Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Fluorescent tracer of dopamine enables selective labeling and interrogation of dopaminergic amacrine cells in the retina of living zebrafish

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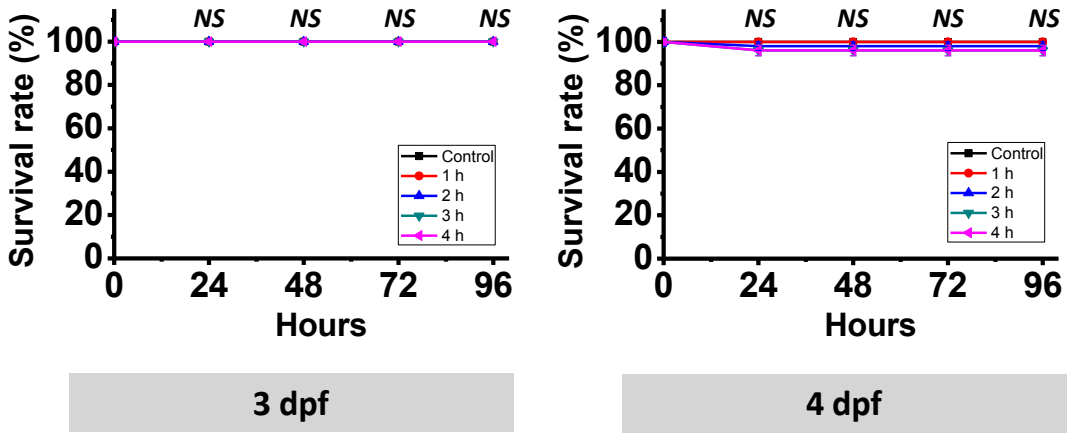
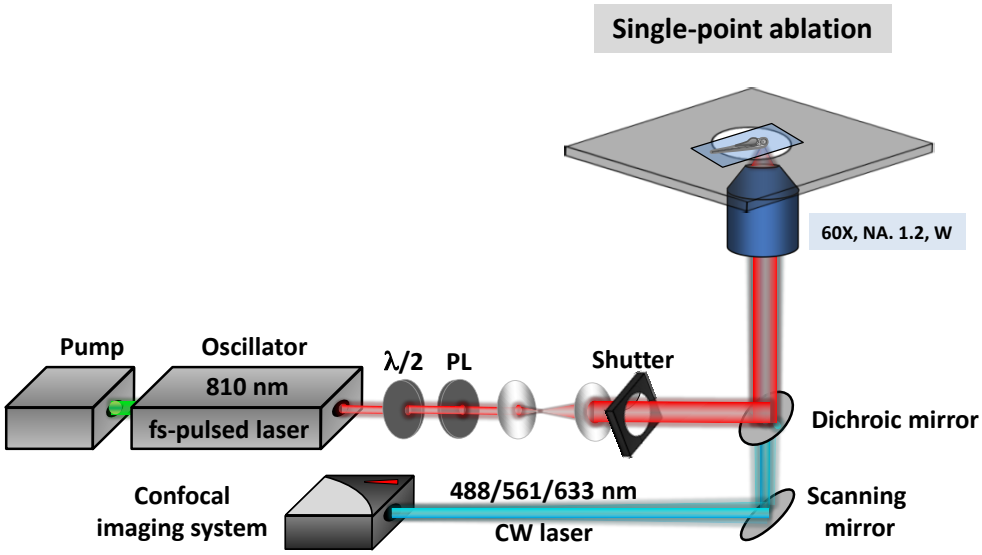
SUPPORTING DATA



Supplementary Figure S1. Examination of the toxicity of FITC-DA on the growth of larval zebrafish.

To examine whether the treatment of FITC-DA would impose toxicity to larval zebrafish by impairing their growth, wild-type embryos (3 and 4 dpf) were treated with FITC-DA (2 mM) for varied duration (1, 2, 3 and 4 h). A total of 50 embryos were tested for each group (10 embryos per test, 5 repeated tests). The control group was prepared on incubating embryos with PBS solution for the same duration. The percentage survival rate for each group was determined at designated time points and was displayed in the plots. The survival rate of the control group for all incubation times remained 100% throughout the observation time (96 h). For simplicity, only one line was shown to represent the result of the control group. The result shows that the FITC-DA treated groups continued to grow comparatively with nearly all embryos survived regardless of the duration of treatments. This observation demonstrates that FITC-DA imposed negligible toxicity to larval zebrafish and did not impair its growth.



Supplementary Figure S2. Schematic of the setup for femtosecond-laser ablation on dopaminergic amacrine cells.

The setup comprised a laser scanning confocal microscope (Leica TCS SP5 II, Leica) and a femtosecond (fs)-pulsed laser (λ = 800 nm, pulse-width = 80 fs, rep. rate = 80 MHz, approximate power = 340 mW; Tsunami, Spectra-Physics) is designed for ablation of single target cell and for confocal imaging. The beam of the fs-pulsed laser was directed to the side port of the optical microscope, merging with light beam of the imaging laser with a short-pass dichromatic mirror (780 DCSX, AHF Analysentechnik). The merged beam was then focused to the sample with an objective lens (HC PL Apo CS2 63XW, NA 1.2, Leica).

Before ablation, we first acquired confocal images near the retina of a larval zebrafish. A target cell was then identified from the confocal images, and positioned to the focus of the ablation laser with a translation stage. A mechanical shutter was employed to control the duration of the ablation laser (typically 10 ms). After ablation, time-course confocal fluorescent images were then acquired near the target region.

Depending on the fluorescent indicators in use, an imaging laser of varied excitation wavelength was employed. For instance, to excite the fluorescence of FITC-DA, a blue laser (488 nm) was employed as excitation and the signal between 495 and 555 nm was detected. Transmission and fluorescence images were recorded with a pixel density of 512 × 512 and a frame rate of 22 frame/s. The power of the imaging laser was kept below 1 mW.