

Carpe lucem: harnessing organic light sources for optogenetics

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With the advent of optogenetics, numerous functions in cells have been rendered responsive to the experimental delivery of light. The most common implementation of this technique features neurons genetically modified to express light-sensitive ion channel proteins, which open specifically in response to pulses of blue light, triggering electrical impulses. Optogenetics has now matured to a point where in addition to answering fundamental questions about the function of the brain, scientists are beginning to consider clinical applications. However, further progress in this field will require new ways of delivering light. One of these involves the use of organic light-emitting diodes (OLEDs), a display technology increasingly common in modern-day smart phones, for the optical stimulation of cells.

Making neurons receptive to light

In the last decade, the field of neuroscience has been transformed by newfound capabilities to control and monitor neuronal biochemistry with light, using a suite of techniques collectively referred to as optogenetics. The unifying feature of these experiments is their use of either light-sensitive 'actuator' proteins (these drive changes in the cell upon absorption of light, e.g. a change in membrane voltage) or light-emitting 'sensor' proteins (these provide a readout of the cell state through changes in the intensity or wavelength of emitted light, e.g. in response to intracellular calcium concentration). Optogenetics is arguably most widely identified with the first of these strategies, where genetic tools to sensitize neuronal tissues to light are combined with optical technologies to deliver precise illumination to neurons, with the combined effect of changing the patterns of electrical signals generated by neurons. When applied at the animal level, e.g. in mice or rats, this allows controlling the behaviour of the animal with light delivered through optical fibres that are surgically implanted in the brain (Figure 1).

At the heart of many optogenetics experiments is the light-sensitive actuator protein Channelrhodopsin 2 (ChR2). ChR2 was originally identified as a key protein required for phototaxis (movement in response to a light stimulus) in the green alga *Chlamydomonas reinhardtii*. Detailed characterization of ChR2 established that it is a light-gated ion channel, specifically mediating the flow of ions such as sodium and calcium into cells that are exposed to blue light (Figure 2a). Fortuitously for neuroscientists, when the genetic code for ChR2 was copied into neurons, its response to blue light was found to trigger neurons to fire the electrical impulses (action potentials) that ordinarily form the basis of

communication in the nervous system (Figure 2b)¹. These findings were the prelude to the widespread adoption of optogenetics by neuroscientists around the world. Nowadays, neuroscientists have developed a huge library of channelrhodopsins (ChRs), with variants capable of either activating or silencing neurons, in response to light of wavelengths all along the visible spectrum, acting on timescales extending from milliseconds to hours.

The combined power of optogenetics emerges from the ability to target cells of interest based on their genetic make-up, plus the comparative ease with which individual cells can be targeted with light. Genetic information for ChRs can be safely packaged inside viral particles, which neurons internalize and dutifully process to produce the light-sensitive proteins. In this way, tissues in live animals can be genetically modified to express ChRs where they are required. When genetically modifying cells to produce ChRs, their genetic code can be delivered alongside sequences that encode fluorescent proteins, thus allowing the easy identification of ChR-producing cells by fluorescence microscopy before delivering excitatory pulses of blue light. Likewise, they can be linked to sequences that limit the production of ChRs to certain cell types, or tied to the action of specific enzymes, chemical compounds or other factors. These properties can be combined like a programming language, enabling complex experiments. For instance, ChRs could be produced only by neurons in a particular circuit in the mouse brain that is activated when the animal performs a specific task. Subsequently reactivating the same circuit with blue light could make the animal recapitulate the learned behaviour. Using such strategies, researchers have identified

assemblies of neurons in the brain that are involved in processing information during certain activities.

Delivering optical stimuli to neurons

In parallel to these innovations in molecular, cellular and behavioural biology, the widespread adoption of optogenetics has also driven technological advances in optics that increase the specificity with which light can be delivered to individual neurons. For basic experiments, on cell cultures, for example, the same light sources used to illuminate samples in fluorescence microscopes are often repurposed to deliver optical stimulation for optogenetics. Light stimulation in optogenetics should usually be delivered as sequences of pulses with a length in the 1 to 10 millisecond range in order to emulate naturally occurring patterns of electrical activity in neural circuits. In addition, the spectrum of light should match the absorption of the used ChR and enough light needs to be delivered so that a sufficient number of ChRs per neuron are opened and electrical impulses are triggered. Light-emitting diodes (LEDs) and laser diodes meet all the above requirements and are indeed widely used in optogenetics.

Another challenge is how to direct light to certain cells or groups of cells without exposing neighbouring cells. Such targeted light delivery can in principle provide a massive improvement in the specificity with which cells can be activated. Genetically, one can programme certain types of neurons to produce ChR, but optically one could address individual neurons (or at least very small, localized groups of neurons). Available options to achieve this involve the use of sophisticated confocal or two-photon microscopes which use complex arrangements of mirrors and lenses to shape and distribute light provided by pulsed lasers. However, due to the bulky size of these microscopes, they are usually limited to studies on tissue explants or on head-fixed animals. In addition, microscopes deliver light from the outside and so can only reach a few tenths of a millimetre into the brain, which limits their usefulness, in particular, for future clinical use. Scientists have therefore turned to inserting optical fibres, fine bundles of fibres or other miniaturized optics into the brain to deliver light to deeper brain regions². While this has yielded exciting results, it is fundamentally difficult to miniaturize these devices sufficiently to prevent damage to the surrounding brain tissue. Researchers have now developed arrays of microneedles decorated with tiny LEDs along the needle shaft. These so-called 'LED shanks' can be extremely thin and have enabled optogenetic control of free-moving awake mice^{3,4}. The vast complexity of the brain means that ideally one will have hundreds to thousands of individually addressable LEDs on each shank, with each LED being small enough



Figure 1. Optogenetics allows the manipulation of animal behaviour through light-mediated activation of neurons in the brain. This facilitates a range of novel and controlled neuroscience experiments which use light to better understand the function of the brain. (Reprinted with permission, Society for Neuroscience 2014, from M.J. Robinson, *J. Neurosci.* **34**, 16567 (2014).)

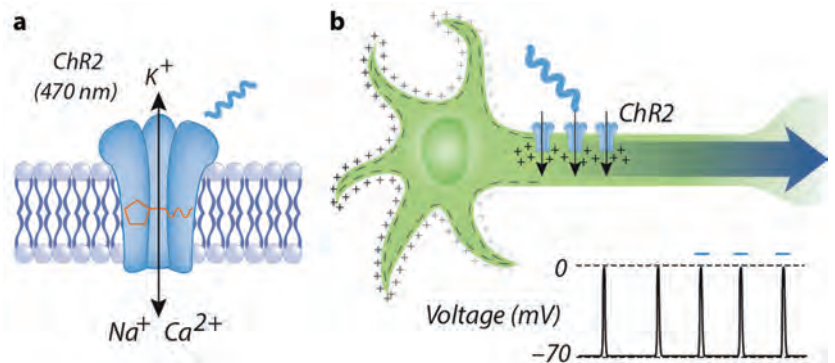


Figure 2. (a) Schematic of the ion-channel protein ChR2 which opens in response to irradiation with blue light. (b) When embedded into the membrane of a neuron, ions flowing in through the ChR2 lead to a change in membrane potential that can trigger the neuron to fire an action potential. (Reprinted with permission, Nature Publishing Group 2011, from E. Pastrana, *Nat. Meth.* **8**, 24 (2011).)

to address, at most, a handful of neurons. Controlling such a vast number of LEDs without an unmanageable number of connections and wires would require placing the LEDs directly onto a small electronic microchip. Unfortunately, however, the presently available LED technology, which is based on gallium nitride, is not directly amenable to integration with today's mostly silicon-based microchip technology (ultimately this is due to the crystal lattices of gallium nitride and silicon having different dimensions). This incompatibility between microchips and LEDs is one of the reasons we recently began to repurpose a display technology which is used in many modern-day smart phones for the delivery of light in optogenetics experiments.

Repurposing mobile phone displays for optogenetics

Organic light-emitting diodes are light sources based on plastic-type hydrocarbons containing large π -conjugated electron systems. In an OLED, thin layers of different organic compounds are sandwiched between two thin electrodes, at least one of which

Shine a Light

is semi-transparent (Figure 3). When an electrical current is passed through this thin stack – the total thickness is typically less than 200 nm – light of a specific colour is emitted. Although OLEDs were first described over two decades ago, it is only in recent years that refinements to fabrication processes and device designs have seen OLEDs more routinely being deployed in everyday devices. Today, they form the light-emitting structure in displays of mobile phones – and for the wealthier among us they are increasingly used in TV displays – and due to their high efficiency they may well become light sources of choice for general illumination in the future.

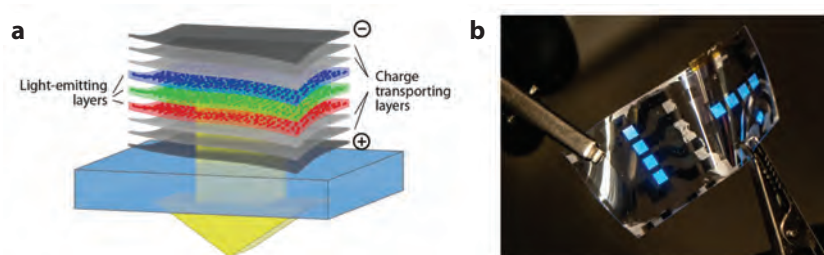


Figure 3. (a) Schematic structure of an organic light-emitting diode (OLED) consisting of multiple vertically stacked layers of plastic-type organic materials sandwiched between two electrodes. When passing a current through this stack, the organic material efficiently generates light of a specific colour. (b) When deposited on a flexible substrate, OLEDs can be bent and flexed and could thus be made to conform to the surface of the brain. (Credit: IAPP, Dresden.)

The organic materials used in OLEDs share many properties with inorganic semiconductors such as silicon or gallium nitride. However, in contrast to these, they are amorphous rather than crystalline which means they are mechanically flexible and can be readily deposited on a large variety of different substrates, including silicon-based microchips. This makes them a prime candidate for microchip-based OLED shanks that feature a large number of individually addressable light sources and may also allow the design of conformable and bendable implants that would be less disruptive to brain tissue.

Our efforts to demonstrate the usefulness of OLEDs as light sources for optogenetics have somewhat mirrored the path followed by biologists when they first developed optogenetics. The first demonstration that OLEDs can control light-mediated responses in cells used the green *Chlamydomonas reinhardtii* alga, from which ChR2 was originally extracted. We placed these cells onto a dense array of OLEDs that contained over 100,000 individually addressable OLEDs and found that the alga cells swam towards whichever OLEDs on the array were turned on⁵. When we first succeeded in making this happen, we joked that this was a bit like having cells watch TV!

Next, we demonstrated that cells can be grown directly on an OLED array. This time, we used cells that are not normally light sensitive and that were genetically programmed to produce ChRs. Because of the small dimensions of each OLED and the short distance between OLEDs and cells, this indeed allowed us to address the ChRs in individual cells by turning on the OLEDs just underneath each cell⁶. Figure 4 shows pictures of the used OLED arrays and data from a measurement that compares the response of cells with light from OLEDs located at different distances from the cell.

As the OLED technology was originally developed for TVs and displays, the brightness they provide is normally in a range suitable for human vision. However, our eyes are considerably more sensitive to light than cells that are rendered light sensitive via optogenetics; in fact, the light intensity required for an optogenetics experiment is 100–1000-fold higher than the brightness of a typical computer screen or TV. We have recently seen that this requirement can be accommodated by resorting to special OLED designs that make use of principles similar to the doping used to adjust the conductivity of conventional semiconductors. With such devices, it is possible to control the motion of fruit fly larvae programmed to produce ChR in their motor neurons and this represents the first demonstration of OLED-mediated optogenetic control for a multicellular organism⁷.

Despite these promising initial results on the use of OLEDs for optogenetics, further research is required for the potential of OLEDs to be fully exploited. Specifically,

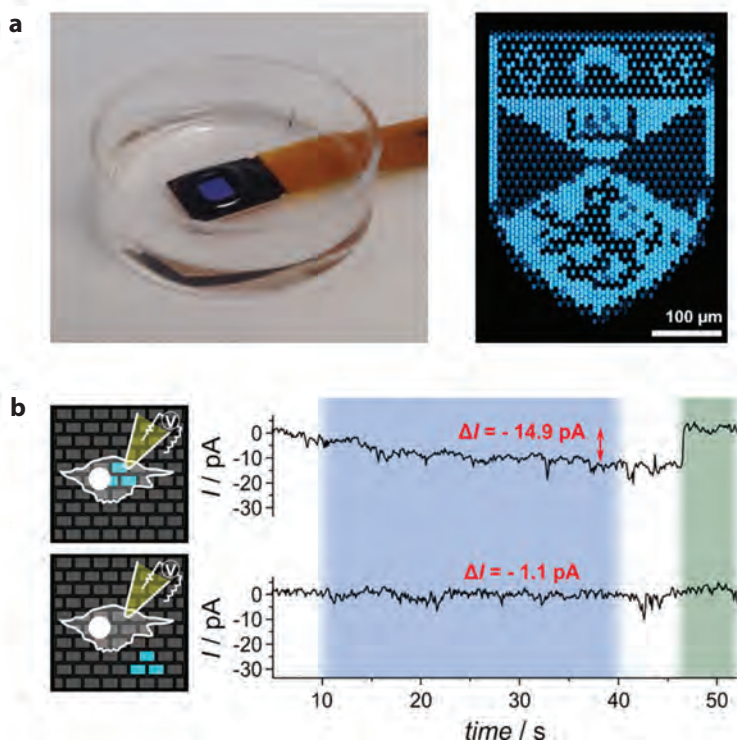


Figure 4. Use of an array containing many thousands of individual OLEDs for optogenetic control. (a) Picture of an OLED array embedded into a Petri dish (left) and microscope image of the array in operation, with individual OLEDs programmed to show the logo of the University of St Andrews (right, reprinted with permission, Wiley-VCH 2015, from Ref. 5). (b) Response of a cell producing ChR to direct light exposure from OLEDs underneath (top) versus no response to light from OLEDs next to the cell (bottom). (Reprinted with permission, AAAS 2016, from Ref. 6.)

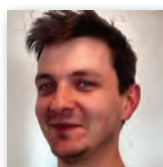
customized microchips in shank format that can operate OLEDs in pulsed mode and at high brightness will need to be developed. Besides further increasing OLED brightness and efficiency, the possibility of designing OLEDs that are mechanically flexible should be exploited for minimally invasive bio-implants that conform to the surface of the brain. Finally, in particular for chronic implants or for any future clinical use – where OLEDs would be left in the brain for weeks, months or even years – their durability needs to be enhanced by better protection against the ingress of water.

Devices of the future

Because OLEDs can effectively be printed on any scale, they lend themselves well to being integrated as light sources in many different types of devices. In lab-based settings, this may enable their integration as a light source into multi-well plates to facilitate high-throughput screening applications, the development of OLED shanks or flexible OLED sheets. Although further advances in light-delivery technologies and optogenetics as a whole will undoubtedly be central to many future insights into the functioning of the nervous system, the question remains, if and how such efforts can and should be leveraged to benefit human health directly. For optogenetics to be implemented in therapeutic efforts, one of the main questions relates to the viability and safety of sensitizing human tissues to light, e.g. via gene therapy. One US-based company is currently carrying out the first clinical trials in humans: for patients suffering from certain types of blindness, ChRs are being virally delivered to the retina, with the aim of restoring some components of light sensitivity in an otherwise degenerated visual system. There are also plans for a clinical trial to use optogenetics for treating patients suffering from chronic pain. Other ideas include the development of optogenetic cochlear implants which could surpass their electronic analogues in terms of frequency range covered or the design of optical pacemakers. While efforts to restore vision through optogenetics may ultimately lead to solutions not requiring artificial light-delivery systems, the success of many other optogenetic-based neuronal interfaces will likely require further innovation on the device level.

Like many other new technologies, optogenetics, and in particular its clinical application, triggers ethical questions about whether we should develop a technology that could be used to alter, augment or even enhance the function of the human brain. And as with other technologies, there are no easy answers. Is it ethical to develop or to refrain from developing a technology that would improve the quality of life for patients with severely disabling neurological diseases? Where should we draw the line between a medical

treatment and a cybernetic enhancement that could provide unfair cognitive advantages – possibly only to those of high social status – or that may affect our judgement or values? Such questions are of the utmost importance and they need to be posed again and again as the technology develops. However, as with other fields of science, categorically banning research that may enable us to understand our own mind is probably a poor choice. From our perspective on optogenetics research, ethical questions are being taken very seriously by the scientists involved so that we are carefully optimistic that the benefits of these new tools for neuroscience research and neuromedicine will greatly outweigh their risks. ■



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