Hyperspectral two-photon excitation microscopy using visible wavelength: supplemental document

This document provides supplementary information for "Hyperspectral two-photon excitation microscopy using visible wavelength". Some experimental data to investigate the photo-damage in visible-wavelength two-photon excitation (v2PE) imaging using slit-scanning confocal microscope is presented.

1. Photodamage in hyperspectfal imaging using v2PE

To evaluate the damage on cells in our method, we performed time-lapse observation of living HeLa cells expressing mTFP1 in Golgi apparatus by using a line-illumination v2PE microscope. We imaged the same cells every 5 minutes by a bright-field microscope and the v2PE microscope.

Figure S1 shows the result of the time-lapse imaging. We did not observe an apparent morphological change in the images obtained at minute 0 to 30. However, in the image after 30 minutes, we confirmed a bubble formation that indicated photodamage. The distribution of the Golgi apparatus visualized by mTFP1 did not show a significant change during the observation, but photobleaching occurred gradually with the observation time.

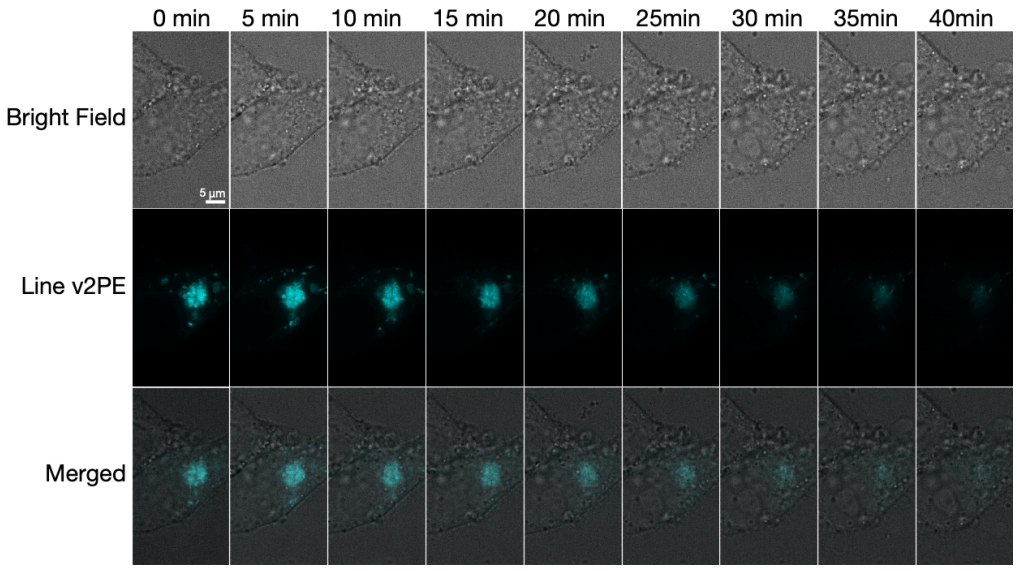

Fig. S1. Time-lapse images of living HeLa cells expressing mTFP1 in Golgi apparatus. The wavelength of the excitation pulsed laser was 530 nm, and the intensity was 170 kW/cm^2 at the object plane. The exposure time was 7.5 ms per line. A silicone-immersion objective lens with an NA of 1.3 was used for the observations. The images were taken every 5 min at an imaging rate of 3.8 s per image.