

## One-color photoswitching of fluorescence of diarylethene derivatives and its application to localization microscopy

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Recently, super-resolved fluorescence microscopy has been attracting considerable attention as a new nano-imaging method while keeping the advantages of far-field optical microscopy such as noninvasive access to the inner structures of wet samples. Fluorescent molecules with appropriately designed properties are indispensable for the super-resolved fluorescence imaging. In particular, the switching ability of fluorescence intensity takes a crucial role both in coordinate-stochastic methods, such as PALM (FPALM)<sup>[1,2]</sup>, STORM<sup>[3]</sup> and dSTORM<sup>[4]</sup>, and in coordinate-targeted methods, such as GSD<sup>[5]</sup> and RESOLFT<sup>[6]</sup> microscopies.

In general, PALM requires multiple light sources with different emission colors for the activation, excitation, and deactivation of fluorescence. Such an optical setup with multiple light sources is somewhat complicated and hence several approaches for the ON  $\rightleftharpoons$  OFF switching of fluorescence with single light source have been demonstrated by using e.g. metastable dark states and fluorescence fluctuation. In this study, we propose a new approach for the ON  $\rightleftharpoons$  OFF switching of fluorescence using the so-called “Urbach tail” of a photo-switchable fluorescent diarylethene derivative (fDE, Figure 1)<sup>[7]</sup>.

Thin films of poly(2-hydroxyethyl acrylate) (polyHEA) including small amount of the fDE in the open-ring isomer (non-fluorescent) were prepared on cover slips by spin-casting. The open-ring isomer undergoes cyclization reaction upon photoirradiation at a wavelength of < 430 nm<sup>[8]</sup>. On the other hand, the closed-ring isomer of the fDE has absorption bands in both visible and UV wavelength range. The closed-ring isomer shows fluorescence ( $\Phi_f = 0.78$ ) with an emission peak at ca. 590 nm; the quantum yield of the cycloreversion reaction is < 10<sup>-5</sup>.

Fluorescence images of the single fDEs in the polymer film were obtained by using a wide-field microscope consisting of an inverted optical microscope (IX70, Olympus), a CW 532-nm laser (Excelsior 532, Spectra-Physics), an objective lens ( $\times 100$ , NA 1.35), and an electron-multiplying CCD camera (ImagEM C9100-13, Hamamatsu Photonics).

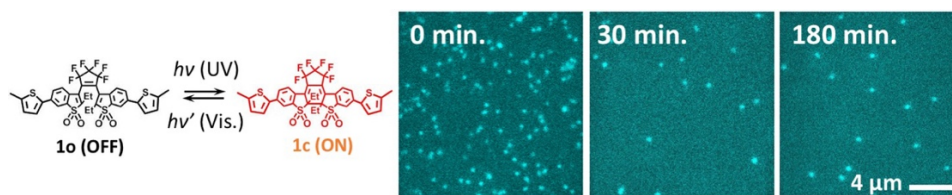


Figure 1. (Left) Structures and photochromic reactions of the fDE. (Right) Wide-field fluorescence images of the closed-ring isomers of the fDE in a polyHEA film under continuous irradiation for 180 min with a 532-nm CW laser.

Figure 1 shows a time evolution of fluorescence images of the single fDEs in the polymer thin film under irradiation with the CW 532-nm laser at  $0.88 \text{ kW/cm}^2$ . At the time origin of the irradiation ( $t = 0 \text{ min.}$ ), many fluorescent spots were observed. This can be ascribed to the presence of the trace of the closed-ring isomer. After a few minutes from the beginning of photo-irradiation, the number of fluorescent spots decreased to a certain value (ca. 10 to 20) owing to the cycloreversion reaction. It should be noted that the number of fluorescent spots remained almost constant for 180 min, indicating that the number of photo-activated fDEs balanced with that of photo-deactivated ones. This fluorescence caused by the irradiation at 532 nm was safely attributed to the closed-ring isomer of the fDE by the measurement of fluorescence spectrum. These results indicate that both ON-to-OFF and OFF-to-ON switching take place under the one-color photo-irradiation at 532 nm.

The photoswitching and fluorescence excitation with a one-color laser was applied to photo-activation localization microscopy. We have successfully visualized nanostructures of dried polymer gel and PMMA fabricated by using electron-beam lithography. These results demonstrate that this single-beam photoswitching and fluorescence excitation pave the way to PALM imaging with a more simple optical setup.

At the conference, we will discuss the mechanism of this one-color photo-activation, fluorescence excitation, and photo-deactivation on the basis of spectroscopic measurements.

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