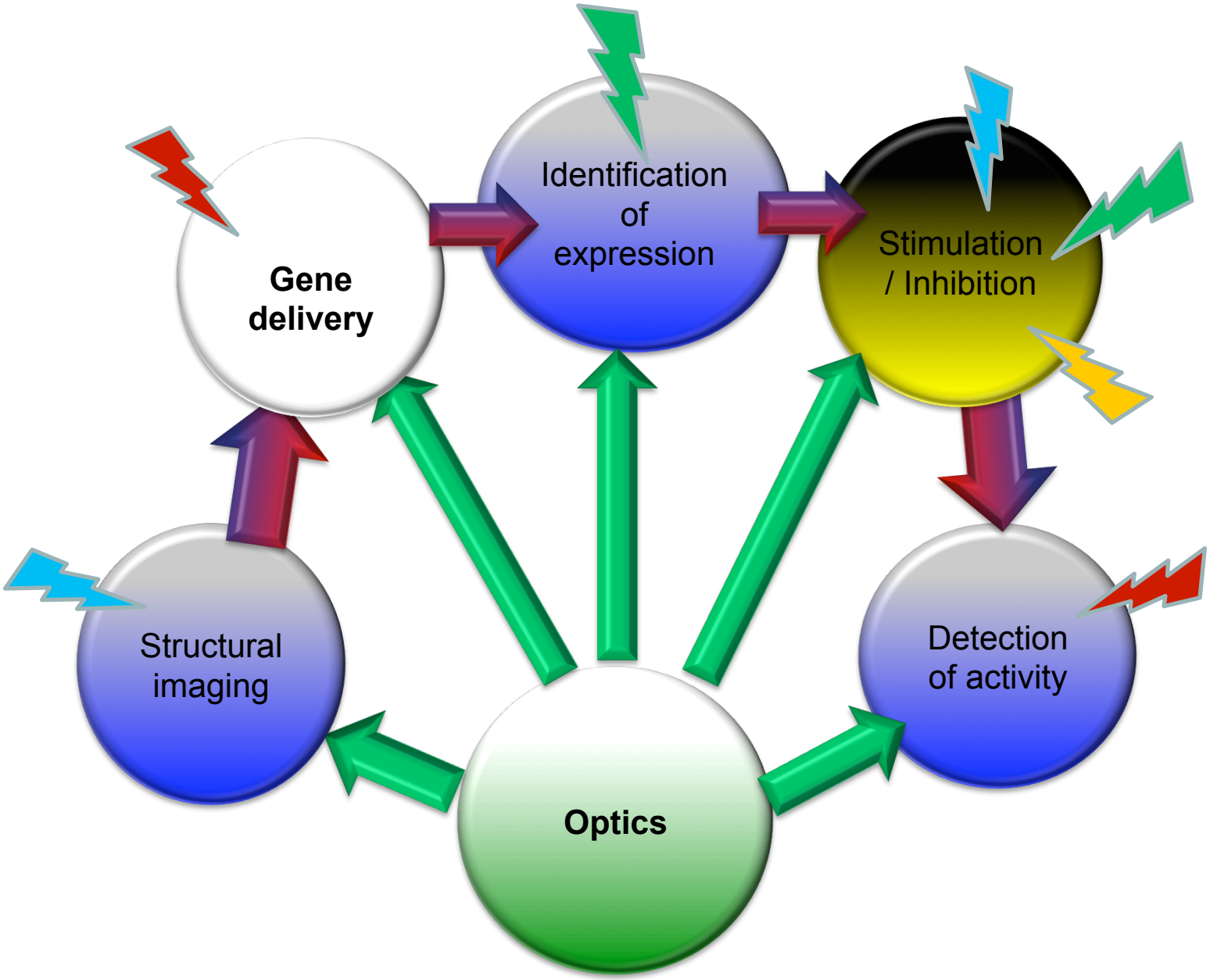
White-light sensitivity of white-opsin. Variation of inward current in HEK cells transfected with white-opsin stimulated using different white-light intensities. N= 30 for each intensity, average ± s.e.m.

White-opsin: enhancing white-light sensitivity

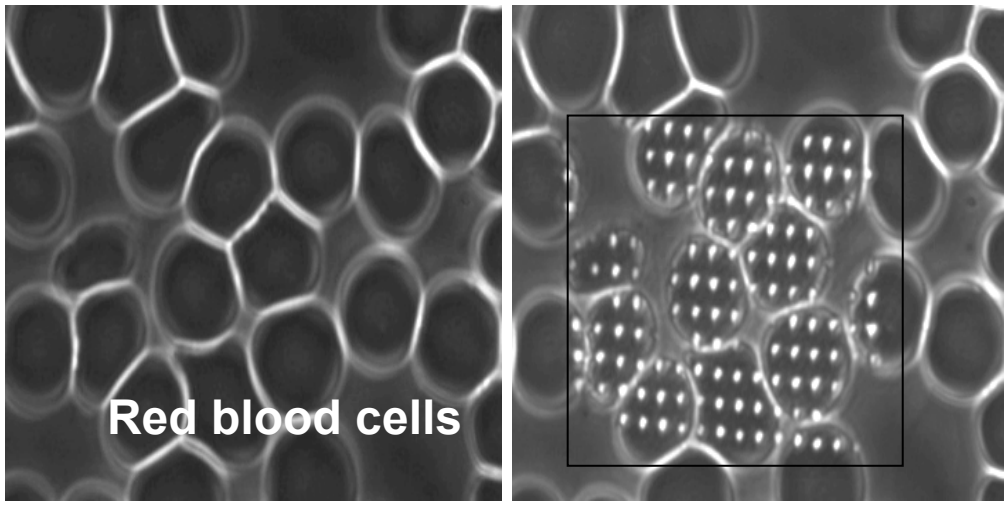


Wavelength-dependent activation of white-opsin sensitized cells. Left: Histogram of current responses of white-opsin sensitized HEK cell stimulated by different color light binned with different bands of peak current. Right: Total number of current profiles between 500-1000 pA, evoked by four different colors of 70 light pulses (pulse width: 10 ms).

All-optical technologies for vision restoration



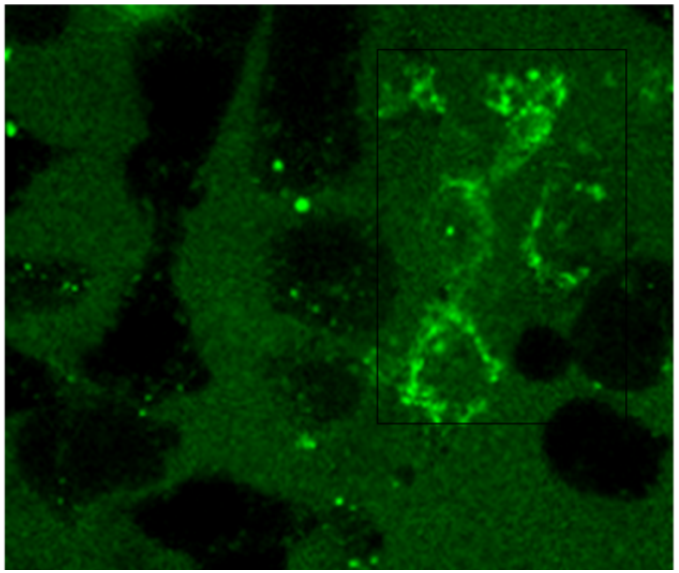
Controlled non-viral delivery by fs laser microbeam



Goal/advantages:

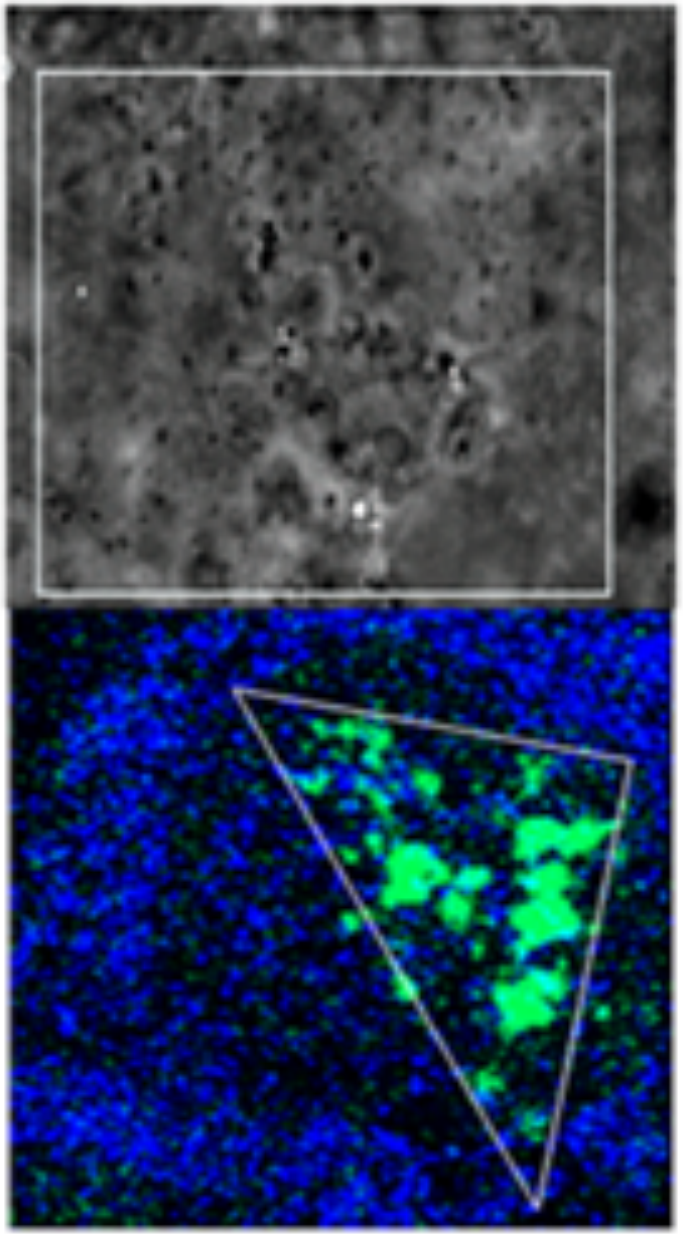
-The delivery of large white-opsin plasmid (10.5 kb) by non-viral method.

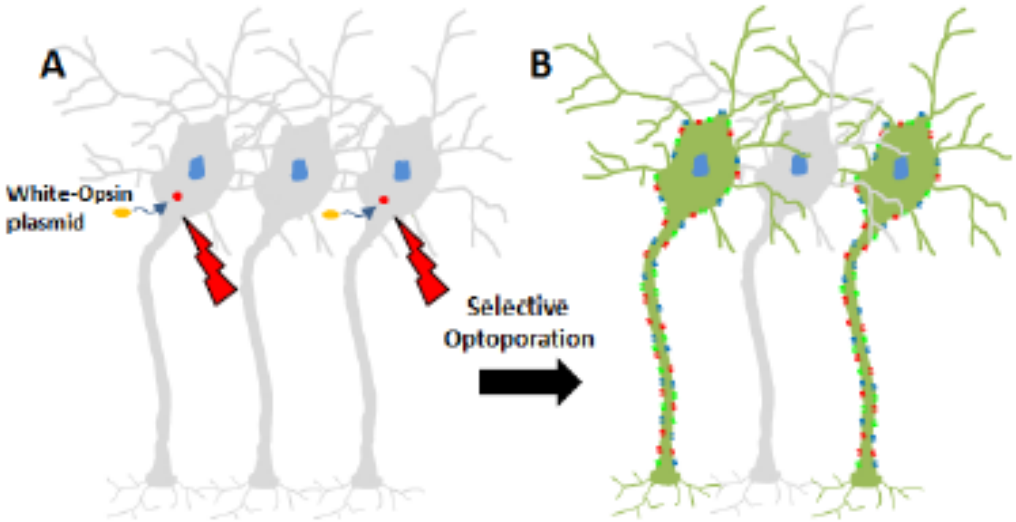
-Delivery to peripheral retina (Loss of peripheral vision in RP)



Optoporation at single-cell resolution. Anti-mitochondrial antibodies introduced into the targeted cells through localized pore formation by fs laser microbeam in the rectangular area. Fluorescence of spatial localization pattern in mitochondria.

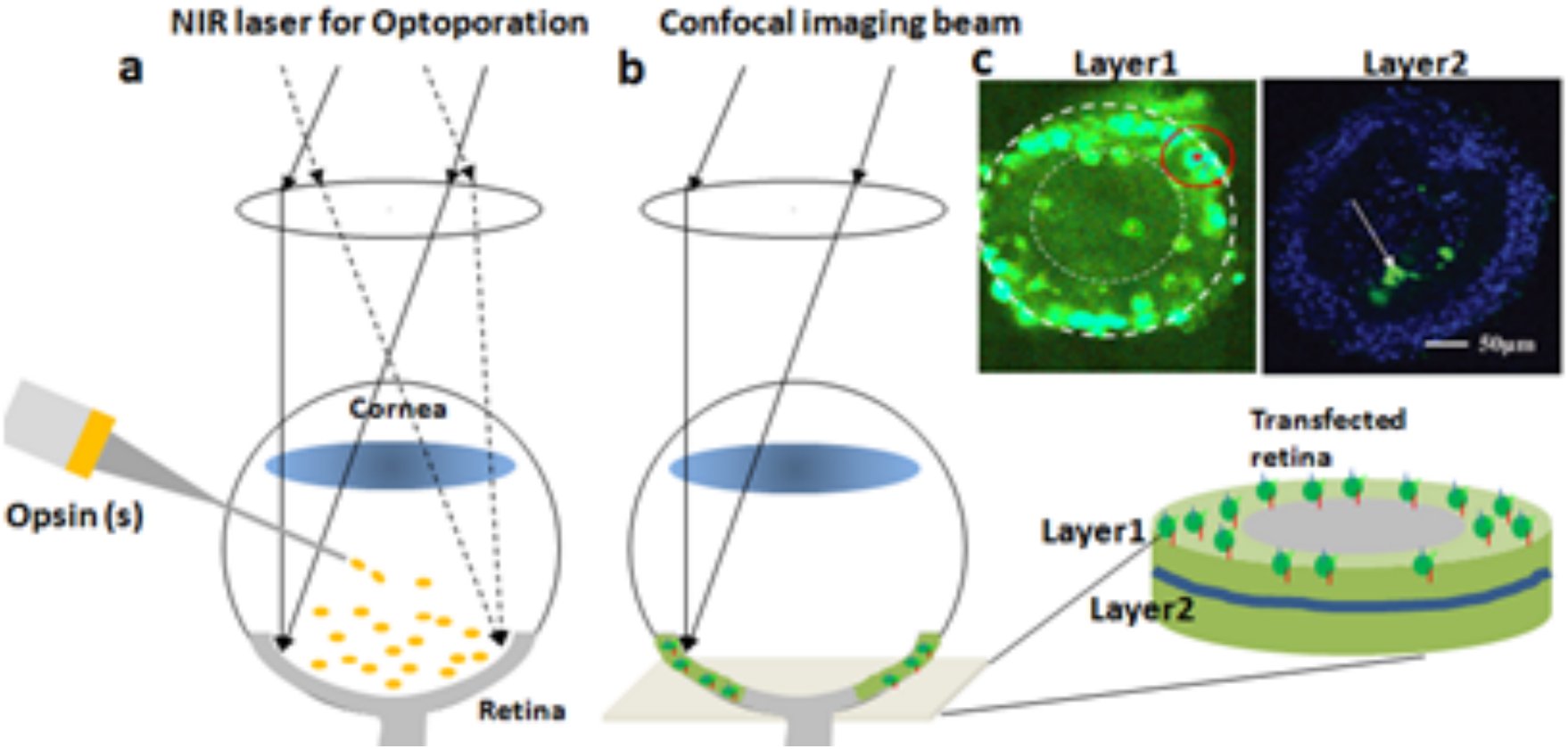
Controlled gene delivery by fs laser microbeam





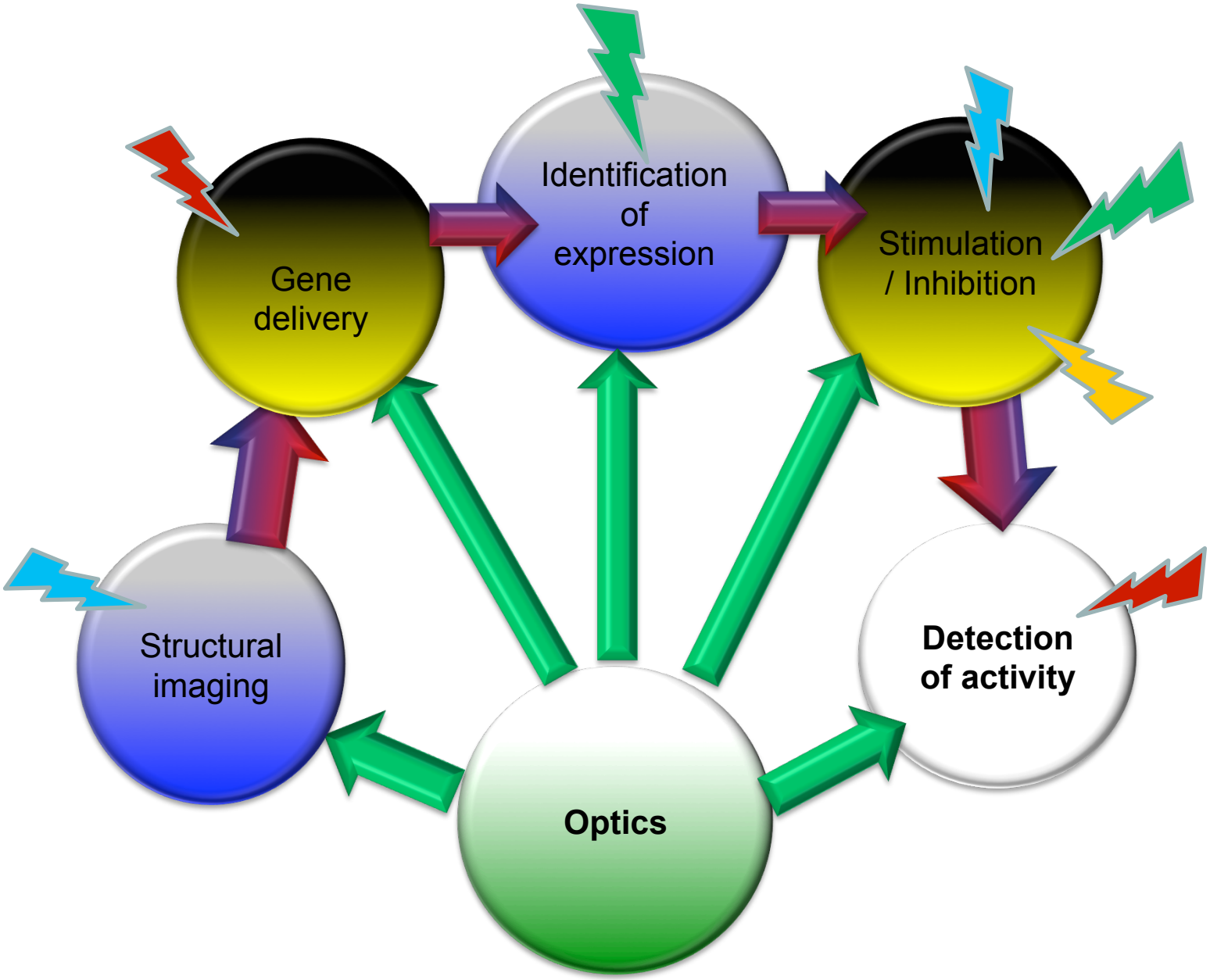
Light-controlled delivery of opsin-gene into targeted area of retina-explant. Top: Nano-perforations (dark spots) made during optoporation in rectangular area. Bottom: Retina tissue, immuno-stained for YFP and nucleus co-stained with DAPI. Triangle indicates region of ChR2–YFP transfection by NIR fs laser.

Delivery to peripheral retina by fs laser microbeam

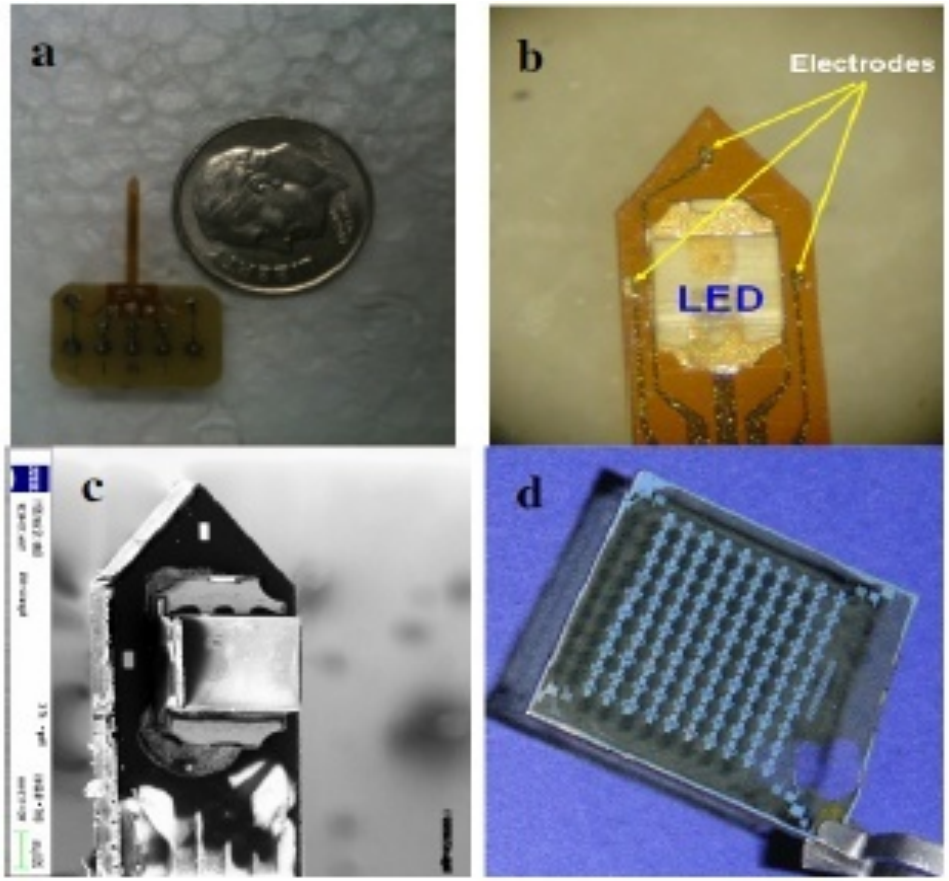


***In-vivo* laser-based delivery and monitoring of expression of opsin(s) in targeted regions and layers of retina.** (a) The opsin encoding plasmids injected into the vitreous through sclera. Scanning near-infrared laser focused for transient perforations on outer cell membrane of retinal cells in different layers to allow delivery of opsin plasmids. (b) Confocal imaging of retina one week after laser transfection (green depicts expression in peripheral retina regions). (c) Fluorescence spectral imaging of the laser-transfected retina showing targeted YFP-expression in different layers: (1) annular peripheral region (concentric circles) and (2) single cell.

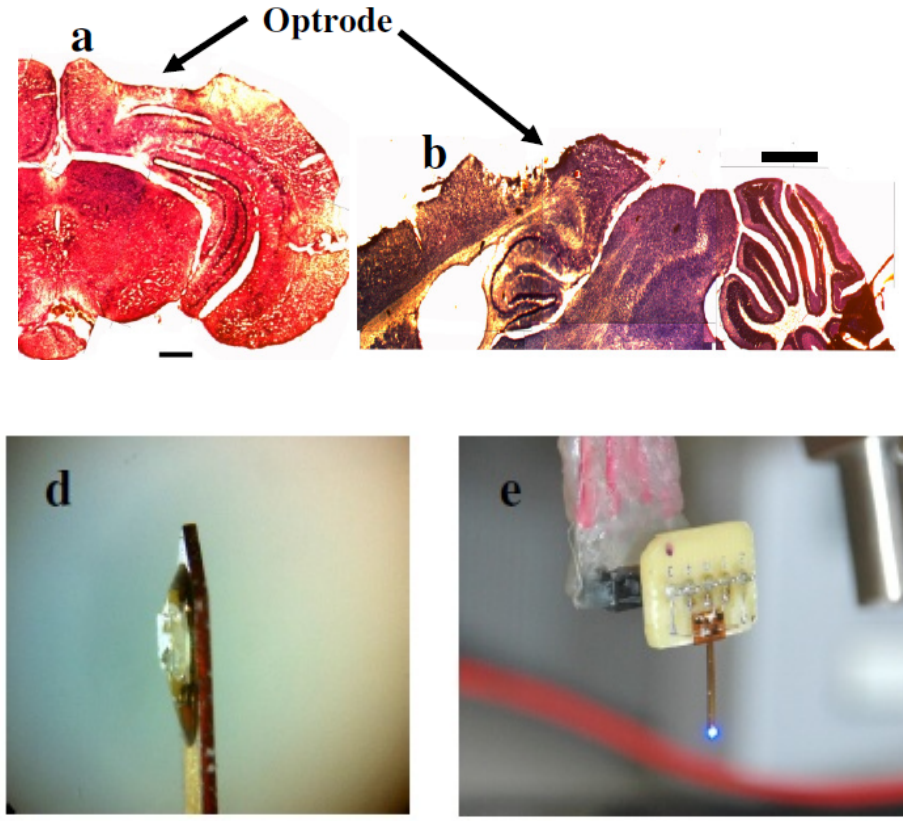
All-optical technologies for vision restoration



Detection of optogenetically-controlled neural activity

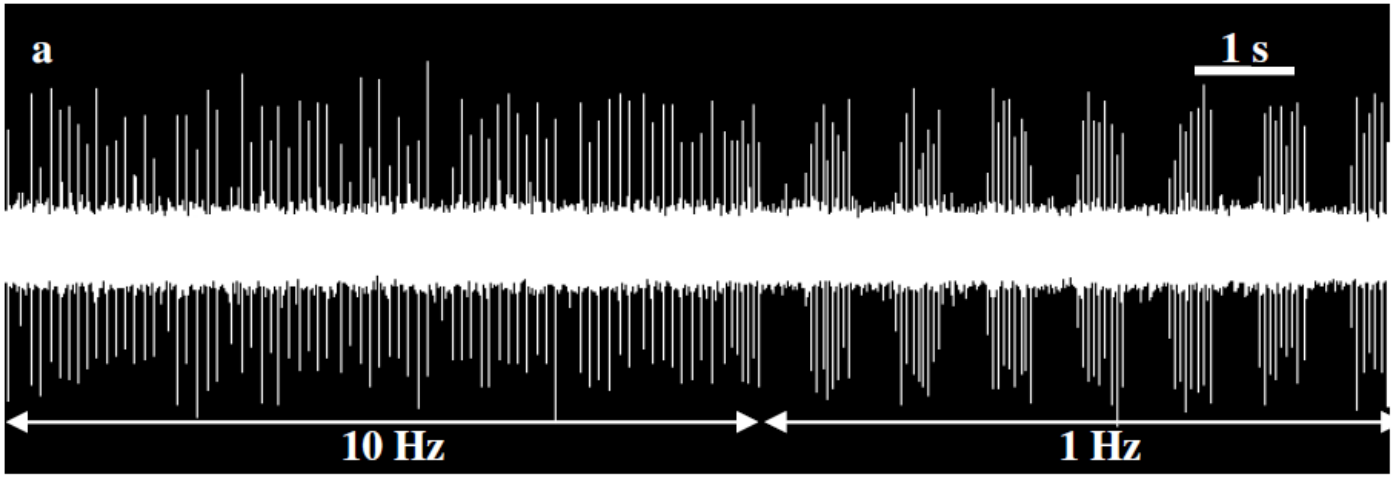


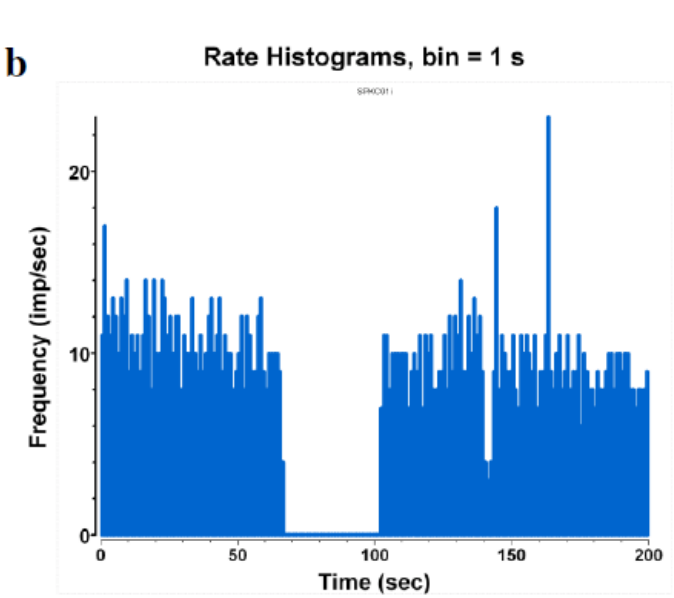
Optrode for optical stimulation and electrical detection

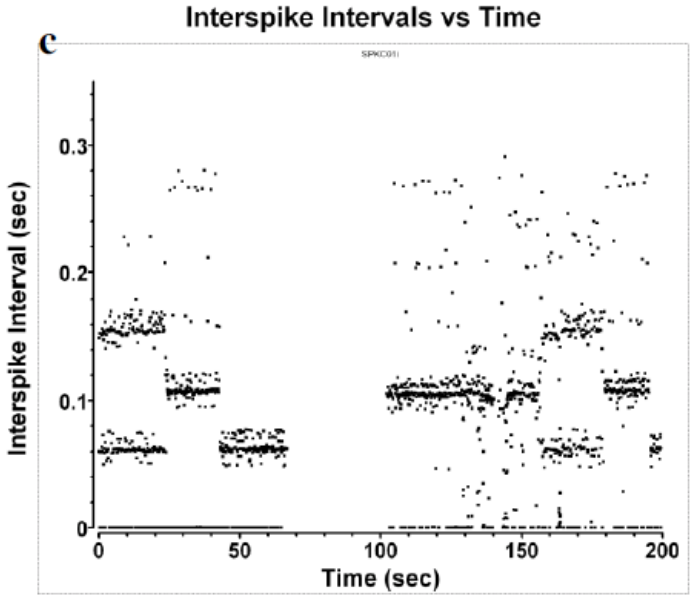


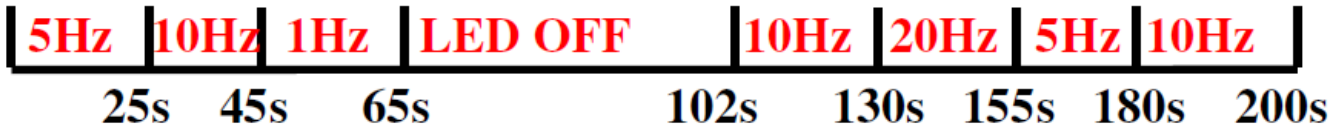
Damage to the neural tissue during electrical detection

Stimulation-detection by µLED-electrode array



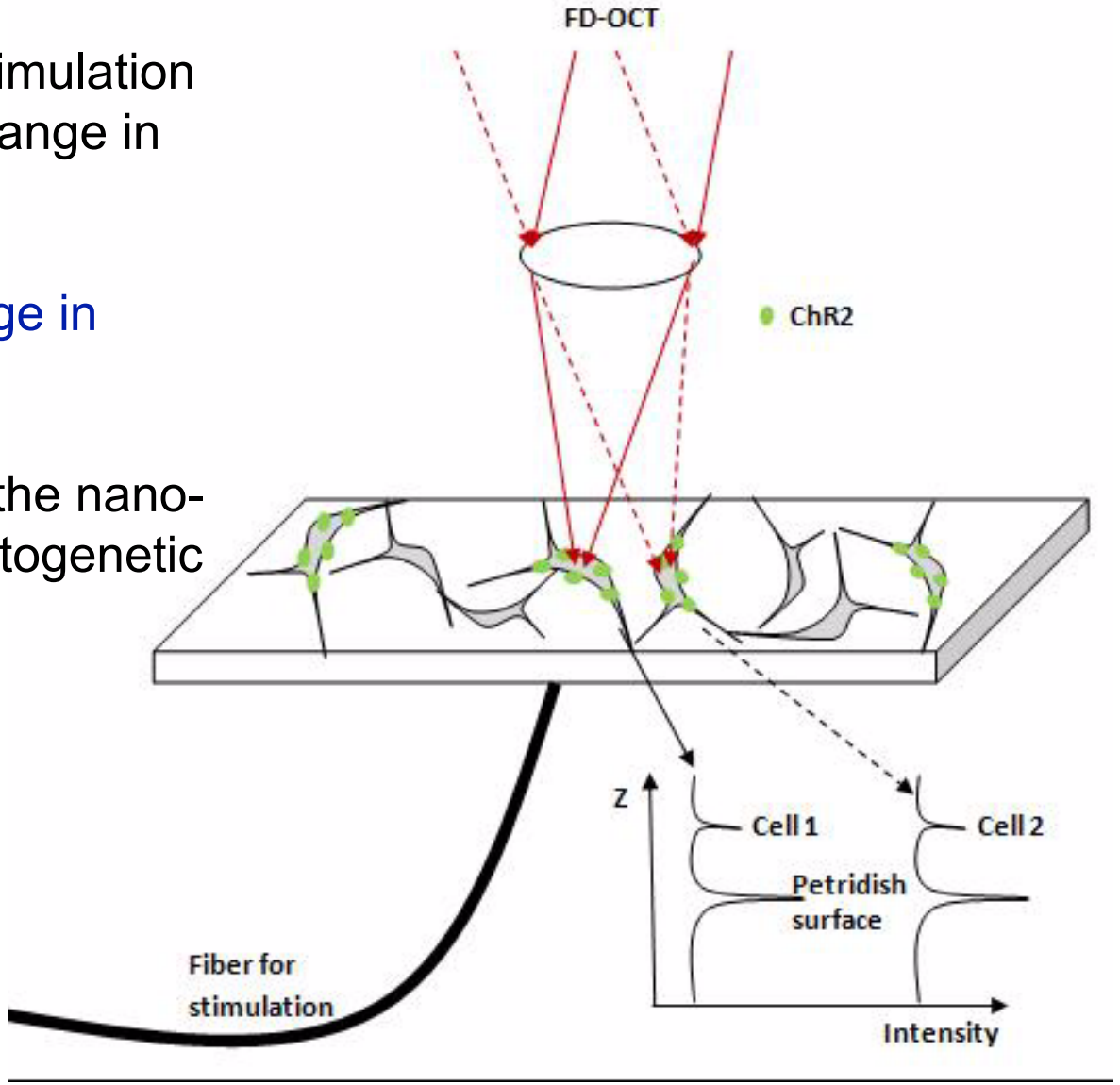




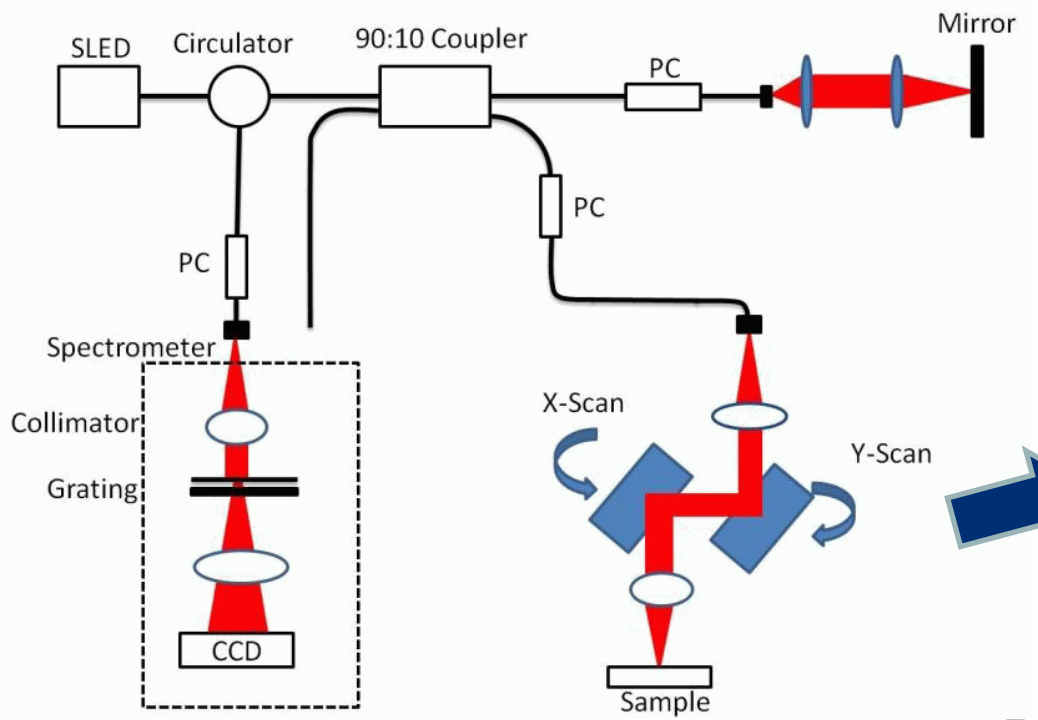


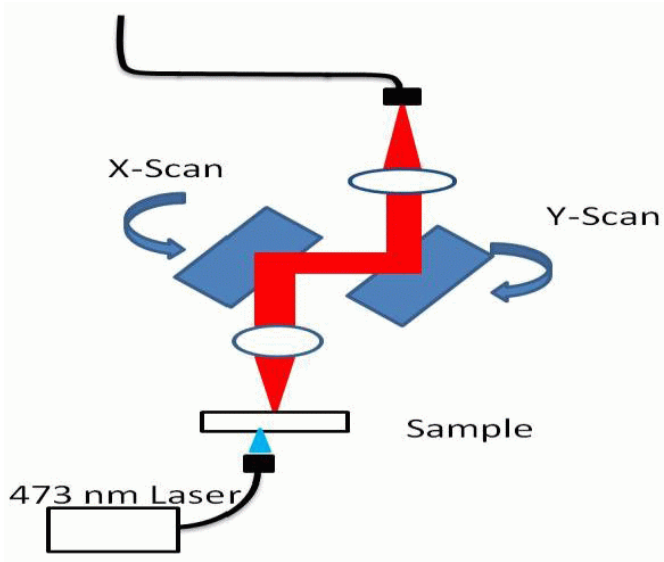
Optical label-free detection of activity of optogenetically controlled cells

- Hypothesis: Cellular stimulation leads to nano-scale change in volume of cell.
- How much is the change in thickness?
- Is it possible to image the nano-level change during optogenetic stimulation.



Phase Sensitive Fourier Domain Optical Coherent Tomography (PSFD-OCT)

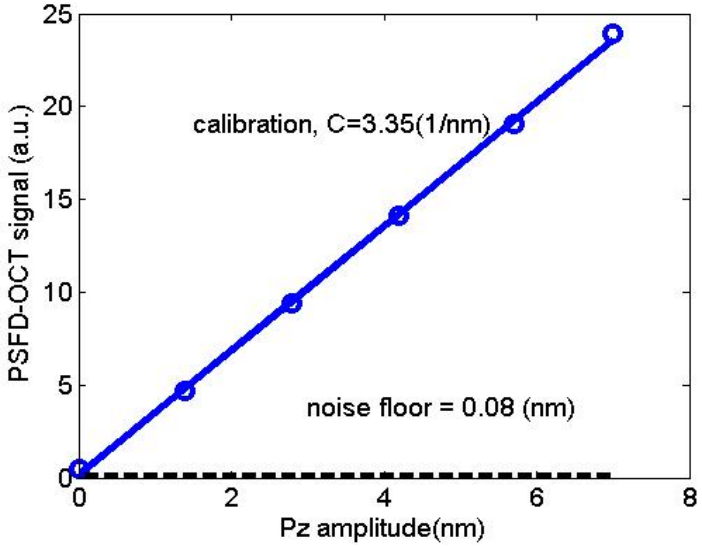




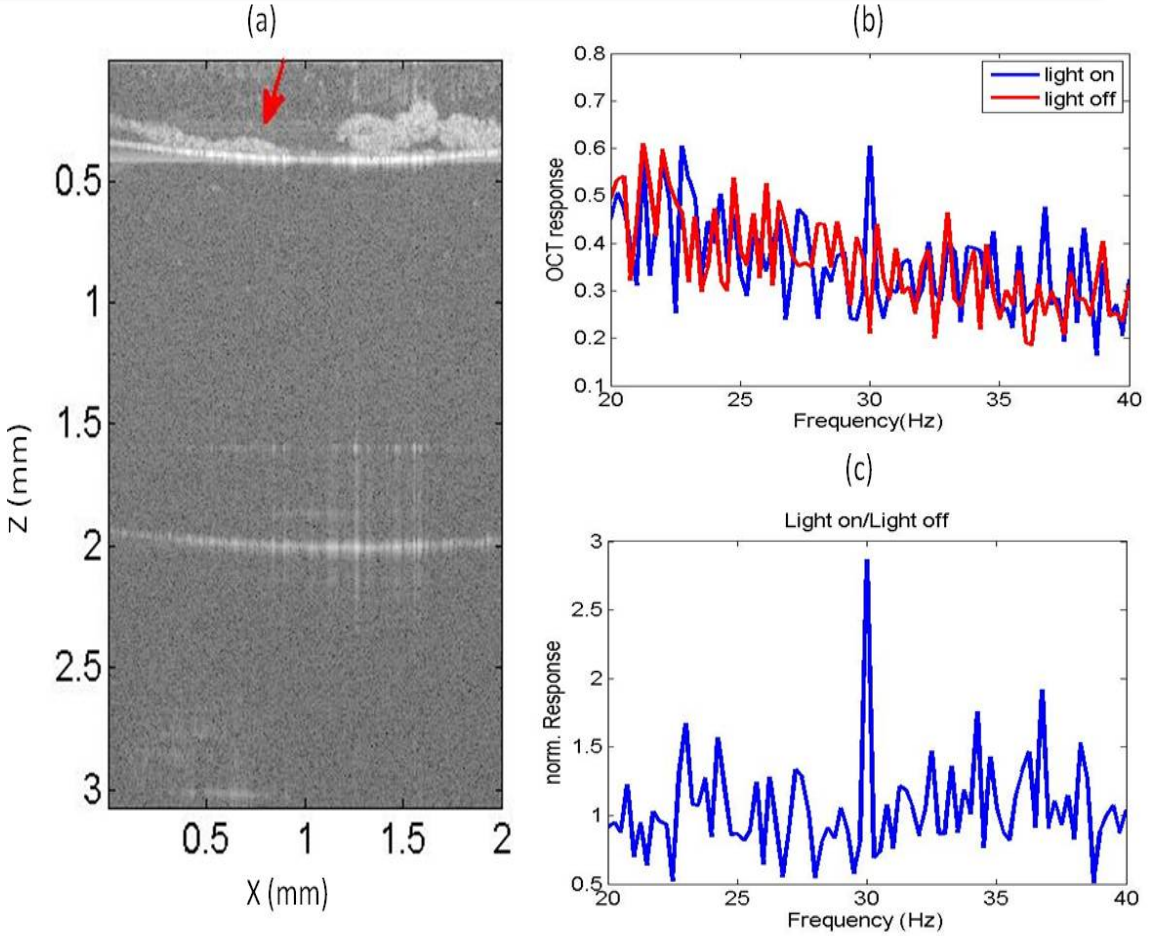
- Conventional **Michelson interferometer** with broadband super luminescent diode as a light source.
- Wavelength **1316 nm** & **80 nm** Bandwidth
- Axial resolution of **~10 µm** in air.

- Possible to image the nanometer level change in cells under stimulation.
- Study the Nano-change of a cell in 3D volume
- Broadband light source with **> 10 µm** of coherent length

PSFD-OCT measurement of optogenetically activated cells

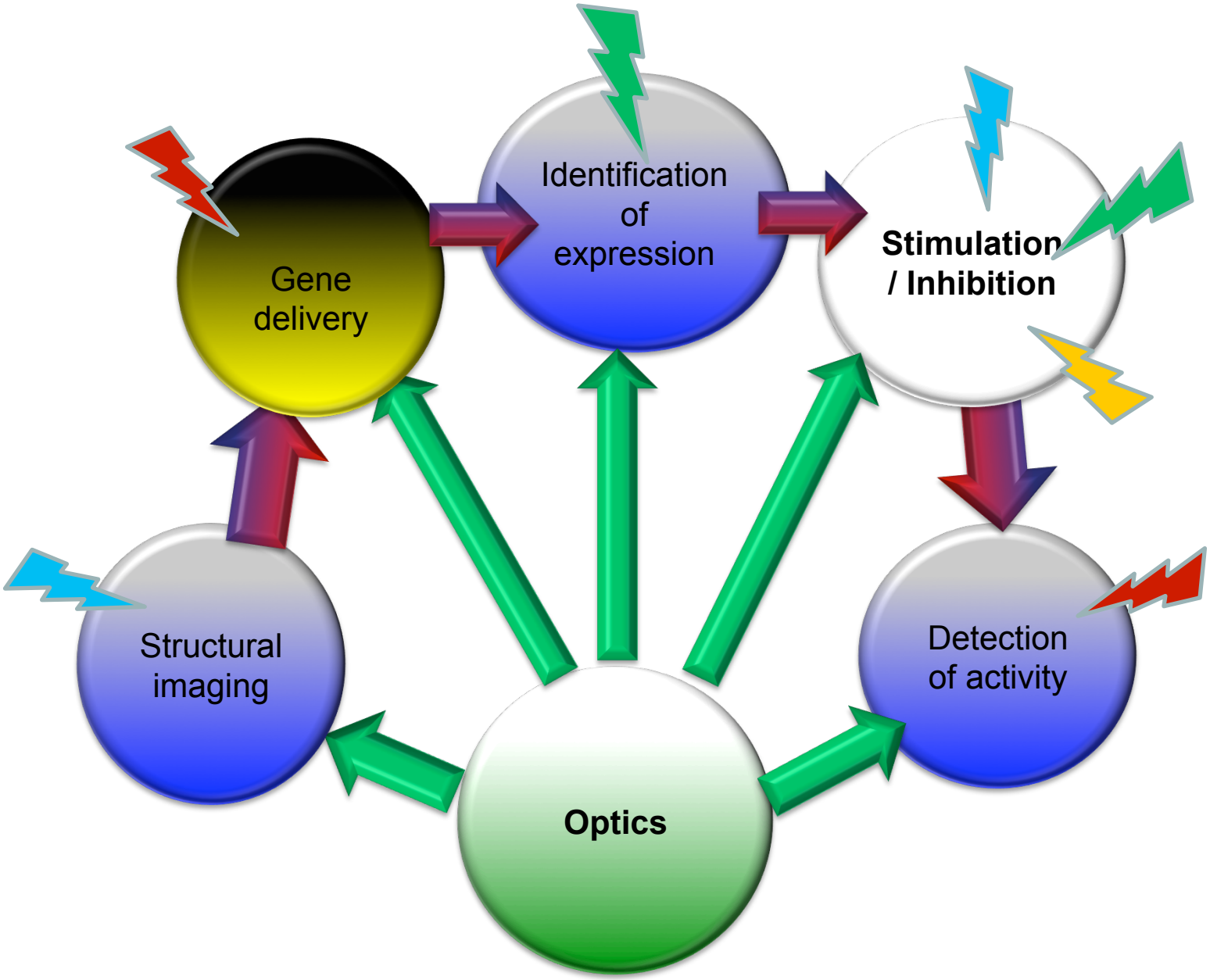


Measured PSFD-OCT signal at different displacements of a piezo-stack vibrating at 30 Hz. Dashed line: measurement from a stationary piezo.



(a) Cross-sectional view of the ChR2-expressing cells in a cell-culture dish. (b) PSFD-OCT response measured with the stimulation light ON and the stimulation light OFF. (c) Normalized response obtained by dividing the light ON response by light OFF response.

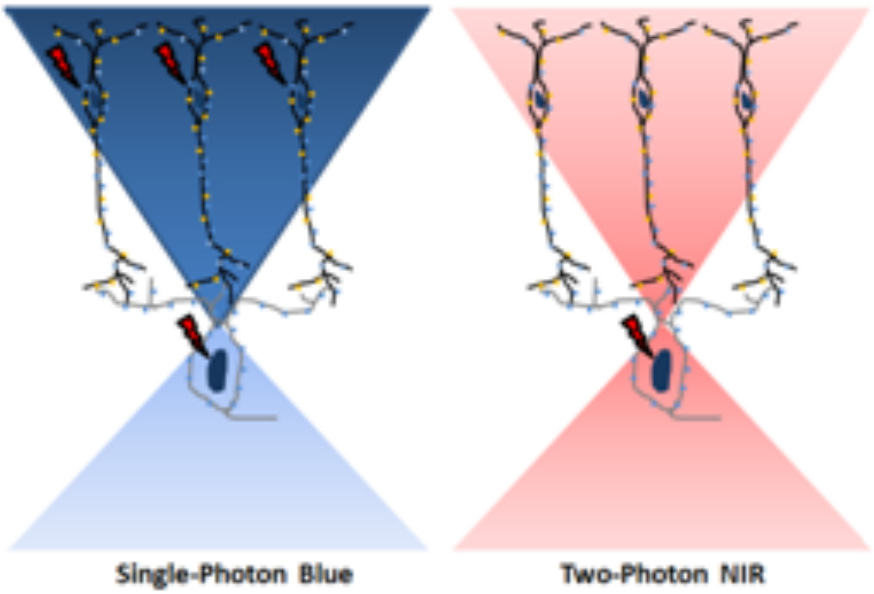
All-optical technologies for vision restoration



Non-linear Optogenetic excitation: Motivation

→Selected activation with high spatial resolution not possible with 1ph

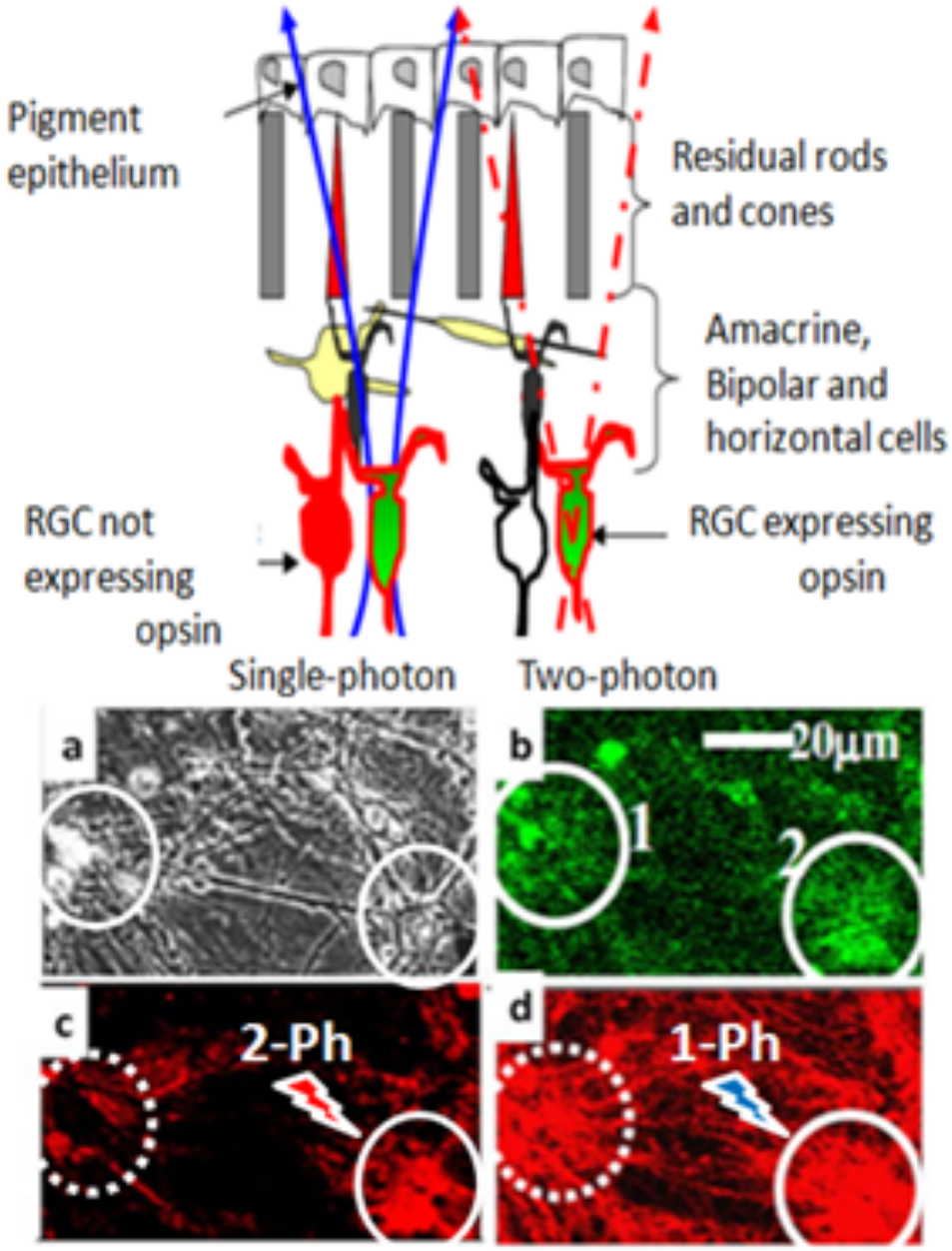
→Need for two-photon activation



Focused two-photon beam increases target-specific stimulation ( ) as compared to single-photon.

- Non-localized (out-of-targeted retinal layer) stimulation occurs while using one-photon (e.g. blue) light (left panel), it poses significant limitation on attributing the role of specific neural elements of retina in processing visual signals.
- By virtue of non-linear nature of light-matter interaction, high spatial precision in optical stimulation can be achieved using NIR ultrafast beam.
- We have developed both microscope objective based as well as fiber-optic based two-photon (near-infrared) activation of opsin-expressing cells.

Probing neural circuitry by two-photon activation



Schematic of the 1-ph vs 2-ph optogenetic stimulation for probing neural circuitry of retina. 1-Ph (left) stimulates both opsin sensitized RGCs and residual photoreceptors, leading to indirect-stimulation of non-sensitized RGCs. 2-Ph (right) stimulates only RGCs expressing opsin and thus measurement in V1 provides exclusive mapping of optogenetically-stimulated RGC-activity.

(a) Bright field of two RGCs (encircled) in a retina explant. (b) ChR2-YFP expression. (c) Focused 2-ph only stimulates targeted cell-2 (marked by solid circle) as detected by increase in Calcium fluorescence. (d) Focused 1-ph illumination though confined to cell-2, out-of-focus stimulation of photosensitive cells lead to indirect stimulation of non-targeted cell-1 (marked by dashed circle) as evidenced by increase in Calcium fluorescence.

Conclusions

- Non-viral based approach for light-sensitization of specific neurons in retina presents a unique opportunity for treatment of RP, as demonstrated by electrophysiology and behavioral assays in mouse models.

- Enhancing the light-sensitivity by wide-spectral opsin has promise to eliminate the requirement of active illumination source for vision restoration in RP patients.

- Use of ultrafast lasers will enable delivery of genes (including that encoding opsins) to targeted areas of degenerated retina (e.g. periphery in RP).

- Detection of neural activity in a label-free manner is possible by phase-sensitive interferometry and thus all-optical control and evaluation of light-based therapeutics of RP.

- Use of localized two-photon stimulation of retina will allow study of neural circuitry, function of specific cells, leading to better understanding of signal processing in retina and perturbations during disease progression.

Acknowledgements

Prof. Y. Kim, UTA Bioengineering

Prof. J-C. Chiao, Univ. of Texas-Arlington

Prof. E. Wong, Univ. of California, Irvine.

Grieshaber Foundation

Thank you for your attention