High-resolution 3D imaging of cell cortex structure by multi-angle total internal reflection fluorescence microscopy

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Abstract: Total internal reflection fluorescence microscopy (TIRFM) provides high optical sectioning capability and superb signal-to-noise ratio for imaging of cell cortex structures [1]. Using the relationship between illumination angle and penetration depth of the evanescent field, recent researches have demonstrated that multi-angle (MA) TIRFM enhances the axial resolution of the subcellular structure imaging down to a few tens of nanometers [2,3]. However, one challenge of MA-TIRFM imaging is to achieve lateral high-resolution and 3D reconstruction of cell surface structures. In order to elevate the lateral resolution of TIRFM, typically it was integrated with other super-resolution imaging modalities such as SIM and STED. In this study, we used custom-designed MA-TIRFM setup with dedicated components for high-speed imaging, and developed a stochastic photobleaching and single molecule localization method to enhance the lateral resolution of TIRFM imaging. Furthermore, in order to visualize and quantitatively analyze the cytoskeleton network in cells, we proposed novel algorithm to perform automated filament detection, segmentation and 3D reconstruction from MA-TIRFM images. The application of these methods permits 3D high-resolution imaging and reconstruction of the surface cytoskeleton structure in cell. We achieved a lateral resolution of ~150 nm and an axial resolution of ~40 nm in cell samples. Finally, we studied the 3D distribution of cortical actin and microtubule structures in U373 cancer cells. We found that cortical actin localizes ~27 nm closer to the plasma membrane when compared with microtubules. In addition, anti-cancer drug nocodazole treatment disassembled microtubules and caused single filaments localized more towards the cell surface. We believe these methods have the potential to be used in imaging and analyzing other biological parameters in cell and advance our understanding of cellular structures and their function.

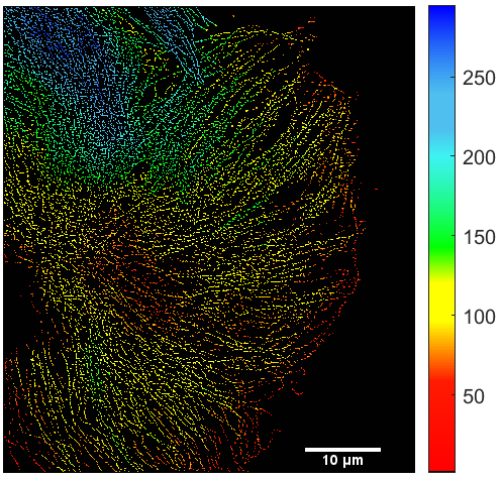

Figure.1: 3D reconstruction of microtubules in U373 cell.

References

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