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Wavefront shaping empowered high-resolution optical focusing at depths in tissue and its application for single neuron optogenetics

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Abstract: Wide field single neuron activation on primary neurons was achieved by wavefront shaping-enabled multimode fiber-based optogenetics. The capability through intact skull layer opens a new horizon for noninvasive single neuron optogenetics at depths.

1. Introduction

Recently, optogenetics has been proved to be a powerful tool in neuroscience, especially in exploring the function between behavior and neural circuits. Selective stimulation of neuronal cells with cellular resolution and high temporal resolution is a major goal in neuroscience [1,2], because the precise neuron manipulation with the specific time sequence will help us to understand the working principle of the brain or the pathology of some brain disease [3,4].

Nowadays, optogenetics can be implemented on free-moving animals to study the role of a class of neurons within a certain area or involved in specific brain functions using an optical fiber to deliver light energy. This method could avoid the strong scattering effects of the skull and brain tissues and also be minimal-invasive compared with other invasive methods like graded-index (GRIN) lenses [5,6] or fiber bundles [7]. However, there still a technical barrier to achieving selective stimulation with a single optical fiber, because the light from the fiber is diverse which will result in a divergent large spot which cannot follow the spatial variant of the neurons in the subset of chosen neurons, thus it could be difficult for recall a behavior involved in the circuits as it biases the temporal sequence of the firing pattern of the specific functions [4,8]. Thus, increasing the penetration and spatiotemporal resolution is urgent.

Here, we propose an endoscopic approach by utilizing a multimode fiber (MMF) of 120 μm aperture which is combined with wavefront shaping techniques to achieve cellular scale spatial resolution and high temporal resolution. A digital micromirror device (DMD) was utilized to manipulate the light propagation through the MMF at a very high speed (23k Hz). Based on the recent wavefront shaping theory, the output field of MMF can be fully controlled and changed without an optimization process after the measurement of the transmission matrix (TM). Besides, our system has also been proved that can generate a sharp focus through the MMF and skull, indicating the possibility of non-invasive activation of neurons through the skull with high spatiotemporal resolution.

2. Results

In order to demonstrate that the wavefront shaping of excitation beams enables the generation of selective focus through the MMF, and thereby the single neuron excitation, an *in vitro* model system was built and tested on primary neurons. Fig 1 shows the tinny focus after the MMF (Fig.1a-b), attached skull layer (Fig.1c-e), and dynamic focusing (Fig.1f). The feasibility of our system was tested via single neuron

stimulation on primary cortical neurons (ChR2 expression confirmed) harvested from embryonic mouse brains (E16). The results indicated our system could activate a single neuron or two separated regions targeting two individual neurons (Fig. 2).

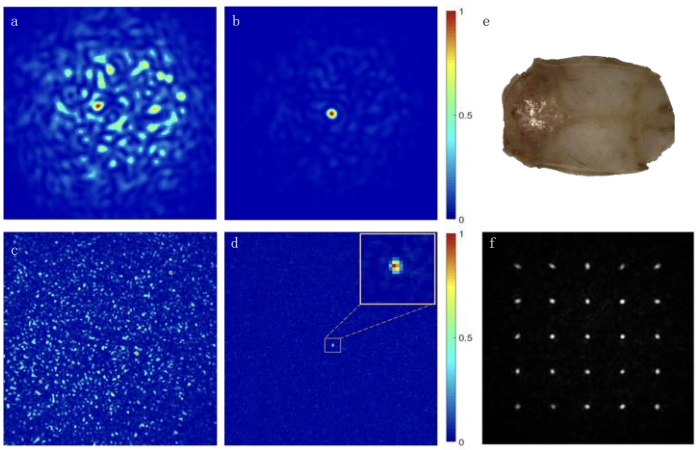

Fig.1. high spatial temporal resolution optical focusing through MMF based on wavefront shaping techniques. Intensity images of MMF without wavefront shaping (a), and with the wavefront shaping (b). intensity distribution of light transmitted after MMF and skull (c) and foci generated by wavefront shaping techniques (d). The skull layer dissected from a mouse without any thinning procedure (e). Combined focus at working plane (f).

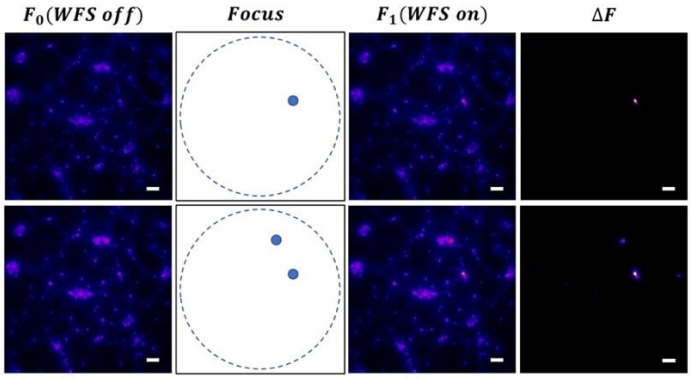

Fig.2. *in vitro* experiment results. Calcium imaging acquired by epifluorescence imaging system. Based on the wavefront shaping technique, the mask of one focus (top line) and two focus (bottom line) could be calculated and upload to DMD chip. $\boldsymbol{F_0}$ and $\boldsymbol{F_1}$ are the fluorescence images of the cultured neurons before and after DMD display the masks separately. The stimulations corresponding to focus were highlight by remove the background ($\Delta\boldsymbol{F}$). The scale bars are 50 μm.

3. References

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