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News & Highlights

Development of a Wireless-Controlled LED Array for the Tunable Optogenetic Control of Cellular Activities

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1. Introduction

In order to decipher a complex biological process, tools are required to perturb the various players involved to gain information about the important parameters. Optogenetic modules are genetically encoded molecular reagents that, when expressed in cells, allow a specific biological process to be precisely controlled by light in a spatiotemporal manner [1]. Optogenetics thus offers cell biologists an unprecedented new way to perturb cellular activities. The application of optogenetic approaches in cellular biology and synthetic biology research has evolved tremendously in the last few years [2–4].

2. Using light to control cellular activities

Compared with the chemicals that regulate cellular activities, light offers some unique properties; for example, it is easily deliverable, reversible, and tunable. Most importantly, light does not interfere with cellular activities. In order to attain precise optogenetic control of cells, light sources are required that are spectrally matched to the activation spectrum of the light-sensitive protein module. To date, many light sources have been used in sophisticated ways in optogenetics experiments, including standard arc lamps [5], lasers [6], and light-emitting diodes (LEDs) [7].

Single-cell or subcellular-level optogenetic manipulations are usually performed under a microscope that is equipped with appropriate laser lines and optical control modules (e.g., galvo mirrors), which enable precise light illumination [6,8]. However, in other biological applications—such as when manipulating and analyzing the cellular activities of a large quantity of cells (e.g., in a cell culture dish with tens of thousands or millions of cells), or when simultaneously activating cells under multiple different conditions for high-content screening studies—a practical, tunable, and portable illumination light source becomes extremely necessary.

3. The design of a wireless-controlled LED array for optogenetic studies

A novel wireless-controlled LED array system has been developed for the tunable control of light illumination through wireless communication. This system permits the precise modulation of light for optogenetic studies. The wireless-controlled illumination system consists of a master computer, a wireless Bluetooth communication module, and a slave computer. The core of the slave computer is a printed circuit board (PCB) outfitted with electric components that include a microcontroller unit, 12 shift registers, an 8×12 rectangular array of LED beads, and 96 corresponding chip resistors (Fig. 1(a–c); also see Appendix A). The physical size of the PCB is 124.46 mm × 86.36 mm and the distance between each LED is 9 mm; thus, the slave computer can fit a 96-well cell culture plate (Fig. 1(b)). The emitting wavelength of the LEDs is (470 ± 10) nm, so the system can be applied in blue-light-induced optogenetic studies [6].

In addition, the on/off light switch and the brightness of the LEDs can be accurately regulated by pulse-width modulation (PWM) (see Appendix A), which allowed us to display a "ZJU" (an acronym for Zhejiang University) pattern on the LED array with individual letters having different intensities (Fig. 1(d)). We calibrated and calculated the intensity of individual LEDs and determined that the full power of a single LED was 60 mW and the luminous intensity was 2500 mcd. On our developed LED illumination system, the theoretical brightness and measured light intensity fit perfectly with a linear regression (Fig. 1(e)), which further demonstrated the precise light-control capability of our system. The software we used for sophisticated control of the light illumination was custom developed on the Visual Studio 2015C# environment and optimized for user application (see Appendix A for details).

4. Using light to quantitatively control phosphoinositide 3-kinase activation in cells

Although most previous optogenetic research focuses on turning a specific signaling molecule on or off using light, a very small

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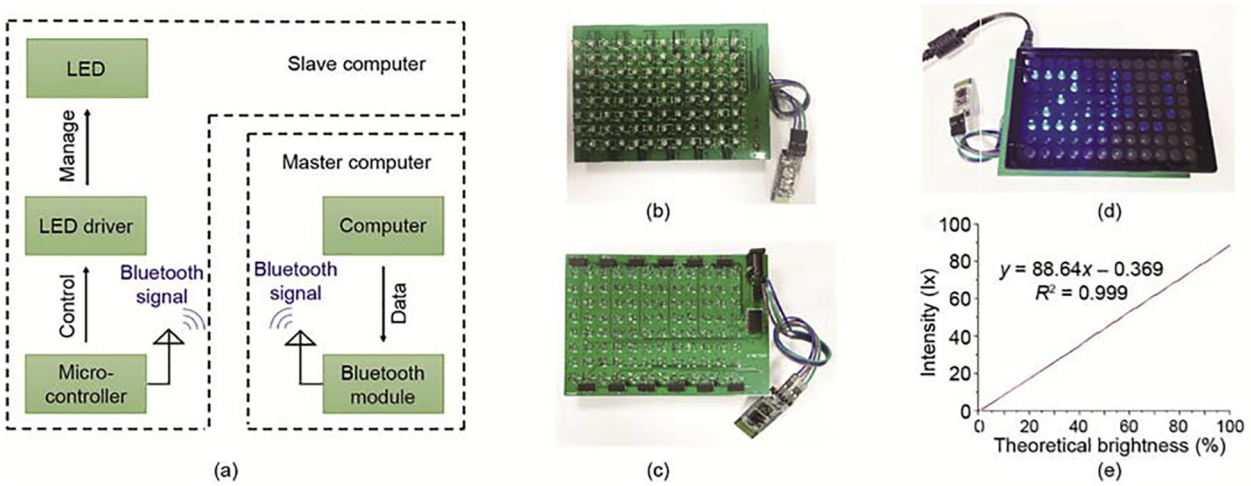

Fig. 1. Design of the wireless-controlled LED array system. (a) Schematic diagram of the system; (b, c) back view and front view of the slave computer part of the system, which has a PCB outfitted with electric components and an 8 × 12 rectangular array of LEDs; (d) display of the acronym "ZJU" (for Zhejiang University) on the LED array under the control of the master computer, in which different letters emit different doses of light; (e) precise control and measurement of the light intensity from individual LEDs.

number of studies were able to quantitatively modulate signaling activation in cells. We have demonstrated that our wireless-controlled LED system is able to remotely manipulate phosphoinositide 3-kinase (PI3K) signaling in cells—a task that is impossible without this sophisticated light-control system.

The optogenetic module we used is based on blue-light inducible protein heterodimerization between cryptochrome 2 (CRY2) and the N-terminus of CIB1 (CIBN) [9]; we modified this system to be able to control PI3K signaling [6]. Upon blue-light activation, CRY2-iSH2 is recruited to the plasma membrane that expresses a CAAX-tagged CIBN binding partner; this results in the recruitment of PI3K to the cell surface to activate downstream Akt signaling (Fig. 2(a, b)). HeLa cells were transfected with CIBN-CAAX and CRY2-iSH2 plasmids, and blue-light-induced Akt phosphorylation was studied by western blotting (see Appendix A). The cells in the incubator were illuminated with different on/off frequencies of blue light for a total of 30 min. The frequencies used were 5 s/1 min (i.e., light on for 5 s and off for 1 min), 1 min/1 min (i.e., light on for 1 min and off for 1 min), and 30 min (i.e., light continuously on for 30 min). Based on the biochemical results (Fig. 2(b)) and on quantitative analysis (Fig. 2(c)), Akt phosphorylation at both the Thr308 and Ser473 residues was increased upon illumination with blue LED light. The folding of Akt phosphorylation was increased with elevated blue-light exposure, with 30 min of light illumination having the most potent effect on Akt activation (Fig. 2(c)). In essence, this experiment demonstrated that using the wireless-controlled LED array allowed us to use light to quantitatively control endogenous PI3K signaling in cells.

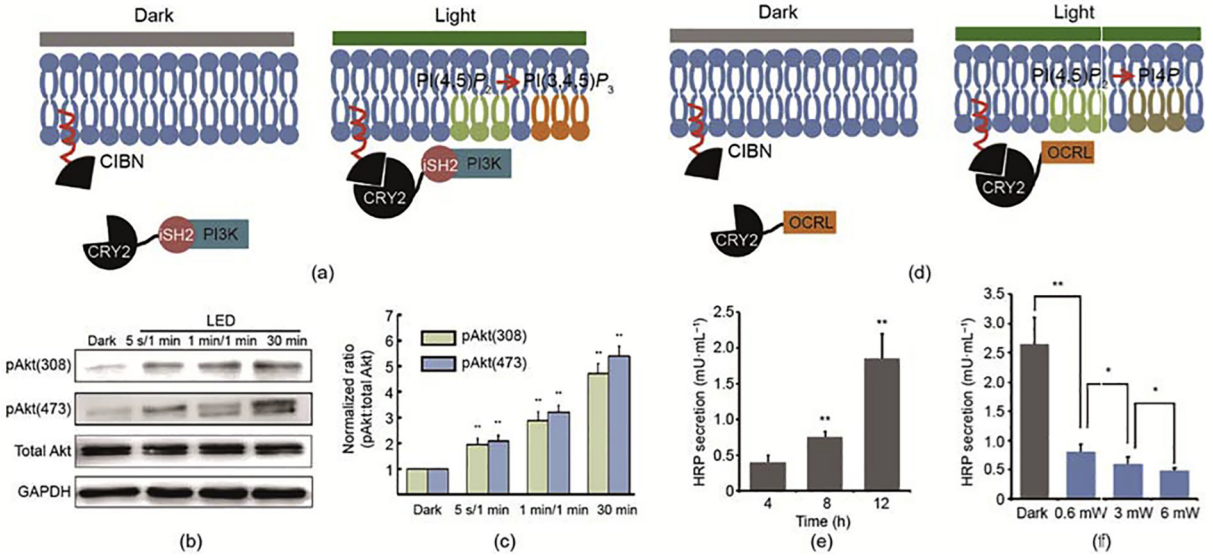

Fig. 2. Using the wireless-controlled LED array to quantitatively control PI3K signaling in cells and to perform optogenetics-assisted cell screening of PI(4,5)P_2 alternations on constitutive horseradish peroxidase (HRP) secretion on a 96-well cell culture plate. (a) Schematic of PI3K recruitment to the plasma membrane using CRY2-iSH2, which constitutively binds to endogenous PI3K; (b) immunoblot analysis of Akt phosphorylation at both the Thr308 and Ser473 sites after different light-illumination conditions; (c) plot of quantifications of phospho-Akt (pAkt); (d) schematic of the 5-phosphatase OCRL recruitment to the plasma membrane using CRY2-OCRL to dephosphorylate the 5 position on the inositol ring of PI(4,5)P_2; (e) validation of the HRP secretion assay and quantitative quantification of HRP in cell culture media at different time points after HRP-GFP transfection; (f) depletion of PI(4,5)P_2 by the recruitment of OCRL to the cell surface, which contributes to decreased HRP secretion in HeLa cells. * indicates $P < 0.05$ and ** indicates $P < 0.01$.

5. High-content optogenetics-assisted screening of cell secretion

We have demonstrated that this novel light-illumination system was able to perform an optogenetics-assisted, cell-based screening experiment in a 96-well cell culture plate. We used a CRY2-OCRL fusion protein to manipulate the cellular phosphoinositide metabolism and probe for its effect on constitutive horseradish peroxidase (HRP) secretion in cells [8,10]. Upon blue-light activation, CRY2-OCRL is recruited to the plasma membrane that expresses a CAAX-tagged CIBN binding partner, which results in the dephosphorylation of the 5 position on the inositol ring of PI(4,5)P_2, and thereby reduces its presence on the cell surface (Fig. 2(d)).

We measured HRP secretion by the expression of HRP-GFP in HeLa cells. HRP-GFP has been shown to incorporate into vesicles and undergo a constitutive secretion pathway [11]. The cells were transfected with CAAX-CIBN, CRY2-OCRL, and HRP-GFP constructs, and the HRP secretion was quantified (see Appendix A). Without blue-light activation, we detected an increase in HRP secretion in the media in a time-dependent manner (Fig. 2(e)). Next, we performed optogenetic control on these transfected cells with one LED per well in a 96-well plate, and illuminated different columns with different light intensities. It was found that the inhibitory effect of OCRL on HRP secretion correlated with the illuminating blue-light intensity. We demonstrated that 3 mW of light illumination has a more potent effect than 0.6 mW of light, and that 6 mW of light has the most potent inhibition when compared with the effects of 3 mW and 0.6 mW light illumination (Fig. 2(f)). As the control, blue-light LED illumination had a negligible effect on HRP secretion in HeLa cells, which suggested that the inhibition was caused by optogenetic control (see Appendix A). Therefore, this experiment demonstrated that using the wireless-controlled LED array allowed us to use light to quantitatively manipulate cells *in situ* with high precision, and to attain high-content analysis of cellular functions.

6. Conclusions

In recent years, synthetic biology has been successful in rewiring cellular signaling pathways and engineering new biological circuits to control cell functions. Optogenetics has been an excellent extension and complement to these approaches due to its unique characteristic of using light as a tunable and noninvasive media to control light-responsive protein activities. In a given optogenetic study, light is applied as a cellular input signal or virtual stimulus to trigger the responses of optogenetic modules expressed in cells; thus, light is used to regulate intracellular molecular networks or signaling pathways [1]. A tunable light source becomes extremely important when studying how cells perceive complex and time-varying input signals and when attempting to understand the quantitative input–output relationships between signaling transduction and its induced function in the cell.

Our novel wireless-controlled LED illumination system permits tunable optogenetic applications within cells; it enabled the quantitative activation of PI3K signaling in cells and the use of optogenetics-based cell screening to dissect the important role of PI(4,5)P_2 in cell secretion. We anticipate that this novel system will have a vast range of applications in cell biology and synthetic biology research in the future, and that it will be used to solve complex basic research puzzles and design quantitative synthetic approaches for light-guided biomedical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eng.2018.08.005.

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