Electronic Supplementary Information

A Near-infrared I Emissive Dye: Toward the Application of Saturable Absorber and Multiphoton Fluorescence Microscopy at the Deep-Tissue Imaging Window

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Sample Characterization. The linear absorption spectra of BODIPY dye in methanol with a concentration of 1×10^{-5} mol/L were measured using a UV-vis spectrometer (Lambda 950, PerkinElmer, Inc.), while one-photon excited fluorescence spectra were measured using a fluorescence spectrophotometer (SENS-9000, Zolix). The absolute fluorescence quantum yield of BODIPY dye in methanol was determined using the spectrophotometer (SENS-9000, Zolix) equipped with an integrating sphere.

Open-aperture Z-scan measurements. The BODIPY dye in methanol solution was filled in 1 mm quartz cells, with a concentration of 1.01×10^{-4} mol/L. The dye solution was excited with 680 nm femtosecond pulses (1000 Hz, 100 fs) from an optical parameter amplifier combined with traveling-wave optical parameter amplifier system (TOPAS, Spectra-Physics, Inc.) with a tunable wavelength range. In the measurements, the input laser beam was first passed through a beam chopper with an open ratio of 50/50 and a rotating speed of 315 rounds per second. Then the Gaussian beam was tightly focused into the sample by a convex lens (f = 30 cm). The radius of focal spot size was 50 μm, needed for obtaining peak intensity. The transmitted beam was detected by a silicon photodiode using the standard lock-in amplifier technique.

Measurements of multiphoton excited fluorescence spectra and MPA cross-sections. The BODIPY dye was dissolved in methanol and filled in a 1 cm quartz cell, with a concentration of 1.01×10^{-4} M. Room-temperature multiphoton excited fluorescence spectra of the solution were excited with laser pulses (1000 Hz, 100 fs) from an optical parameter amplifier (TOPAS, Spectra-Physics, Inc.) with tunable wavelengths (250-2600 nm). The amplifier system was seeded by a femtosecond Ti: Sapphire oscillator (100 fs, 80 MHz, Mai Tai HP, Spectra-Physics). The input laser beam was focused into the sample by a convex lens (f = 10 cm) and the emission signals were dispersed by a 750 mm monochromator combined with suitable filters, and detected by a photomultiplier tube (Hamamatsu R928) using a standard lock-in amplifier technique. Prior to emission measurements, wavelength sensitivity was calibrated. The MPA cross-sections of BODIPY dye in methanol were investigated via the fluorescence comparison method with the same excitation source as that used in the measurements of the multiphoton excited fluorescence spectra.

Cell culture: In growth media (DMEM supplemented with fetal bovine serum (10%), streptomycin (100.0 mg/L) and penicillin (100 IU/mL)), 4T1 cells were cultured and maintained in a humidified atmosphere of 5% carbon dioxide at 37 °C for following use.

Three-photon fluorescence imaging: 4T1 cells were seeded in glass-bottom dishes and grown till 70 ~ 80% confluency. Subsequently, cells were further incubated in medium containing different doses of BODIPY dye (5, 10 and 20 μg/mL) for 10 h. After that, Cells were then washed three times with PBS for imaging purpose. The multiphoton microscope system used here is the same to that used in Ref. 1. Briefly, the 1665-nm soliton source consists of a photonic-crystal (PC) rod (SC-1500/100-Si-ROD, NKT Photonics) pumped by a 1550-nm femtosecond laser (FLCPA-02CSZU, Calmar) at 1 MHz. The soliton is generated through soliton self-frequency shift (SSFS) in the anomalous region of the PC rod.[2] After long-pass filtering, the residual 1550-nm pump was removed, allowing only the 1665-nm soliton pulse to be delivered to the multiphoton microscope (MOM, Sutter).

1 Y. X. Wang, W. H. Wen, K. Wang, P. Zhai, P. Qiu, and K. Wang, *Appl. Phys. Lett.* 2016, **108**, 021112.

2 K. Wang, N. Horton, K. Charan, and C. Xu, *IEEE J. Sel. Topics Quantum Electron.* 2014, **20**, 6800311.