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Single objective tilted lightsheet for three-dimensional localization microscopy

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Abstract: Optical sectioning technologies achieve high precision localization by reducing the background photon count. We use tilted light-sheet microscopy to achieve optical sectioning in localization microscopy, enabling thick sample observation and low background photon count images. A deformable mirror was incorporated to generate a tetrapod point spread function (PSF), enabling high resolution 3D localization. DNA-PAINT was imaged with 15 nm transverse and 60 nm axial resolution.

1. Introduction

Single molecule localization microscopy has been shown to surpass the diffraction limited resolution and has become an important technology for biology research [1–6]. By localizing the single molecule PSF spots with the maximum likelihood estimation, the localization uncertainty is the Cramér-Rao lower bound (CRLB), which is the theoretical minimum localization microscopy resolution. The higher background photon counts increase the CRLB, which means reducing the localization microscopy resolution [6].

One way to reduce background photon counts is by using optical sectioning technologies. For example, total internal reflection microscopy (TIRF) can overcome the high background issues in the biological imaging [7–9], but the evanescent wave illumination in TIRF microscopy limits the axial observation range to within 100-200 nm.

An alternative solution to reducing image background but nevertheless possessing long axial observation range is selective plane illumination microscopy (SPIM). The optical sectioning of SPIM reduces the axial illumination volume, thereby lowering the image background counts [10, 11]. Most SPIMs are designed with two objective lenses, one for illumination and the other for detection. The two objective lens design makes microscope alignment and assembly more complex and usually a customized sample holder is needed to position the excitation objective lens and detection objective lens.

To overcome the above problems, researchers have developed single objective lens oblique light-sheet microscopy [12, 13]. In oblique light-sheet microscopy, a tilted light-sheet is launched from an objective lens and the resulting fluorescence signal is detected by the same objective lens. The combination of excitation and detection avoid the usage of special sample holders and can be used with most commercial microscopes. The inclined light-sheet illumination can achieve sub-micron optical sectioning.

In this research, we achieve three-dimensional optical sectioning localization microscopy at arbitrary depth of sample by single objective lens oblique light-sheet localization microscope combined with a deformable mirror for PSF engineering. We validated the optical sectioning performance of oblique light-sheet microscopy by the observation of DNA-Paint nanoruler samples, which is a high background fluorescence sample. The single objective lens design avoid the complex system alignment and make oblique light-sheet microscopy more accommodating towards the non-expert.

2. Results

We use DNA-PAINT nanoruler sample(Gattaquant) to validate the optical sectioning performance of oblique light-sheet microscopy and measure the localization uncertainty. Each ruler in a nanoruler sample is 160 nm in length with three fluorescent binding sites spaced 80 nm apart. The medium of the nanoruler samples is full of fluorescence molecule donors which randomly bind and leave the binding sites, generating single molecule blinking. Thus, the nanoruler is a high background photon sample. With our oblique light-sheet microscope, we can clearly detect single blinking molecules, proving optical sectioning ability. We quantify the localization microscopy resolution by calculating position standard deviation of each localization cluster, obtaining 15 nm (x-axis), 20 nm (y-axis), and 40-100 nm (z-axis) resolution.

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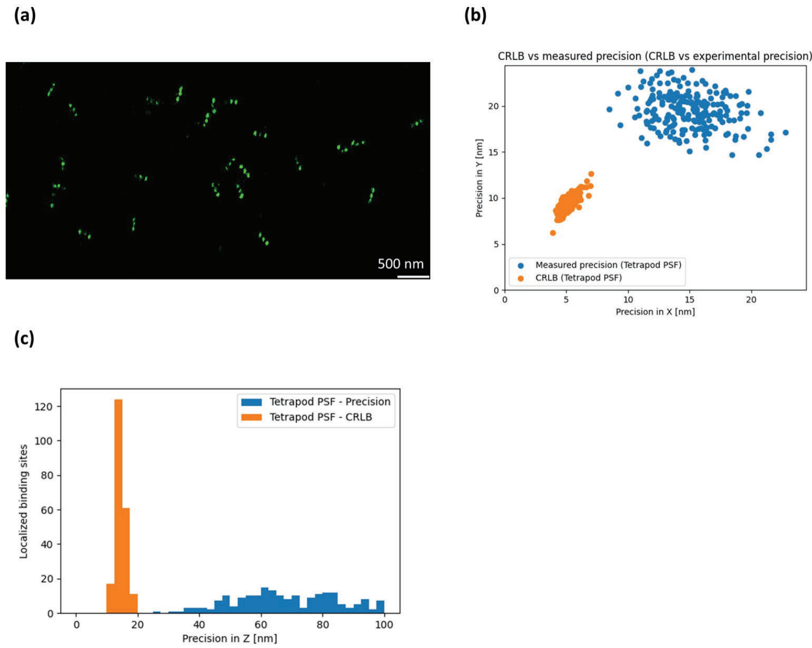

Fig. 1. (a) Localization image of 80 nm nanoruler. (b)(c) Localization precision analysis of 80 nm nanoruler calculated by the standard deviation of resulting localization cluster. The resulting localization precision is 15 nm x-axis, 20 nm y-axis, and 40-100 nm z-axis.

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