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Super-resolution optical fluctuation imaging with high spatiotemporal resolution

Xuanze Chen[1], Zhiping Zeng[1], and Peng Xi[1]

[1] Department of Biomedical Engineering, College of Engineering, Peking University, China
E-mail: xipeng@pku.edu.cn

1. Introduction

Super-resolution microscopy opens up a new era for visualizing the dynamic subcellular structure and interaction in a whole new scale[1]. The methods to achieve super-resolution can be largely divided into two categories: (1) targeted modulation; and (2) stochastic modulation/fluctuation analysis. In stochastic single-molecule localization microscopy, the spatial resolution is gained through the scarification of temporal resolution, to ensure the sufficient sampling of the specimen. However, in super-resolution microscopy, the high spatial resolution is generally at a price of the imaging speed[2]. Super-resolution optical fluctuation imaging (SOFI) is a new technique which can allow multiple emitters within one diffraction limited region to be at on state simultaneously, and using the fluctuation dynamics to achieve super-resolution. Thus, it provides high spatial and temporal resolution simultaneously.

Here we report several advances of SOFI: (1) to enable high spatiotemporal resolution of SOFI (via JT-SOFI) [3]; (2) better 3D imaging capability (via 3D-MUSIC)[4], and (3) improved in vivo imaging capability with Skylan-S [5].

2. Results

JT-SOFI

Firstly, we report on an optical super-resolution imaging scheme implementing joint tagging using multiple fluorescent blinking dyes associated with SOFI (JT-SOFI), achieving ultra-high labeling density super-resolution imaging. Two advantages are obtained by this scheme: (1) In each channel because of the decrease of the labeling density, the reconstructed structure is more continuous in high-order SOFI, leading to a high resolution, high fidelity reconstruction; (2) JT-SOFI can significantly decrease the frame number required for SOFI, enabling fast super-resolution microscopy through simultaneous data acquisition. Experimentally, we demonstrated the faithful reconstruction of the continuous microtubule structure through collection of only 100 frames per channel, or temporal resolution of 3 seconds with spatial resolution of 85 nm.

3D-MUSIC

Secondly, we present a three-dimensional multimodal sub-diffraction imaging with spinning-disk (SD) confocal microscopy (3D-MUSIC), which not only takes fully advantages of spinning-disk confocal microscopy, such as fast imaging speed, high signal-to-noise ratio, optical-sectioning capability, but also extends its spatial resolution limit along all three dimensions. Both axial and lateral resolution can be improved simultaneously by virtue of the blinking/fluctuation nature of the modified fluorescent probes, exemplified by the quantum dots (QDs). Further, dual-modality super-resolution image can be obtained, by SOFI and bleaching/blinking assisted localization microscopy (BaLM).

Skylan-S

Thirdly, we report the development of a new reversibly switchable fluorescent protein (RSFP) that can be effectively used for super-resolution optical fluctuation imaging (SOFI) based on the switching and fluctuation of single molecules. Several properties of RSFPs strongly influence the quality of SOFI images. We term it Skylan-S, which features very high photostability, contrast ratio and averaged fluorescence intensity in the fluctuation state, making it applicable for long-time SOFI imaging with high spatial-temporal resolution.

3. Conclusions

The simultaneous emission collection capability has made SOFI with improved temporal resolution. To further enhance its imaging speed without sacrificing the spatial resolution, we have implemented joint-tagging scheme, in which multiple QDs were labeled on the same cellular organelle toward super-resolution. We found that not only the speed is m-fold improved, but the spatial fidelity is dramatically improved due to the decreased labeling density of each spectral channel.

We have also demonstrated improved 3D imaging capability taking advantage of the parallel detection of the spinning disk confocal microscopy. The inherent optical sectioning capability of the spinning disk and the high signal-to-noise ratio improved the axial resolution of SOFI.

To improve SOFI's performance in live cell imaging, a new type of RSFP monomer, termed Skylan-S, have been developed, with much better fluctiona and brightness over Dronpa.

References

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