Drift-free single molecule localization microscopy provides artefact-free super-resolution imaging

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Introduction

The development of single-molecule localization microscopy (SMLM) has facilitated the application of light microscopy at macromolecular resolution beyond that of the classic diffraction limit. The main principle of SMLM is the identification of the centroids or centers of mass of the sparsely distributed point spread functions (PSFs) of fluorescent molecules followed by fitting a 2D-Gaussian function to each PSF to enable high-precision localization [1]. The key commonality across SMLM methods is the temporal separation of the fluorescent signals from single molecules. Effectively, SMLM sacrifices temporal resolution in exchange for high localization precision and spatial resolution. During the long image acquisition in SMLM, typically around 30 minutes [1], samples can drift away from their initial locations due to the mechanical instability of the microscope [2] and laser-induced heating [3], both of which are detrimental to the resolution and fidelity of super-resolution images [4].

In this paper, we present a novel method for achieving SMLM with negligible sample drift using innovative reinforced optical cage systems (ROCS). In the ROCS, tungsten steel rods seamlessly connect and support each optomechanical component holistically, forming reinforced mechanical constructions. ROCS-SMLM eliminates lateral sample drift by deploying an ultra-stable sample stage, obviating the need for additional hardware, sample preparation, and image post-processing. Our experimental validation of this approach reveals the sample drift in ROCS-SMLM is too small to adversely affect the image resolution, demonstrating a straightforward, inexpensive, open-source, and state-of-the-art solution that makes high-performance super-resolution microscopy easily accessible to biomedical scientists and beyond.

Results

A bench-top microscope was constructed based on ROCS (Fig. 1B). The optical arrangement of the microscope (Fig.1A) employed a standard epi-fluorescence microscopy system, and all lenses were aligned and positioned as 4f systems. The backbone of this microscope comprised of a few groups of interconnecting tungsten steel rods and some φ30 mm single-hole cage plates mounted directly on to the optical bench. A silver reflective mirror, turning the direction of the optical path for illumination, was housed in a right-angle kinematic mirror mount, which was perforated and supported by two groups of two tungsten steel rods perpendicular to each other. All lenses were mounted in cage plates that were translatable along the rods. Two φ1.5" mounting posts were vertically mounted on to the optical bench, serving as the reference of the vertical optical path (Fig. 1C). All cage components were interconnected by the tungsten steel rods and featured an adjustable clamping force between the component and the rods through some φ 2.5 mm screws. The sample stage sub-system (Fig. 1C) was vertically mounted onto the optical bench through four interconnecting tungsten steel rods while fixed constraints were placed between three φ60 mm cage plates and the breadboard.

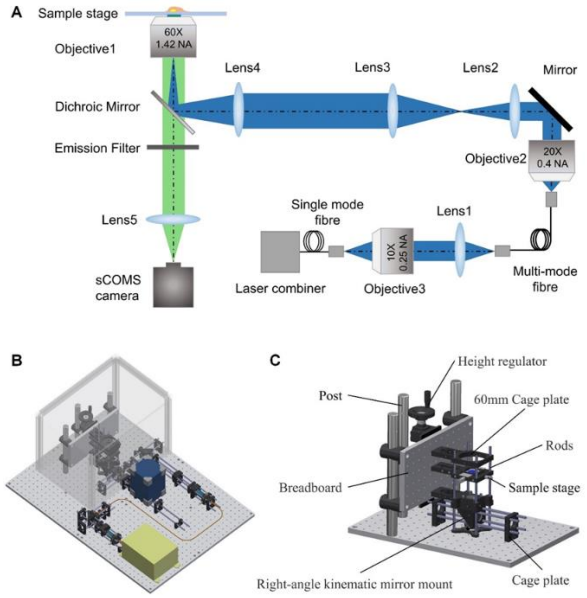

Fig. 1 Schematic of the optical setup and 3D CAD rendering of the ROCS microscope. (A) Schematic of the optical setup. (B) 3D CAD rendering of the microscope and (C) the sample stage sub-system.

We implemented SMLM imaging of the microtubule network in fixed COS-7 cells in the ROCS-SMLM system. Microtubules are formed by rigid hollow fiber spools with a diameter of approximately 25 nm [5], and so have been widely used as biological reference standards for quantifying the resolution in STORM [6]. Super-resolution images (Fig. 2B) revealed details of the microtubule network that cannot be discerned from conventional wide-field images (Fig. 2A). Quantitatively, the mean localization precision of the SMLM image was 14.7±5.3 nm (Fig. 2C) and the Fourier ring correlation (FRC) resolution, at a correlation threshold of 0.143, of a region-of-interest (ROI) was 75.57 nm (Fig. 2D). To investigate the impact of any potential sample drift on the resolution, we also applied drift correction on the STORM image using cross-correlation in ThunderSTORM [7], resulting in an FRC resolution of 72.5 nm. From a practical view, the difference in resolution before and after drift correction was negligible, demonstrating that the ROCS-STORM system exhibited minimal sample drift.

To quantify the sample drift in SMLM, we analyzed the drift of single AF647 dye molecules that were non-specifically bound to the cover slip (Fig. 2H). From the measurement of 10 molecules, we concluded that the drift was 11.96 nm and 12.42 nm along the x- and y-axes, respectively (Fig. 2I). The sample drift in SMLM was insignificant as the value was lower than the localization precision.

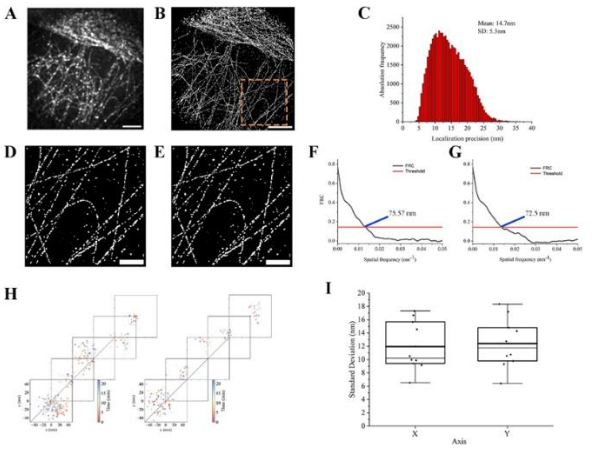

Fig. 2 Super-resolution imaging of Alexa Fluor 647-labelled microtubules in fixed COS-7 cells using the ROCS-STORM system. (A) Wide-field image of the microtubules in COS-7 cells. (B) Super-resolution STORM image of the microtubules. Scale bar: 5 μm. (C) Localization precision histogram of the STORM image. (D) Enlarged image of the region enclosed by the orange, dashed border box in (B). (E) Drift-corrected STORM image following cross-correlation drift correction of (D). Scale bar: 2 μm. (F) FRC curve of the region shown in (D). The resolution was 75.57 nm at a correlation threshold of 0.143.(G) FRC curve of the region shown in (E). The resolution was 72.5 nm at the same correlation threshold as (F). (H) Scatterplots of the drift from 10 AF647 molecules in the STORM image sequence. (I) Box plot of the standard deviations of the drift of the AF647 molecules along the x- and y- axes.

Conclusions

We demonstrated a novel and straightforward approach to nearly drift-free SMLM using the ROCS. This method effectively and efficiently prevents sample drift caused by mechanical instability, circumventing the need for additional hardware or image post-processing. In SMLM imaging, the bench-top microscope produced a sample drift of 12 nm along both the x-axis and y-axis over 30 minutes, resulting in negligible resolution degradation. This work is an exemplary biological imaging method in which the ROCS facilitates superior imaging performance and capabilities without the need for additional hardware or image post-processing. We anticipate that the ROCS will emerge as a valuable tool for many researchers to simplify the quest for ultra-stable, high-performance scientific instrumentation.

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