EPI-CONESHELL LIGHTSHEET SUPER RESOLUTION MICROSCOPY

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ABSTRACT

Super resolution microscopy, based on the development of direct stochastic optical reconstruction microscopy (dSTORM), has been proven to be a powerful approach in biological imaging through sequential activation and time-resolved localization of photo-switchable fluorophores. [1] The conventional manner of dSTORM super-resolution imaging is in the structure of the epi-fluorescence setup. Even though epi-fluorescence microscopy has been one of the most popular microscopic technology in the biological imaging field due to its non-invasive nature, biomolecular specificity, 3D imaging and multi-colors capability, high N.A. objective lens availability, and simple optical path; However, fluorophores bleaching or quenching issue holds the development back so hard, especially for 3D imaging or deep tissue. The excitation beam shines through the whole tissue all the way down and even the portion where is not at focused plane, which results the waste and depletion of blinking fluorophores, and it is insufficient consequently for reconstructing features in images.

In this study, we demonstrate a compact, efficient, and economic epi-cone-shell light sheet super-resolution microscopy; With the aid of the special excitation design in the setup, it allows us to reduce bleaching issue while obtaining most benefits of epi-fluorescence setup, yet most importantly, imaging a large volume could no longer be a problem. The cone-shell light is achieved by converging a ring beam, and this special excitation shape could prevent the depletion of out of focusing region from directly changing the route of excitation light as well as forming a disc-shape of excitation area. In order to retain the field of view and minimize the depth of cone-shell neck, the ring beam should focus at the back focal plane of objective lens while maintaining the radius of ring beam within the back-aperture size of objective lens. By adjusting the laser power, the dimension of excited portion where exceeds the bleaching threshold could be down to 50x50x2 μm^3 while using a Zeiss LCI Plan-Neofluar objective (63x N.A. 1.3).

REFERENCE

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