Two-photon excited ReaChR by a three-stage femtosecond optical parametric amplifier

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Abstract: A three-stage optical parametric amplifier is built to produce 1 kHz, 31 fs, ~200 μJ signal pulses with tunable wavelengths. Red-activatable channelrhodopsin in fruit fly is optimally two-photon excited to copulation behavior at 1250 nm.

OCIS codes: (190.4970) Parametric oscillators and amplifiers; (190.4400) Nonlinear optics, materials; (320.7090) Ultrafast lasers; (320.7110) Ultrafast nonlinear optics; (100.0118) Imaging ultrafast phenomena; (000.1410) Biography;

1. Introduction

Two-photon excitation (TPE) via femtosecond near-infrared (NIR) laser pulse is attractive in light-tissue interaction due to deeper penetration and less heat accumulation. Channelrhodopsin-2 (ChR2) [1,2,3] is a light-gated ion channel containing light-isomerizable chromophore all-*trans*-retinal that permits minimally invasive, genetically targeted and temporally precise photo-stimulation in fruit flies. Images in the depth of ~60 μm have been successfully taken via TPE ChR2 [2]. Red-activatable ChR [4,5] (ReaChR), a variant of ChRs, can be optimally excited with orange to red light (590~630 nm). It is expected to optically control the fruit fly behaviors by depolarizing neurons via TPE of ReaChR if a femtosecond NIR (1200~1300 nm) light source with sufficient peak power is available.

In this work, we build a Ti:Sapphire amplifier-pumped three-stage optical parametric amplifier (OPA) [6] to produce ~200 μJ signal femtosecond pulses with central wavelengths ranging from 1150 nm to 1565 nm. By shining pulses on the fruit flies with ReaChR embedded in the *Crz-Gal4* neuron, we observe high bending rate at optimized central wavelength and peak intensity.

2. Experimental setup and results

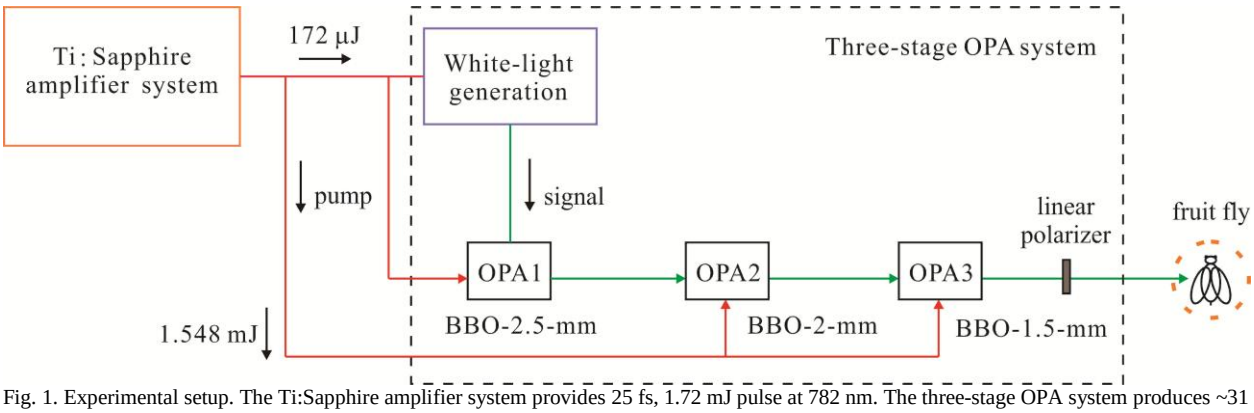

Fig. 1. Experimental setup. The Ti:Sapphire amplifier system provides 25 fs, 1.72 mJ pulse at 782 nm. The three-stage OPA system produces ~31 fs, 90-203 μJ signal pulses at wavelengths of 1150-1565 nm.

Figure 1 shows the experimental setup. A Ti:Sapphire amplifier system (Femtopower™ HE PRO CEP) produces 1 kHz, 25 fs, 1.72 mJ pulse train centered at 782 nm. About 10 μJ of the Ti:Sapphire pulse energy (controlled by a variable neutral density filter) passes through a 4-mm-thick YAG window to generate white-light (907-1365 nm at -20 dB level), seeding the first stage OPA (with a 2.5-mm-thick BBO and pumped by 86 μJ Ti:Sapphire pulse). A 10-mm-thick ZnSe plate chirps the white-light pulse such that the signal wavelength could be selected by controlling the relative delay between the pump and white-light pulses. The produced signal pulse is seeded to the second (with 2-mm-thick BBO and 310 μJ pump energy) and third (with 1.5-mm-thick BBO and 1.24 mJ pump energy) OPA

stages in sequence, producing ~31 fs, 203 μJ pulses (at 1200 nm) while the signal central wavelength can be tuned from 1150 nm to 1565 nm.

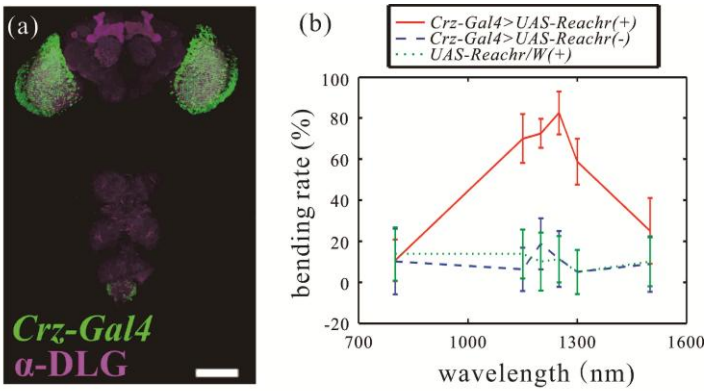

Fig. 2. The expression of Fruit fly neuron map and bending rate as a function of wavelength. (a) Corazonin neurons (green) are preferentially labeled in the *Crz-Gal4>UAS-GFP* fly. The brain and thoracic ganglia are immunostained with anti-discs large antibody (magenta). Scale bar represents 100 μm. (b) Wavelength-dependent bending rates of *Crz-Gal4* driver *UAS-ReaChR* fruit flies [with (+) or without (-) all-*trans* retinal] and *UAS-ReaChR/W* fruit flies (with all-*trans*-retinal) during 10 cycles of 3-s on/5-s off test.

Figure 2(a) shows the expression pattern of *Crz-Gal4* transgene flies. Activation of the corazonin neurons induces abdominal bending and ejaculation [7], which can be used to verify our TPE experiment. Figure 2(b) shows the bending reflex assay. Laser pulses with 694 GW/cm^2 peak intensities irradiate on *Crz-Gal4>UAS-ReaChR* at different wavelengths. Bending rates are determined during 10 cycles of "3-s light spotting and 5-s rest". We genetically express ReaChR proteins on the *Crz* neuron. The experimental fruit flies (*Crz-Gal4>UAS-ReaChR*) fed with 100-μM all-*trans*-retinal (red solid line) exhibit significantly higher bending rate than control fruit flies without all-*trans*-retinal feeding (blue dash line) or without *Crz* expression (green dotted line). The ReaChR [2] protein does not function properly in fruit flies that are not fed all-*trans*-retinal. In this experiment, it is expected that ReaChR protein can be stimulated by TPE in the range of 1180 nm to 1260 nm and activates *Crz* neuron to trigger bending reflex. It is found that the two control groups present low bending-like behavior because of astonishing aversive visual effects when the fruit fly are shined by the laser pulse. These results can prove that ReaChR protein can be optimally stimulated by high-intensity light at 1250 nm.

3. Conclusions

We demonstrate TPE of ReaChR that can lead to bending behavior of fruit flies. A three-stage OPA system, producing 31 fs, 694 GW/cm^2 signal pulse in the wavelength range of 1150 to 1565 nm, is a proper tool for biological TPE experiments. Optimal excitation wavelength of 1250 nm is found by the highest bending rate of fruit flies. This work is supported by the Ministry of Science and Technology in Taiwan under grants MOST 103-2221-E-007-056-MY3, 102-2112-M-007-025. The authors acknowledge A. H. Kung for supporting the Ti:Sapphire laser; and Klemens F. Störtkuhl, Roger Y. Tsien, and David J. Anderson for providing fruit flies.

4. References

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