SUPER-EASY SUPERRESOLUTION IMAGING BY SPONTANEOUSLY PHOTOSWITCHABLE FLUORESCENT PROTEIN

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Superresolution imaging breaks the diffraction limit of light and enable us to explore nano meter world in living cells [1]. Among them, photoswitchable fluorescent proteins (PSFPs) play an important role for superresolution imaging such as single molecule localization microscopy (SMLM). For SMLM, optimal timing control of the illumination of switching on, off, excitation light, and camera exposure is required to detect single molecules localization data. However, such controls complicate the microscopy system. Here, we developed novel PSFP, named SSFP. SSFP can be recovered immediately from its fluorescence off-state to that of on-state spontaneously. Therefore, once the state of SSFP is switched off, only continuous illumination of excitation light is enough to observe single molecules stochastically switched-on and reconstruct superresolution images. With SSFP, we demonstrated superresolution imaging of cellular architecture at a 33 nm spatial resolution, 1 s time resolution (1 ms × 1,000 frames for single image reconstruction), and time-lapse imaging.Our system enables to lower the hurdle for researchers who perform superresolution imaging.

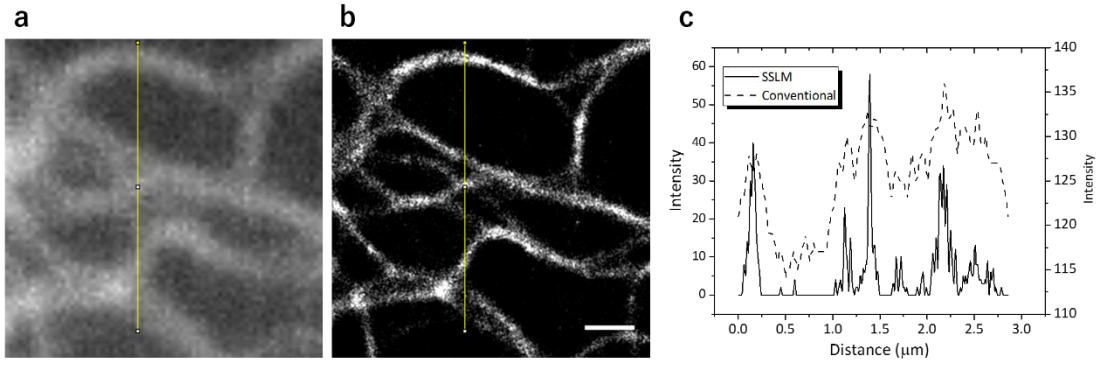

Figure 1. Comparison of conventional and superresolution image. (a) Averaged image of single molecule imaging data. (b) Reconstructed image. Scale bar 500 nm. (c) Line profiles of conventional (dashed line) and superresolution image (solid line).

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