SUBDIFFRACTION IMAGING THROUGH THE SELECTIVE DONUT-MODE DEPLETION OF THE FLUORESCENT PROTEIN DRONPA

Peter Dedecker[#], Jun-ichi Hotta[#], Cristina Flors[#], Ryoko Ando[§], Hideaki Mizuno[§], Atsushi Miyawaki[§], Johan Hofkens[#]

[#]Katholieke Universiteit Leuven, Celestijnenlaan 200F, 3001 Heverlee, Belgium
[§]RIKEN, 2-1, Hirosawa, Wako, Saitama 351-0198, Japan
E-mail: peter.dedecker@chem.kuleuven.be

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The fast and reversible on/off switching of the fluorescence emission of the GFP-like fluorescent protein Dronpa [1,2] has attracted considerable interest for applications in subdiffraction imaging. In this paper we study the use of a donut-mode beam in combination with two more overlapping laser beams to increase the imaging resolution through selective switching to the nonfluorescent photoswitched state. We devise and run a series of numerical simulations to determine suitable photophysical parameters of prospective, thermally stable photoswitchable molecules, in terms of photoswitching quantum yields, fatigue resistance, and possible presence of transient nonfluorescent states. Many of our findings [3] are applicable to other measurements that make use of donut beams, and these guidelines can be used in the synthesis and screening of novel photoswitchable compounds. We experimentally demonstrate the possibility of obtaining increased resolution by making use of the efficient and thermally stable Dronpa photoswitching, using equipment that is commonly available.

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