

All Optical Interface for Parallel, Remote, and Spatiotemporal Control of Neuronal Activity

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ABSTRACT

A key technical barrier to furthering our understanding of complex neural networks has been the lack of tools for the simultaneous spatiotemporal control and detection of activity in a large number of neurons. Here, we report an all-optical system for achieving this kind of parallel and selective control and detection. We do this by delivering spatiotemporally complex optical stimuli through a digital micromirror spatiotemporal light modulator to cells expressing the light-activated ionotropic glutamate receptor (LiGluR), which have been labeled with a calcium dye to provide a fluorescent report of activity. Reliable and accurate spatiotemporal stimulation was obtained on HEK293 cells and cultured rat hippocampal neurons. This technique should be adaptable to in vivo applications and could serve as an optical interface for communicating with complex neural circuits.

The human brain is an organized interconnected network of more than 100 billion nerve cells, whose activities underlie perception, thought, decision-making, and action. A primary challenge of neuroscience is to understand how groups of cells in the massive neural networks of the brain communicate and dynamically regulate their connections.^{1–4} As a result, there is a need for tools that permit the organized activation and monitoring of activity in groups of cells that represent discrete components of large networks. Developed and perfected in the last few decades, patch clamping has been one of the most useful tools for accurately stimulating and recording electrical activity in neurons,^{1,5} but it requires a high degree of skill and is difficult to scale up to more than a few patch clamps at a time.⁶ Microfabricated arrays of electrodes, or field effect transistors, have been used for parallel stimulation and recording,^{7–9} however, these methods cannot control activity in select target cells within densely

packed neural circuits. As a result, most experimental research still focuses on the function of one or a few neurons or synapses.¹⁰ In addition, the established methods for electrical stimulation and recording are invasive and involve contact with neurons. In contrast, optical recording and stimulation do not require physical contact with cells and are inherently noninvasive. Fluorescent probes, such as voltage-sensitive and calcium indicator dyes, provide powerful means to monitor neural activity and synaptic transmission on a population basis when cells are situated within neural circuits.^{11–13} Recent developments on optical stimulation and inhibition of neural activity^{14–20} have now made remote and noninvasive control possible.^{14–29} One powerful method among these is to engineer ion channels conjugated with a synthetic light switch molecule, an approach that offers several unique advantages, including rapidly reversible photo-switching, sustained activity in the dark, and the ability to optically depolarize or hyperpolarize by engineering different types of ion channels with light switchable molecules.^{14–16,19} Hence, we proposed a parallel optical scheme for the remote and spatiotemporal control of neural circuit activity.³⁰

In this paper, we achieve and report this kind of all-optical platform for the parallel control and recording of activity in

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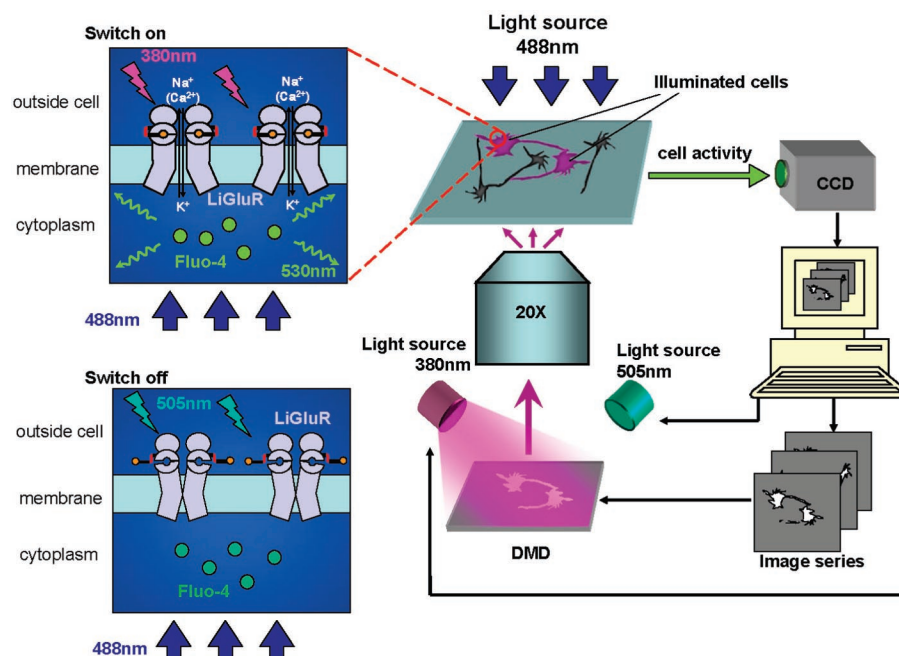


Figure 1. An all-optical platform for parallel and remote control of neuronal cells. Cells transfected with iGluR6 and chemically modified with the chemical photoswitch MAG to generate the light-activated ionotropic glutamate receptor (LiGluR) are loaded with calcium dye (Fluo-4) and placed on the stage of the optical microscope. Two LEDs at different wavelengths serve as sources of illumination of a DMD, which projects spatially designed patterns of the light through an objective lens onto selected cells within the field. Illumination at 380 nm opens the LiGluR channels and illumination at 505 nm closes them, thereby exciting the cells or turning the excitation off, respectively. The image sequences displayed by the DMD were synchronized with light pulses from the LEDs. Activation of the cells, detected by Fluo-4 (illuminated at 488 nm) due to calcium entering the excited cells, was imaged using a CCD.

large numbers of cells in culture by using a spatial light modulator that contains 800 000 micro-mirrors to activate the light-sensitive excitatory ionotropic glutamate receptor (LiGluR)^{14–16} while using a calcium dye to optically record activity. This remote control and detection method has a spatial resolution at the micron scale and a temporal resolution on the scale of milliseconds, and it avoids the invasiveness and spatial constraints of electrode placement. The method enables simultaneous and selective stimulation and recording in a large number of cells and could in principle be used to control either information flow through neural circuits, or network plasticity, over long periods of time. Combined with a dynamic light moderator and a zoom lens, this optical platform is capable of stimulating neurons from the subcellular level to the level of large-scale circuits.

The system configuration is illustrated in Figure 1. Two light emitting diodes (LEDs) with center wavelengths at 380 and 505 nm were used to switch on and off LiGluR channels that were expressed in cultured postnatal hippocampal neurons, following attachment of the maleimide–azobenzene–glutamate (MAG) photoswitch^{14–16} and loading of the Fluo-4 calcium dye. Illumination at 380 nm switches MAG from its trans isomer to the cis isomer and activates the channel, while illumination at 505 nm triggers the opposite isomerization and deactivates the channel.^{14–16} The 380 and 505 nm LEDs were coupled to the system and combined into the same beam path. The design makes it possible to modulate light at the two wavelengths using the same digital micromirror device (DMD) thus avoiding a problem of registry of the fine patterns of the two wavelengths of light.

The patterned light was projected onto cell cultures using a 20× objective in an optical microscope. The optical addressable area on sample is 0.87 mm × 0.65 mm with spatial resolution 0.85 μm (this can be adjusted by using different reduction objective lens). The measured output power density on the sample plane after the objective lens was adjustable up to 2 mW/mm². The Fluo-4 calcium dye was excited with a separate light source at 488 nm, and emission was imaged following a bandpass filter centered at 530 nm and collected through a charge-coupled device (CCD) camera. The illumination intensity for calcium imaging was low (approximately tens of microwatts per square millimeter) to minimize an effect on LiGluR.

Experiments began with imaging of the distribution and geometry of the cells. Optical patterns were designed to selectively stimulate specific cells, and these were displayed by the DMD and projected onto the cells through the objective lens. Preceding and following optical stimulation at the activating and deactivating wavelengths, fluorescence images of the Fluo-4 signal were acquired from the entire field of cells and were analyzed in real time. The system was capable of spatiotemporal modulation of the light stimulation pattern with switching times as short as milliseconds and with a submicron resolution within a projection area of approximately one millimeter.

We first used HEK293 cells as a model to examine the fidelity and accuracy of the parallel optical stimulation and detection method (Figure 2). Like neurons, HEK293 cells expressing iGluR6 and labeled with MAG respond to optical stimulation in a manner that can be detected both electro-

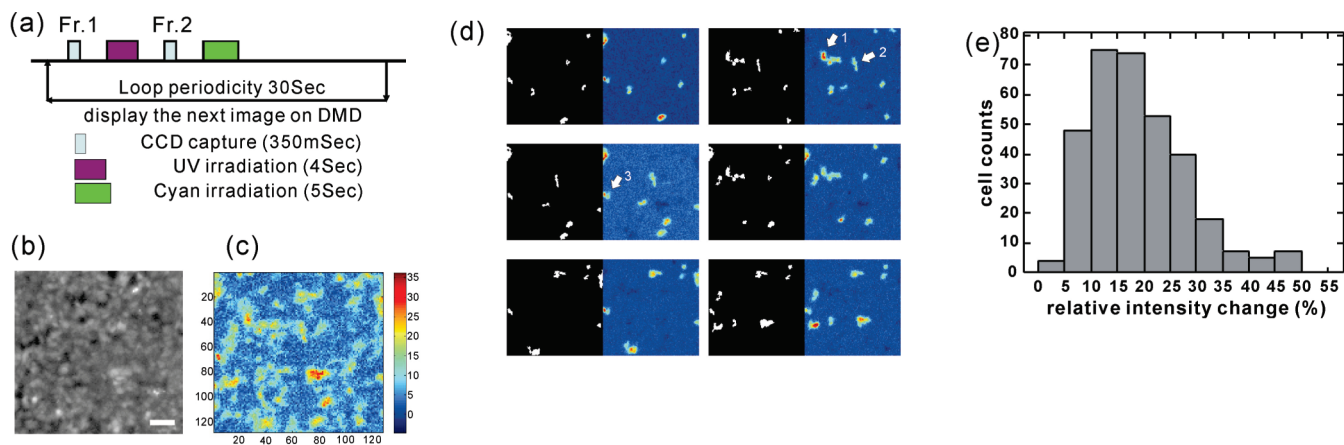


Figure 2. Parallel optical stimulation of multiple HEK 293 cells. (a) Optical stimulation protocol. Within one optical stimulation and imaging loop, 380 and 505 nm irradiation were applied to open and close LiGluR, respectively, and two captured CCD images (before and after 380 nm irradiation), were used to determine the cell response as the difference in Fluo-4 intensity. (b) Resting Fluo-4 image of the HEK cells (scale bar = 60 μm). (c) Percent Fluo-4 intensity change using flood exposure of entire field. (d) Six example frames showing accurate parallel optical stimulation. Black and white images indicate the DMD displayed patterns. Color images on right are cell responses corresponding to exposure patterns on left (Fluo-4 intensity change increases with color scale from blue to red). (e) Cell response distribution for all the stimulated cells over 33 frames using distinct illumination patterns (see Supporting Information, Movie.1). The pixel with maximum intensity change in each cell region was picked up to judge the cell response. The percentage of successful stimulation was 98.8% using the threshold of 5.1% for the Fluo-4 intensity change ($P < 0.05$, one-tailed t-test, $n = 99$).

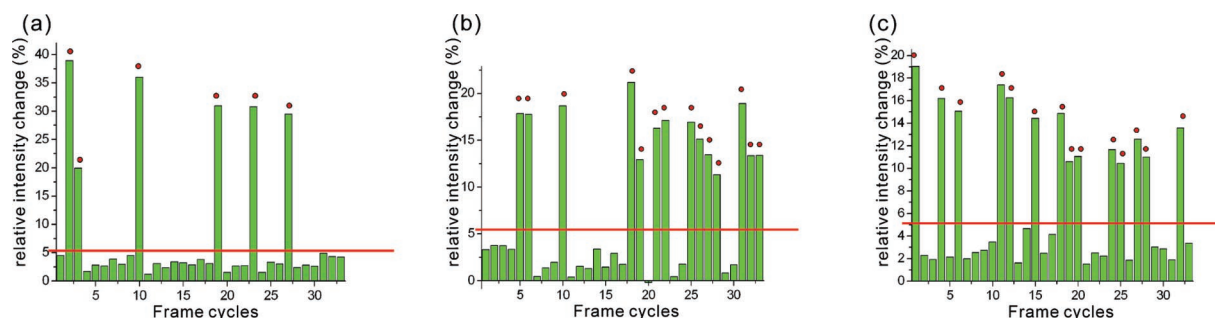


Figure 3. HEK cell responses. Responses to patterned optical stimulation in three cells (a–c) labeled 1, 2, 3 in Figure 2d. Red dots at top of frame cycle response columns indicate that the cell was optically addressed in those cycles. Threshold of 5.1% ($P < 0.05$) is indicated by the red bars. Responses show that optical stimulation of cells has a high fidelity.

physiologically and via calcium imaging, but the responses are simpler because the cells are nonexcitable, have no synaptic connections, and exhibit only passive responses.^{14–16} The methods for HEK293 cells preparation, maintenance, transfection, and MAG labeling were as described previously.¹⁴ Following expression of iGluR6 and labeling with MAG, the HEK293 cells were preloaded with Fluo-4 by incubating with 10 μM Fluo-4AM for 30 min at room temperature. The bath solution was (in mM) 135 NaCl, 5.4 KCl, 0.9 MgCl₂, 1.8 CaCl₂, and 10 Hepes, pH 7.4. The transfection rate of HEK293 cells is about 30%. Successfully transfected light-sensitive cells were identified by optical screening in which cultures in the entire view field were alternately flood exposed to 380 and 505 nm light to open and close LiGluR, respectively, transiently allowing calcium to flux into the cell via the open channel, leading to an increase in Fluo-4 fluorescence (Figure 2a–c). A variety of optical illumination patterns were designed for selectively stimulating small groups of light-sensitive cells (around 10 cells on average per frame).

By synchronizing LED illumination, DMD pattern formation, and CCD imaging (Figure 2a), these designed video

frames were displayed on the DMD, and each exposure pattern was alternately applied at 380 and 505 nm light before and after acquiring Fluo-4 images from the entire field. A high accuracy and fidelity of optical addressing was demonstrated over multiple repeats of the different illumination patterns (see Supporting Information, Movie 1). Examples of different exposure patterns displayed via the DMD and corresponding images of Fluo-4 intensity changes are shown in Figure 2d in which significant fluorescence changes can be observed only in cells that were optically excited. As shown in Figure 2e, we obtained successful stimulation 98.8% of the time, representing a high degree of spatial accuracy. The responses of three individual cells exposed to different temporal patterns of light over the course of 33 cycles were examined (Figure 3). Large increases in Fluo-4 fluorescence precisely correlated to cycles that included optical stimulation with 380 nm light. The above results indicate that parallel optical stimulation can be achieved with high fidelity and spatial accuracy on HEK cells.

Because calcium imaging is a useful tool for analyzing synaptic connections and neural circuit activity,¹³ we next applied our system to the spatiotemporal optical stimulation

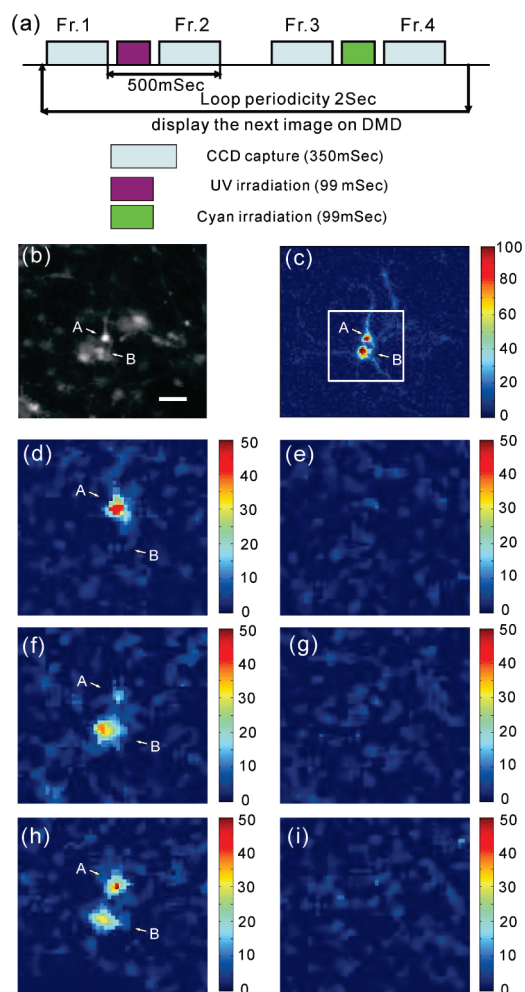


Figure 4. Patterned optical stimulation of cultured hippocampal neurons. (a) Optical stimulation protocol. Optical activation of neurons calculated as intensity difference between Frame 3 and Frame 1. Difference between Frame 4 and subsequent Frame 1 in next loop (LiGluR closed period) was used to determine if LiGluR activity was successfully shut off by irradiation at 505 nm. Cell calcium images were averaged from 10 loops with the same spatial stimulation pattern and normalized to enhance signal to noise and extract optical stimulation elicited activity over spontaneous activity on the cells. (b) Resting Fluo-4 image. (c) Cell responses to flood exposure of entire field at 380 nm triggers activity in two cells (A and B). Scale bar = 60 μ m for (b,c). (d–i) Magnified images of region in white box in (c). (d,e) Neuronal calcium responses when only cell A was optically stimulated ((d) at 380 nm; (e) at 505 nm). (f,g) Neuronal calcium responses when only cell B was optically stimulated ((f) at 380 nm; (g) at 505 nm). Neuron A had some activity when B was stimulated, but there was no response in B when A was stimulated, possibly indicating a synaptic input by B onto A. (h,i) Neuronal calcium responses when both A and B cells were optically stimulated together ((h) at 380 nm; (i) at 505 nm).

of cultured rat hippocampal neurons. The methods for postnatal hippocampal neurons preparation, maintenance, transfection, and MAG labeling were as described previously.¹⁵ Typically, a few transfected cells can be found in the view field through 20 \times objective lens. The bath solution was (in mM) 138 NaCl, 1.5 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 10 glucose, and 5 HEPES, pH 7.4. Stimulation protocols were similar to those used on the HEK cells (Figure 4a). Similar optical screening was applied, then two neurons exhibiting

optical responses were shown (Figure 4b,c). Selective optical stimulation directed at the soma of these cells selectively turned activity on and off in the individual neurons (Figure 4d–i). These results show that our optical stimulation system can selectively elicit activity in target neurons within a simple neural network in a noninvasive manner.

The ability to optically stimulate and detect neural activity using a device that can address multiple cells simultaneously is a significant advance over current techniques for investigating neural circuits. The use of light-sensitive proteins to activate neurons is becoming more prevalent, but the approach so far has been usually limited to wide-field illumination of regions containing very large numbers of cells, and detection has been typically coupled to invasive electrode-based methods for detection.^{26–29} On the other hand, photouncaging of glutamate is often combined with calcium imaging to give an all-optical, noninvasive means of stimulating and recording neural activity, but because of the presence of glutamate receptors in virtually all neurons this can only stimulate single cells when the excitation volume is small and is usually used to stimulate one neuron at a time. Now, using our optical method, light-sensitive proteins whose expression can be targeted to desired specific cell types, or caged proteins or compounds, can be activated in parallel at selective multiple sites around a cell, or at multiple cells in any desired spatiotemporal modulated manner, with the ability to rapidly switch between multiple wavelengths and illumination patterns in milliseconds. The micron-scale spatial precision of our method can potentially be used to study responses to subcellular stimulation, while the ability to precisely stimulate multiple cells within neural circuits is well suited to studies and controls of circuits within living animals that can potentially bridge the massive information flow between the man-made computer and in vivo neural circuits. Furthermore, combining the affinity labeling of MAG¹⁶ and micro-stereolithography^{31,32} may inspire a “neural lithography” leading to spatially modulating neural optical sensitivity by spatially biased labeling with patterns of light. In general, when not limited to the neural system our method of spatiotemporal uncaging chemical compounds or activating proteins can be useful for cell regulation, differentiation, and migration study and may even lead to medical and clinic applications.

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Supporting Information Available: Supplementary Video 1. This movie shows dynamic parallel optical stimulation within field shown in Figure 2. In each frame of this movie, the black and white pattern on the left indicates parallel optical stimulation, and the color images on the right show the cell calcium response (QuickTime; 2.3 MB). This

material is available free of charge via the Internet at <http://pubs.acs.org>.

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