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Three Dimensional Super Resolution Fluorescence Imaging of Single Bacterial Cells by Stereo Photoactivated Localization Microscopy

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Abstract:

Emerging optical microscopy based on localization of serially activated single emitters allows observation of biological samples with resolution close to molecular scale [1-3]. Rapid progress has been reported on extending this super resolution imaging technique to three dimensions [4-5]. A common approach is to explore the off-focus pattern to localize single molecules with depth information, and the axial resolution is generally two to three times less than lateral resolution.

We have developed a new imaging technique, Stereo Photoactivated Localization Microscopy (Stereo PALM), which can obtain 3D super resolution fluorescence imaging with equal spatial resolution in all three dimensions. In this method, a microfabricated tilted micro-mirror is used to simultaneously generate side view of emitting single molecules, so the task of axial localization is transformed to lateral localization. By correlating single molecule events in front view and side view, the three dimensional image can be reconstructed with isotropic resolution. This stereo imaging method is also less vulnerable to axial drift, which is otherwise difficult to overcome. Further discussion is presented on the point spread function analysis and error analysis, as well as the effect of angular variation of the tilting mirror.

We present the result of super resolution Stereo PALM imaging of micron-size beads coated with photoactivated chromophores. Moreover, we demonstrate the application of Stereo PALM to probe internal structure of single bacterial cells. Photoactivatable fluorescent proteins have been genetically tagged with specific proteins inside E. coli cells, in which the spatial distribution of target proteins can optically resolved beyond diffraction limit. The results demonstrate that Stereo PALM technique is ideal to approach many microbiological questions, which are otherwise difficult by other techniques, in unprecedented details.

References:

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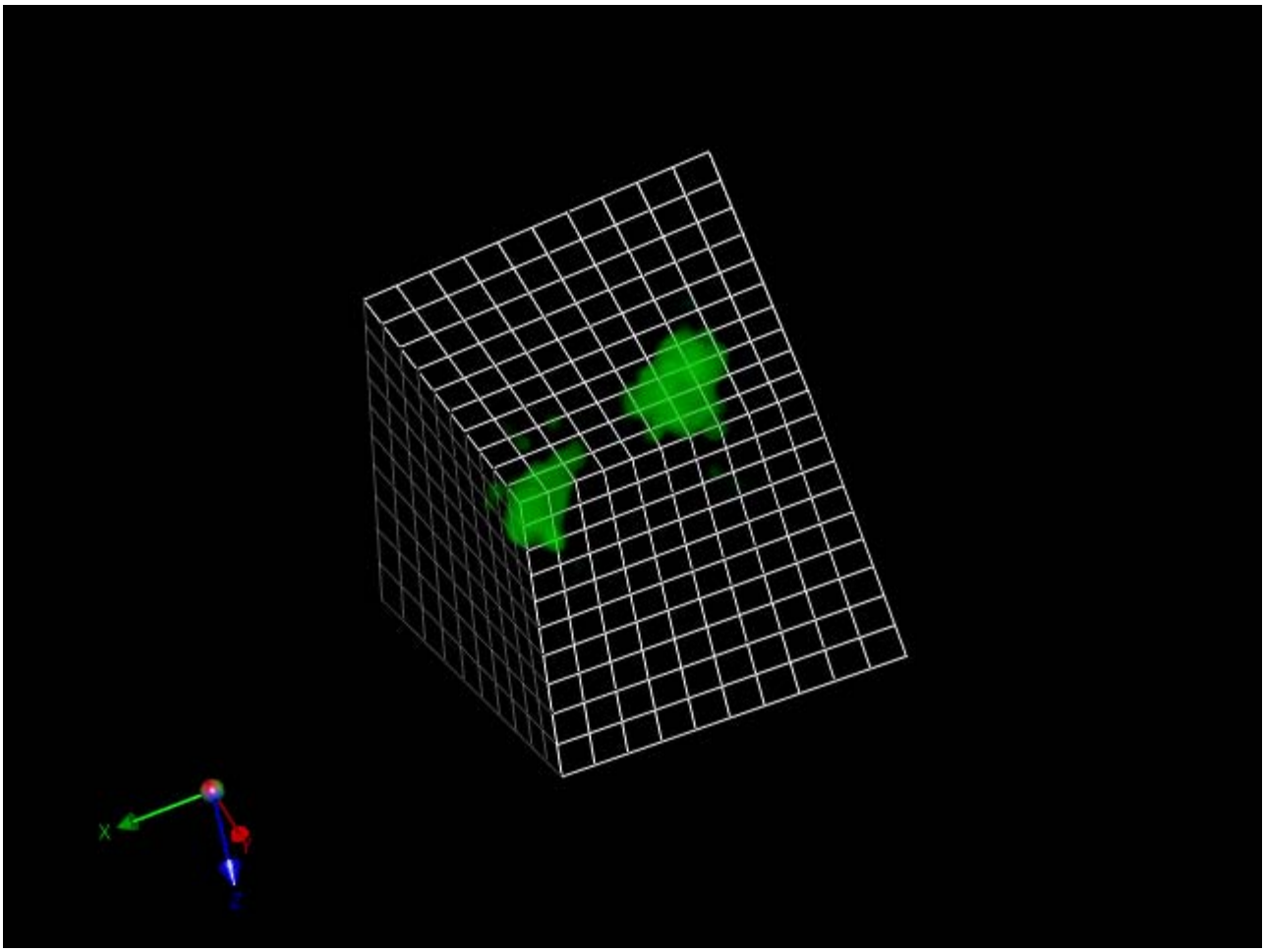

Fig. 1. Three dimensional super-resolution fluorescence image of a single E. coli cell. Photoactivated fluorescent protein Dronpa is genetically tagged with casC protein which is over-expressed in E. coli cells. Each unit in the scale grid is 200 nm.

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