Sub-diffracted Dark Spot Localization Microscopy

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Abstract: Recently, a new technique called MINFLUX was promoted, in which a doughnut-shaped illumination spot moves over an area in diameter size L, and the position of a single molecule in the scanning domain is determined by triangulation based on the detected photon count N for distinct doughnut positions[1, 2]. The localization precision of this process scales as L/√N. As the scan range L can in principle be chosen arbitrarily small, the localization precision could be ultra-high[3]. However, MINFLUX is incapable of discerning two molecules within the diffraction-limited area unless with the help of single molecular localization nanoscopy, like STORM or PALM. Here, we produce a novel kind of focal spot pattern, called sub-diffracted dark spot (SDS), to localize molecules within the sub-diffracted region of interest. The proposed technique is nominated as SDS localization microscopy (SDLM). Two doughnut-shaped beams are involved: one excitation beam and one depletion beam, both of which feature zero-intensity in the center. With the increase of the hollow depletion beam intensity, the SDS dimension is greatly suppressed which thereby could be below the diffraction limit. With the aid of intrinsic illumination pattern rather than extra localization optics, multiple molecules within the diffraction-limited area could be distinguished. Theoretically, the size of SDS could be unlimitedly decreased and possesses higher light sensitivity in contrast to diffraction-limited dark spot. We define the peak diameter of SDS, fwhm, as the characteristic parameter. We have experimentally and numerically presented the SDS modality. And simulation localization framework has been implemented on densely-packed molecule ambient. In the condition of same ratio L/fwhm, SDLM is more advantageous in localization precision compared with MINFLUX. In our perspective, SDLM is held great promise in biological and physical utilizations.

Key words: Single molecular localization nanoscopy, Sub-diffracted dark spot, Localization precision

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