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Instrumentation of a compact random-access photostimulator based on acousto-optic deflectors

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Recently developed optogenetics provides a fast, non-invasive, and efficient method for cell activation. However, it is difficult for the optical stimulators used for optogenetics to realize selective multi-site fast activation. In this paper, we developed a random-access photostimulator based on a pair of perpendicularly oriented acousto-optic deflectors. Precise laser targeting in the x-y plane was verified, and the lateral spatial resolution of laser intensity after the objective was measured as \( \sim 1.38 \mu \text{m} \). Photostimulation of ChETA-expressing astrocytes induced reliable inward currents only if the laser beam was directed onto the targeted cell. In the ChR2-expressing neuron, multiple locations along two dendrites were stimulated, and spatiotemporal integration was observed in the soma with fast multi-site activation. These results demonstrated that this random-access photostimulator would be a powerful tool for selective multi-site fast activation. The compact and modular design of this photostimulator makes it easily integrated with different commercial microscopes, and thus widely popularized in many laboratories. © 2012 American Institute of Physics.

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I. INTRODUCTION

Selective activation of multiple cells or neurites is very significant for investigating integration and computation in neurons. In the last few years, the combination of molecular genetic engineering and optical stimulation has boosted the appearance of a new technology—optogenetics, which provides a fast, non-invasive, and accurate activation.1–3

Cells expressed light-sensitive channelrhodopsins can be activated when they are illuminated by a beam of light. However, light sources used in common fluorescence microscopes are wide-field light sources, which would activate all cells or the whole neural circuits in the field of view (FOV).4 A laser scanning microscope equipped with galvanometer-driven scanning mirrors can realize the activation of multiple selective cells.5,6 Nevertheless, the scanning speed and the targeting positions are limited because of the inherent inertia of the scanning mirrors. Recently, the spatial light modulator (SLM) was introduced to achieve selective activation in a non-inertial way.7,8 However, the low refresh rate (\( \sim 60 \text{ Hz} \)) and diffraction limitation of SLM limit the application of this technology.9,10 In contrast, acousto-optic deflector (AOD) has the advantage of fast random addressing and has been used to deliver laser non-inertia to multiple pre-selected sites for photolysis of caged compounds in cultures.11–14 The good spatial selection will also make AOD suitable for selectively stimulating light-sensitive channels such as ChR2 and a newly engineered channelrhodopsin-ChETA.15

In this paper, the instrumentation of a random-access photostimulation system for selective activation based on a pair of perpendicularly oriented AODs is reported. First, we illustrate the optical and mechanical design of a random-access photostimulator using AODs and its integration with a commercial microscope. The photostimulating process was controlled by a custom-written software developed by LABVIEW. Subsequently, the performances of the photostimulator including targeting accuracy, FOV uniformity, and lateral resolution were evaluated. Finally, selective and fast activation of multiple locations were demonstrated using the ChETA-expressing cultured astrocytes and ChR2-expressing cortical neuron.

II. SYSTEM DESIGN

The optical stimulation system named QuickView-Stim is illustrated in Figure 1(a). The laser source is a diode-pumped solid-state blue laser (Mll-III-473, \( \lambda = 473 \text{ nm} \), Changchun New Industries Optoelectronics Tech, China). The laser was coupled into a single mode fiber (SMF, NA = 0.11, OZ, Canada) which was equipped with two lenses right at the two ends. To enhance the coupling efficiency, an aspheric lens (focal length, 4.5 mm) was used before the SMF (coupling efficiency >50%). An achromatic lens (focal length, 30 mm) was fixed right at the output end for laser…

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collimation, and a laser beam 4.6 mm in diameter was obtained. A halfwave plate was used to adjust the polarization direction of the collimating laser beam. After a pair of perpendicularly oriented AODs (DTSXY-400-473, AA, France) and a scan lens, the laser was directed into the commercial microscope (FN1, Nikon, Japan) by a dual port (Y-QT, Nikon, Japan), which consisted of one dichroic mirror and one emission filter. The dichroic mirror (DM505) was utilized to reflect the 473 nm laser to the microscope and to transmit fluorescence for CCD (CoolSNAP HQ2, Photometrics, USA) imaging together with a bandpass filter. The scan lens and the tube lens in the microscope comprised a relay system to image the AODs onto the back focal plane of the objective. The scan lens with a focal length of 180 mm was used in the system to balance the scanning range with the spatial resolution. Then the scanning beam was focused onto the samples by objective.

The optical elements of the system, except the laser, were all assembled on one raised platform (the scanning unit in Figure 1(b), indicated by the yellow arrow). In order to integrate the scanning unit into the microscope, eight tapped holes were made in the two profiles of the main body of the microscope. Two triangular frames were designed and fixed onto the main body by eight M6 screws. After those preparations, the photostimulation system was firmly fixed onto the microscope and integrated into a uniform unit. In order to control the scanning of the AODs, an AOD driver was built, which consisted of a direct digital synthesizer (DDSPA-B415b-0, AA, France) and a power amplifier (AMPA-B-30, AA, France), which supplied sine electrical signals to the acousto-optic deflector crystal. This driving box design also accorded with the modular concept and so it was easily installed onto the instrument stand.

One application routine termed “random photostim” (RPS) was developed for controlling the photostimulation process using LABVIEW (National Instruments, USA). It worked in association with IMAGE PRO-PLUS (IPP, Media Cybernetics, USA) software (Figure 2(a)), which was used to acquire images from CCD. Before photostimulation, regions of interest (ROIs) were pre-selected on the image taken by IPP. Then the position information of these ROIs was saved as an “.roi” file and imported into RPS (Figure 2(b)). In the RPS, the ROIs position was converted into frequency control words of AODs, which determined the laser delivery and targeting. The stimulation parameters such as stimulation mode, scanning mode, working mode, stimulation power, and stimulation time and interval can be configured in RPS. A multifunction data acquisition (DAQ) card (PCI-6259, NI, USA) was used to accomplish control of the AODs’ drivers or other peripheral devices.

III. RESULTS

A. General performance test

After completing the design and debugging, the functions of the stimulation system were carefully tested. In order to measure targeting accuracy, fluorescence images of laser spot in different positions were synchronously recorded.
while laser beam was moved along a line to illuminate fluorescence solution containing 3.75 μM fluorescein isothiocyanate (FITC) by supplying the signals of intended positions into AODs. Then the actual positions of laser spot were measured from these fluorescence images by IPP software. The relation of actual location and intended location was plotted in Figure 3(a), which showed a highly precise targeting that was an essential parameter for optical stimulation. Next the laser power after the objective was measured by supplying incremental voltage signal into AODs. The correlation between the laser power after the objective and the controlling voltage of the AOD was shown in Figure 3(b). The amplitude of the driving signal affected the diffraction efficiency of the laser traveling through the AOD, and thus determined output power after the objective. Continuously adjustable power was indispensable for the activation of different samples, which is easily achieved in our system.

Because light diffraction efficiency through AODs was dependent on the frequency control word, the laser power after AODs was different for varied controlling frequency in spite of the same voltage into AODs. Since this frequency determined the laser targeting location, the laser power varied at different locations with the same controlling voltage. In order to obtain the uniform illumination throughout the whole FOV, the laser power was adjusted to the lowest value in the FOV by regulating the controlling voltage to AOD in each frequency position. In one example shown in Figure 3(c), the laser power after adjustment in the FOV ranged from 5.90 mW to 6.04 mW with a 2.5% variation. The laser power uniformity in the whole FOV is important for quantitative analysis of cell responses. On the other hand, the sharpness of laser spot after the objective will determine the effect of selective activation of cells. Then the lateral profile of fluorescence spots after the objective was obtained from recorded fluorescence image by IPP software while laser beam illuminated FITC aqueous solution. The lateral resolution (full width at half maximum, with laser power of 0.6 mW) was calculated as 1.38 ± 0.10 μm in Figure 3(d), which was roughly identical at different locations in either the x- or the y-direction. The lateral resolution is mainly dependent on the numeric aperture of the objective. So x-y resolution is identical for varied laser power or controlling voltage into AODs.

**B. Selective excitation of ChETA-expressing astrocytes**

As we know, astrocytes actively participate in neuronal transmission in the central nervous system. Selective
activation of these cells is important for studying the signal processing and communication with neurons. For this purpose, small pieces of hippocampuses of Sprague-Dawley rats on postnatal day 0 were dissected by enzymatic treatment and mechanical dissociation, and cultured in fresh medium. Next day, cells were washed with phosphate buffered saline (PBS) twice for excluding neuronal or other cells, and the culture medium was replaced with the fresh one. After the cells reached confluence, the purified astrocytes were obtained by shaking the 25 cm$^2$ flasks 12 h and the floating cells were removed and the attached astrocytes were kept in culture with fresh medium. Then we used a lentivirus to express a fusion protein of light-activated channel ChETA and enhanced yellow fluorescent protein from the glial fibrillary acidic protein (GFAP) promoter in cultured astrocytes.

In the experiments, one astrocyte whose central region was selected as ROI 1, was sealed by a patch pipettes with a resistance of 3–5 MΩ and whole cell patch-clamp recording was used to measure ionic currents through ChETA channels in response to laser illumination with a MultiClamp 700B amplifier and a Digidata 1440 A data acquisition system (Molecular Devices). The central regions on a neighbor astrocyte and on a cell-free region were selected as ROI 2 and ROI 3, respectively. Then laser beam was controlled to move to the center of three ROIs (Figure 4(a), markers 1–3) for 1 ms illumination and 45 ms break. The patched cell was activated only when laser beam illuminated the center of ROI 1 and did not when laser beam illuminated other two positions (Figure 4(b)). Accordingly, selective cell activation was verified.

C. Fast activation of multiple locations in ChR2-expressing neurons

In virtue of the random addressing of the AOD, fast activation of multiple locations can be realized. To test this, neocortical neuron was transfected with ChR2. Adeno-associated virus vector hSynapsin-NpHR3-EYFP-2A-ChR2-mCherry was stereotaxically injected into the mouse somatosensory cortex to drive ChR2 expression. A strong expression of ChR2 in a limited number of cortical neurons around the injection site after 30 days could be observed in the brain slice, according to the red fluorescence of co-expressed mCherry (Figure 5(a)). Eight positions (numbered 1–8) were selected along two dendrites of this neuron and illuminated in turn by laser beam with different interval (0.01, 0.1, 1, 10, and 100 ms). Whole-cell patch-clamp recording of the soma was carried out to monitor its electrical responses to the phot-
stimulation. As shown in Figure 5(b), with 100 ms interval, individual responses to photostimulation on each position were clearly observed. As the interval was reduced, responses were temporally summed up, forming marked subthreshold depolarization with increased amplitude. When interval was 0.01 ms, the depolarization reached firing threshold, and an action potential was evoked.

These results showed that arbitrary locations were efficiently activated in an ultrafast way, resulting in spatiotemporal integration of multiple inputs at the soma. The fast random addressing of the AOD-based photosimulator could be applied for studying synaptic integration and neuronal computation.

IV. DISCUSSION

We have established a random access photostimulator that allows fast selective activation of cells. We used this system to complete selective activation of astrocytes and neurons, which profited from the choice of AODs. Compared with SLM, AODs have better beam quality, higher diffraction efficiency (>80% for two-dimensional AODs), and faster addressing (<10 μs).

In the system, stimulation time, stimulation intensity, stimulation mode, scanning method, and other parameters can be set up handily and skillfully. So various stimulation patterns can be easily achieved for the activation of different types of cells in multiple sites. The small spot size of 1.38 μm is easy to achieve the selective activation of cells. The lateral resolution in activating cells was measured as 17 μm when laser power after the 40× objective was 30 μW, which demonstrated our system was of good selection in lateral direction. In addition, we also proved that lateral resolution was dependent on laser power and lower laser power could obtain higher resolution. However, the axial resolution was 150 μm, which demonstrated this photostimulator had weaker axial resolution when 473 nm laser was used in this system. Recently, femtosecond pulse laser has been used in a few systems for improving the activation resolution. Therefore, combined with femtosecond pulse laser, our system will also improve the spatial resolution. The performance of our system can be extended by the integration of a second laser and AODs for selective activation and inactivation of multiple neurons.

Our system worked very stably and effectively because of its modular design and compactness. In addition, the graphical development environment based on the LABVIEW language made this application program easy to use. In its current version, the size of the scanning box was only 300 mm × 220 mm × 180 mm, and its weight was only about 3 kg. The small volume and weight allowed it to be easily integrated with different kinds of commercial microscopes produced by diverse microscope producers such as Nikon Corp., Olympus Corp., and so on. These characteristics and advantages are very remarkable and highly beneficial for its popularization in many laboratories.

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