Optogenetics: A Review Study on Biomedical Optics Technique

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Abstract: Optogenetics is a powerful technique utilizing light-sensitive proteins (opsins) for precise control of cellular activity. This concise review provides an overview of optogenetics, covering its principles, tools, applications, and future prospects. Topics include the historical background, basic principles, opsins, expression methods, light sources, and stimulation systems. Applications encompass neuroscience, neurological disorders, and targeted genetic manipulations. Mathematical models and experimental methodologies are discussed. The review concludes with insights into achievements, limitations, and future directions of optogenetics. This concise review serves as a valuable resource for researchers interested in optogenetics and its interdisciplinary applications.

1. Introduction

Optogenetics is an innovative field that has revolutionized the way we study and manipulate cellular activity. By utilizing light-sensitive proteins called opsins, optogenetics enables precise control over specific cells or tissues with remarkable spatiotemporal resolution. This technique has become a valuable tool in biomedical research, providing insights into cellular signaling pathways, neural circuitry, and the underlying mechanisms of various biological processes.

The roots of optogenetics can be traced back to the early 1970s when researchers began exploring light-induced changes in membrane potential. However, it wasn't until the discovery of channelrhodopsin, an opsin derived from green algae, that the field of optogenetics truly took off. In 2005, Karl Deisseroth and his team successfully expressed channelrhodopsin in neurons, enabling the activation of specific cells with light. Since then, the field has rapidly advanced, with the development of new opsins and techniques, expanding its applications in various disciplines.

At the core of optogenetics lies the ability to control cellular activity using light. Opsins, such as channelrhodopsin and halorhodopsin, are light-sensitive proteins that respond to specific wavelengths of light. When exposed to light, these opsins undergo conformational changes, allowing the influx or efflux of ions across the cell membrane. This leads to the activation or inhibition of cellular activity, effectively manipulating the behavior of targeted cells. The precise targeting of opsins to specific cell types, achieved through genetic modification or viral vectors, further enhances the specificity and versatility of optogenetic approaches.

Optogenetics has had a profound impact on biomedical research, opening up new avenues for understanding and treating various diseases. In neuroscience, it has been instrumental in unraveling neural circuitry, studying brain function, and investigating the mechanisms underlying neurological disorders such as Parkinson's disease and epilepsy. Additionally, optogenetics has found applications in cardiac research, optogenetic pain control, retinal prosthetics, and regenerative medicine. The ability to manipulate cellular activity with light offers unprecedented control and precision, paving the way for innovative therapeutic strategies and advancements in biomedical science.

2. Optical Principles and Fundamental Concepts

2.1. Light and Optics

Light is an electromagnetic radiation that exists within a broad spectrum of wavelengths, ranging from gamma rays to radio waves. In the context of optogenetics, visible light is of particular interest due to its interaction with biological systems. Light can be described as a wave, with characteristics such as wavelength, frequency, and polarization. The wavelength determines the color of light, while the frequency represents the number of wave cycles per unit of time. When light encounters a boundary between two media, such as air and a biological tissue, it can undergo various optical phenomena, including reflection, refraction, and diffraction. These interactions with matter play a crucial role in the transmission and manipulation of light in optogenetic applications.

In optogenetics, understanding the principles of light propagation and optics is essential for designing effective experimental setups. Reflection occurs when light waves encounter a surface and bounce back, while refraction refers to the bending of light as it passes through different media with varying refractive indices. These phenomena determine the path of light as it enters biological tissues and interacts with targeted cells. Diffraction, on the other hand, describes the bending and spreading of light waves around obstacles or through narrow openings, and it influences the spatial resolution and focus of light in optogenetic stimulation. Optical components such as lenses, filters, and fiber optics are commonly employed in optogenetics to control the characteristics of light. Lenses are used to focus or collimate light, allowing precise targeting of cells or tissues. Filters are employed to select specific wavelengths or eliminate unwanted background noise. Fiber optics, including single-mode and multimode fibers, provide a means to deliver light deep into tissues or specific anatomical regions.

2.2. Light Sensing Mechanisms in Cells

The eye evolution shows us that the eye spots on algeas enables the living organisms to be effected from light. There are many different photoreceptor proteins which helps the cells to detect and respond to different wavelengths of light. One important group of photoreceptors are opsins. They create conformational changes that triggers cellular signaling upon absorption of specific-propertied photons.

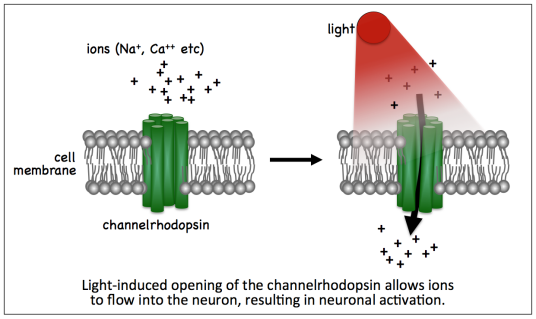

Fig. 1. Channelrhodopsin on a cell membrane (Taken from Indiana University).

Channelrhodopsins and halorhodopsins are the first discovered opsins that later on created the optogenetic field. When activated by a light, channelrhodopsins allow the influx of positive ions into the cell. This influx creates a voltage difference and activates the signaling mechanism of the cell. On the other hand, halorhodopsins allows the efflux of negative ions, such as chloride, within the activation. Therefore, it creates a hyperpolarization and allows the cell to be not activated even there is a signal coming into.

By selecting the right opsins for biological specimens, researches can have precise control over the cells and their signaling mechanisms. This enables a detailed investigation on the inner-cell activities.

2.3. Opsins and their Functions

Channelrhodopsin (ChR) is one of the most widely used opsins in optogenetics for activating cellular activity with a light stimulation. It has been derived from a green algea called Chlamydomonas reinhadtii that lives in small ponds. As an excitatory opsin, blue light creates a conformational change and allows the influx of positive ions such as calcium and sodium for neurons. This leads a initial activation potential for the neuron activity. The activation of ChR has been successfully utilized in various studies to control neuronal firing and investigate neural circuits [1].

In contrast to ChR, halorhodopsin (NpHR) is an inhibitory opsin commonly used in optogenetics. It has been derived from the archaeon Natronomonas pharaonis which lives in very salty mountains. Within the illumination of orange light, NpHR behaves as a light-gated chloride ion pump. It efflux of negatively charged chloride ions from the cell, and results in hyper polarization of the cell membrane. These two opsin proteins works with each other well thanks to their similar light powers and well-separated action spectrum [2].

In addition to these opsins, there are plenty of different ones with diverse properties and functions in the optogenetics field. Due to their activation spectrum, ChR and NpHR cannot be used for deeper tissues. To overcome this issue, opsins engineering becoming a field of optogenetics. C1V1 and Jaws (engineered red-shifted opsins) [3] or Chronos and Chrimson (genetically-developed opsins) [4] can be used with faster kinetics and enhanced light sensitivity. These engineered opsins allows researchers to have more temporal control over cellular activities.

3. Optogenetic Tools and Technologies

3.1. Types and Properties of Opsins

Opsins can be divided into two groups for their functionalities: Excitatory opsins, such as channelrhodopsins (ChRs), enable the activation of cellular responses upon light stimulation. Inhibitory opsins, such as halorhodopsins (NpHR), provide the ability to suppress or modulate cellular activity upon illumination.

When designing an experiment with opsins, there are two must-known properties of the opsins. The first one is their responsiveness to a specific wavelength. Researcher should select or engineer opsins for their specimen selection. The second one is the kinetics of the opsins. Since that activation of ion pumps needs some time, and recovering itself for a new signaling would need another time, researcher should be aware of opsins' response times and recovery rates.

For optogenetics field, it is more common to use microbial opsins because of their nature of their direct ion-pump activation nature. However, there are plenty of other opsins called animal opsins. They connected to G-proteins whose are actually activator proteins of some enzymes. Within those enzymes, the ion-pumps can be opened. Due to this extra layer on the activation process of animal opsins, microbal opsins known for their faster responses and higher modulation frequencies. [5]

3.2. Opsin Expression and Detection Methods

One of the important questions of implementing a real-optogenetics experiment is about embedding the opsins molecules into the interest of region. In neuroscience cases, it would be the having opsins on the nervous cell membranes. Since that a healthy nervous cell won't have any opsins in its synapses, researchers should be insert with a decent methodology.

In gene therapy, viral vectors have emerged as a potent means of delivering opsins into neurons, allowing for precise optogenetic control of cellular activity. It is possible to engineer viruses, such as lentiviruses or adeno-associated viruses (AAVs), to carry opsin genes and effectively and precisely infect target neurons. The opsin genes can be delivered to the nucleus by these viral vectors, which can then integrate them into the cellular genome. The neurons express the opsins after they have been integrated.

Apart from the viral method, there are lipofection, electroporation and optoporation methods available to express the opsins into a cell.

After the expression has been done, the researcher shall check the existance of opsin proteins in the desired cell's membranes. To perform this validation, fluorescence tagging is widely used, which pairs a designed molecule with a fluorophore to make it visible on fluorescence imagining microscopy. There are two different types of experiment procedures to perform detection. They are separated for to in-vitro and in-vivo environments.

3.2.1. Detection in-Vitro Experiments

The principle of in-vitro experiment procedure lies on the confocal fluorescence microscopy. Within the stimulation laser (SL) source, the stimulation light needed for opsins activation is produced. By using neutral density filter, all the wavelengths of the stimulation laser normalized and by using a optical fiber, this laser light transferred into biological specimen.

The experiment procedure can be seen in the Figure 2. The halogen lamp (HAL) is only an extra-illumination for better contrast on the detector. Remaining optical generation components such as fluorescence exitication laser (FEL) and microscope object (MO) generates a wavelength of light to activate opsins' fluorescence tags to have a localization and distribution map of the opsins inside the specimen.

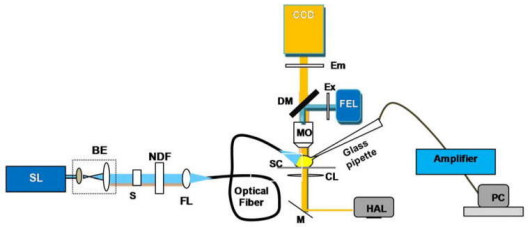

Fig. 2. Opsin Detection Experiment for in-Vitro Environments (Figure 3 on [6].

3.2.2. Detection in-Vivo Experiments

For the experiments on a living animal, in-vivo, the skull must be removed to perform stimulated light. However, the animal must be sacrificed to validate the opsins after the experiment since confocal microscopy is not available for deep-brain tissue monitoring.

On the Figure 3, the experimental procedure can be seen. Within the same methodology of in-vitro procedure, the only difference is to have stimulation and fluorescence excitional laser as the same manner.

3.3. Light Sources and Optogenetic Stimulation Systems

The optogenetic experiments mostly consist of both inhibition and activation processes of cells. Because of this two different processes and their different wavelengths, the experiments may at least have two light sources. The most common source types are LEDs and LASERs. In the case of multiple colored optogenetics experiments, lights that have different wavelengths can be combined in the fiber-cable line. Note that, the for the illumination, the most commonly used

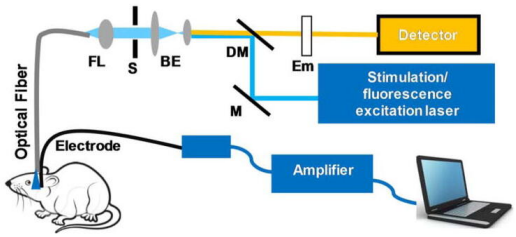

Fig. 3. Opsin Detection Experiment for in-Vivo Environments (Figure 3 on [6].

opsin, ChR-2, expressed neurons needs to be excited $1mW/mm2$ at the maximum absorption wavelength of $470nm$. As a second property, light's temporal resolution is quite important for the particular cases. It means that shutter's on/off cycles should be determined for the experiment will be conducted.

There are two types of illumination techniques on optogenetic researches. On the case of activating or deactivating a group of cells, the selected group members would be expressed with opsins, and all the cells are illuminated. Since the non-expressed cells won't be affected within the light, only desired cells would have formational change. This type of illumination is called **wide-field**.

Understanding brain, cardiovascular system, and eye-sight would need more detailed control over their cells. To perform this spatiotemporal resolution, the experiments may need to be conducted on singular cell level. This type of illumination known as **cellular-resolution**.

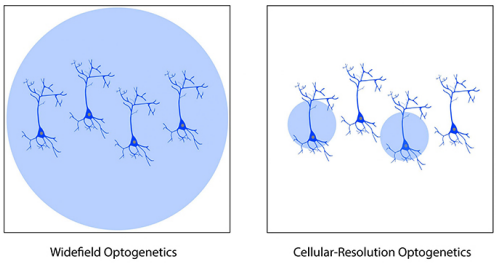

Fig. 4. Wide-field and Cellular-Resolution Illumination (Taken from [7]).

3.3.1. Wide-field Illuminated Optogenetics

It is the first method of optogenetics tools, due to its simplicity, many of the experiments still uses this in-expensive methodology. Also, on some of the papers, illumination is combined with the calcium imagining for simultaneous analysis of the neural activity. [8] Even though its simplicity, and disadvantage on the spatial field, there are lots of studies succeed with the wide-field optogenetics: restoration of visual responses in human ex-vivo retinas (Busskamp et al., 2010; Polosukhina et al., 2012), fear memory recalling (Liu et al., 2012), treatment of sleeping disorders (adamantidis et al., 2007)), understanding of depression with dopamine neurons (Tsai et al., 2009), advancements on developing a treatment for Parkinson's disease (Kravitz et al., 2010).

It should be noted that the wide-field optogenetics still an attractive method for the low SNR needed experiments.

3.3.2. Cellular-resolution Illuminated Optogenetics

Behavioural procedural analysis of cell networks can be achieved by manipulation of singular cells. For those cases, a laser, instead of a LED or a lamp, can be focused directly on a cell. This focusing process can be easily done using the lenses. The focused point size can be seen on the Equation (1) where d is the spot size, λ is the wavelength of the illumination light and NA is the numerical aperture of the lens. However, due to illumination on the out-of-focus cells (out of Rayleigh range), some undesired effect may be applied. Using an intensity just above the opsin activation threshold for the illumination light would be the proper solution. [6]

$$d = 1.22\frac{\lambda}{\mathrm{NA}} \tag{1}$$

It is clear that cellular-resolution optogenetics has an advantage on spatial domain. As a contrast, it would have a disadvantage on temporal domain since illuminating each selected neuron is a slower process comparing to wide-field optogenetics. There are two methods used on the illumination of multiple cells: Scanning and Parallel methods.

$$t_{total} = N(t_{change} + t_{dwell}) \tag{2}$$

Scanning method is a way to rapidly changing of the direction of the light source. The galvonmeter mirrors are heavily used because of their simplicity. Its main drawback is having very long waiting times, therefore longer experiments. The equation of the total time can be seen on Equation (2) where N is the number of cells visited, t_{change} is the duration of change from one cell to another and t_{dwell} is the time needed for activation of an opsin.

The most used tools for scanning the cell networks are resonant scanning mirrors and acousto-optic deflectors. Due to its galvonometer mirror nature of resonant scanning mirrors, the mechanical inertia and the dwell time needed is comparing inefficient against to acousto-optic deflectors. But, on the other hand, less energy consumption on the stimulation light makes them still a valuable choice. It is shown that only a few of kHz can be reached on the frequency within galvonmetric solutions. [9]

Acousto-optic deflectors can go up to tens of kHz which makes them a right choice for investigation of neural circuits. They work by changing the sound frequency as an input, uses the fraction principle and changes the angle of the stimulation light. This intertia-free scanning technique has no mass object, which means all controlled with electronic components, therefore can achieve very small t_{change} duration. However, excitation field is comparably small (150x150 to 200x200) to scanning mirrors.

Simultaneity is very important if the experimental design relies on real-time manipulation. Parallel excitation methods utilises this solution. Within the change of intensity on the light beam, one can mimic multiple light sources within a source. Spatial light modulators are used for this purpose, they may change the amplitude or phase of the beam incident and create desired light.

The amplitude modulation SLMs are most commonly used within the digital micromirror devices (DMDs). Those small mirrors (1920x1080 are available commercially) units as a galvonimetric machine and within the angle change of each pixel-mirror, the amplitude of that incident can be controlled. As an alternative, off-the-shelf LCD projects and μLEDs can be also used. The main advantage is having high-temporal and lateral resolution. For example, studies shows that, DMDs can update their patterns at 1-10kHz. [10] Although, the power loose on the fractions of mirrors are the most important drawback.

The phase modulation, that can be implemented using liquid crystal based SLMs, is the technique where each pixel is controlled for their refractive index with formation change in crystal. The different refractive indexes works similarly as DMDs. As an advantage over amplitude modulation is that it does not have that much of power-loss. Within the computer-generated holographical phase modulation, the t_{total} decreases to only t_{dwell}.

3.4. Light Propagation in Tissues

The most necessary part that has to be considered is the propagation of a light beam in the tissues. Tissues absorbs and scatters light due to their microstructures. These known phenomena attenuates the intensity of the light beam which can be expressed as Equation (3). The light intensity equation shows us that intensity is a decaying function within effective attenuation coefficient where can be expressed as (4).

$$I = I_0 \cdot \exp\left(-\mu_{eff} t\right) \tag{3}$$

It should be noted that I is the intensity in tissue, I_0 is the light intensity and μ_{eff} is the effective coefficient which is a combination of absorption and scattering properties.

$$\mu_{eff} = \sqrt{3\mu_a(\mu_a + \mu_s(1-g))} \tag{4}$$

μ_a is the absorption coefficient and μ_s is the scattering coefficient which can be simulated as a effective attenuation. Note that, g in the equation is the anisotropy factor of the scattering tissue.

4. Application Example: Visual Restoration

Retinal degeneration are very common disease on the today's humanity. It is estimated that 1/4000 people affected from this illness by inheritance. Due to this huge importance, researcher are willingly to try optogenetics on visual treatments. It is already seen that some of the papers found possible ways to cure retinitis pigmentosa (RP) and glaucoma.

The cells connected to eye-vision are rods and cones. The rods cells are responsible of seeing the "shapes" on the low light, and the cone cells are responsible of seeing colors on the bright light. These cells also includes opsins for their functionality. For some diseases, this opsins' functionality decreases or directly disabled. The common sense to solve this issue is an early gen-therapy to make it impossible to happen. Despite its complexity and dependence of gens of illness, the optogenetics creates a new solution of changing the opsins – without following the reason why they become useless.

The most important four opsins used in this field are: Channelrhodopsin (ChR), Halorhodopsin (HR), Melanopsin (OPN4) and Human rhodopsin (RHO). [11] [12] For cone photoreceptors, HR can be used for a replacement for outer retina problems. Also, ChR-2 and RHO are used for inner retinal photoreceptor losses. It is shown that the light responses are more sensitive on RHO replacements comparing to ChR-2 on the experiments conducted with mices. [13]

The approach on the delivery of the opsins into the eyes are similar as we described earlier. The most common technique is the AAV-based viral vectors, because of the approval of LUXTURNA - an AAV gene therapy for humans. As a second method, with increasing popularity, is the nanopartical agents. They have an advantageous over the vectoral delivery. Not only they transmit the genes into retina, but also you can pack them with some drugs. It helps on the researches further.

Four different company has converted the potential of optogenetics on vision treatment into drug industry. On the content they they are still their trail stages, some of the drugs shows very good performance. Note that, all the drugs are for retinitis pigmentosa disease.

- GenSight Biologics: It uses ChrimsonR opsin which is an engineer version of ChR. The treatment needs additional device to change the lights from outer world to the wavelength and intensity that opsin can understand.
- Allergan: The treatment based on ChR opsin whose light stimulation may be toxic for the retina itself. It is shown that there was no significant implact on treatment within this drug.

- Bionic Sight LLC: It is based on ChronosFP opsin. They have a different methodology which combines a encoder and transducer. The encoder converts the visual inputs to some signal that brain can understand, and transducer then stimulates encoded signal into retina. It seems very promising drug.
- Nanoscope Therapeutics: This is the only drug that does not need additional converter light. It is on the next stages comparing to other. The company reported that all the eleven patients had improvements on their visual sight.

5. Conclusion and Future Perspectives

Optogenetics is emerging as a powerful tool and comparatively new field that allows researcher to investigate cellular networks on individual cell level. On some cases, it even helps the researchers to focus on in-cellular level resolution.

5.1. Achievements and Limitations of Optogenetics

Optogenetics increased the knowledge base of the humanity on neurosciences. By using the optics, it let the reseracher to have insights on topics such as Parkinson's, epilepsy, heart diseases, etc. Apart from these advancement, it created an opportunity for the field to understand the behavior and brain relationship. It is a chance of the combining physiology and neurosciences, after understanding the physics of emotions. The further we understand from the brain will help us on creating the in-memory computing devices.

Apart from its valuable points, it is a sure that limitations of optogenetics are exits. The main challenge for now is to having parallel activation, serial activation and increasing the spatiotemporal resolution. As a side challenge, the used technology for the experiments are not suitable for daily-life implementations. The further researches should be done on creating wireless optogenetic devices for daily-life in-vivo environments for the humans.

5.2. Future Potential of Optogenetics

1. **Improved Spatiotemporal Resolution:** Within the increased resolution on spatial domain, we would have the ability to understand inner-cell processes, and organelle-level nature. It would be also useful to understand dentrid and synapsis leveled brain functionality.
2. **Gen Delivery and Better Opsins:** It should be also noted that AI on the engineering opsins are on top nowadays. The advancement would be done on gen delivery will help the researcher on selectiveness of the experimented group of cells.
3. **Reducing the Need of Surgery:** The next step should be decreasing the need of surgergy, or at least, minimizing the change of having tissue damage. Wireless optogenetic devices will be very helpful on the decision-making processes of the humans.
4. **Clinical Disease Treatment:** Optogenetics holds a way to solve many neurological and psychiatric disorders. It may even improved for patient-based approaches.

In conclusion, optogenetics has revolutionized neuroscience research by providing a means to probe and manipulate neural activity with exceptional precision. While facing certain limitations, ongoing advancements in targeting specificity, optogenetic tools, and non-invasive techniques offer exciting prospects for the future of this field. With continued innovation and interdisciplinary collaboration, optogenetics is poised to uncover further mysteries of the brain and pave the way for new therapeutic interventions.

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