

Compact two-photon excitation STED nanoscopy with high-peak-power sub-nanosecond 655-nm pulsed light source

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Two-photon excitation fluorescence microscopy has been widely used for bioimaging at subcellular to tissue levels. It has advantages including superior penetration depth and reduced invasiveness for biological specimens by utilizing near-infrared (NIR) pulsed beam for two-photon excitation. Recently, the technologies to improve the spatial resolution of two-photon microscopy have been requested in the broad biological fields.

We have developed a compact two-photon excitation pulsed STED microscopy with electrically controllable components [1]. A key component is a set of the transmissive liquid crystal devices (tLCDs) directly inserted before the objective lens for STED beam shaping and chromatic aberration compensation. The tLCDs allow the optical paths of the excitation and STED beams combining in advance and make the setup compact and robust compared to general STED setups. However, another key component, the mass-produced laser diode (LD)-based pulsed STED light source, did not have enough pulse energy to achieve the spatial resolution below 100 nm. To solve this problem, we have recently developed a novel light source to generate high-peak-power sub-nanosecond 655-nm optical pulses based on LD-controlling technologies [2]. In this study, by introducing the 655-nm light source developed, we accomplished a novel two-photon excitation pulsed STED nanoscopy. The 655-nm STED pulse had ideal optical properties and sufficient pulse energy to break the limit of the spatial resolution of 100-nm with neither a pinhole aperture nor an image processing of deconvolution algorithms. Even though the setup has been kept compact, the spatial resolution was reached to 70 nm. For biological applications, we screened the suitable fluorescent dyes or proteins and finally realized the sub-100 nm spatial resolution of the presynaptic protein clusters in the fixed primary neuron without severe photobleaching. Our nanoscopy will visualize ultrastructures and dynamics of clusters of biomolecules representing physiological functions in living cells and tissues.

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