Four-dimensional single particle nanometry

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Among many optical microscopic techniques, single particle tracking have been widely used to achieve high spatial-temporal resolution of protein movement both *in vitro* and in cells. Though the resolution of optical microscope is significantly constrained by optical diffraction limits, the two dimensional (2D) position of an individual fluorophore can be precisely determined by fitting its fluorescent image to a Gaussian function. However, the protein in cells not only moves in two dimensions, but also in vertical axis, and further rotates. We extend the spatial dimensions of single particle tracking even further, to four dimensions.

In order to track the 3D movement of single particles, we constructed an optical system for third comportment, Z-position, determination with utilizing intentional astigmatism. The astigmatism is generated by inserting a pair of convex and concave cylindrical lenses into the optical pathway before the camera, resulting in the elliptic deformation of the image of the fluorophore. The ellipticity is a function of the Z-position. By further addition of the optics into the 3D tracking system, we newly developed 4 dimensional single particle tracking with utilizing polarization of fluorophore. The angler position is easily obtained by the polarization. In our 4D tracking system, a polarizing beam splitter is set before the cylindrical lens pair in the 3D tracking optics to divide the fluorescent image into S- and P-polar channels (Fig. 1) (1). In our case, the calculated precisions for the X, Y, Z and θ-positions achieved 5, 7, 9 nm and 1°, respectively.

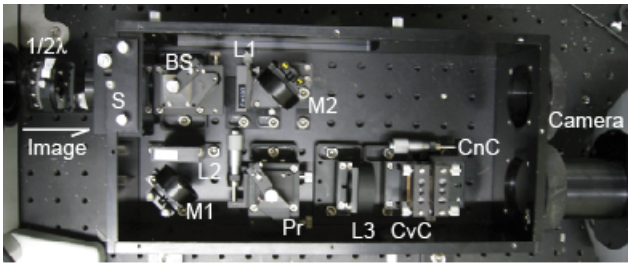

Fig. 1 Optics for 4D single particle tracking. 1/2λ, 1/2 wave plate; S, slit; BS, beam splitter; L, lens; M, mirror; Pr, prism; CvC, convex cylindrical lens; and CnC, concave cylindrical lens.

We synthesized fluorescent polarized semi-conductor nano particles, and applied the 4D tracking to observe the movement of a membrane protein labeled with them. It was showed that a membrane protein in cytoplasm moved along tracks, most likely microtubules, in three-dimensions and slowly rotated helically (Fig. 2). The 4D tracking enables studies of biological phenomena to reveal the relationship between the intracellular orientation of biomolecules and their functions in living cells.

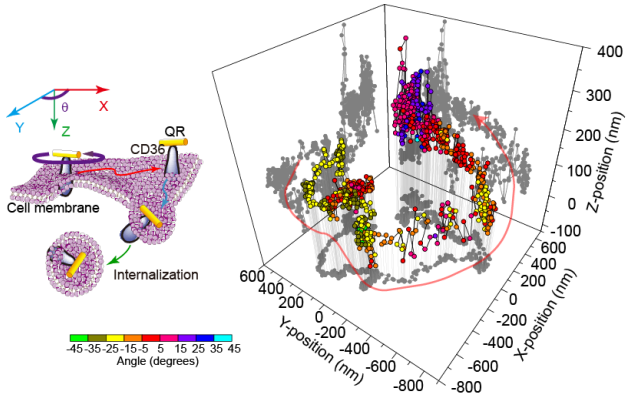

Fig. 2 Schematic and result of 4D single particle tracking of membrane protein. One of membrane protein, CD36, was labeled with polarized nano particle (QR) via antibody affinity. The angle of the QR is indicated by the color bar.

Reference

1. Tomonobu M Watanabe, et al., Biochem Biophys Res Commun.***359***, 1-7 (2007)

A novel technology developed by studies of Biophysics

G-ANG BASE provides your best microscopic system

Hiromichi Oikawa
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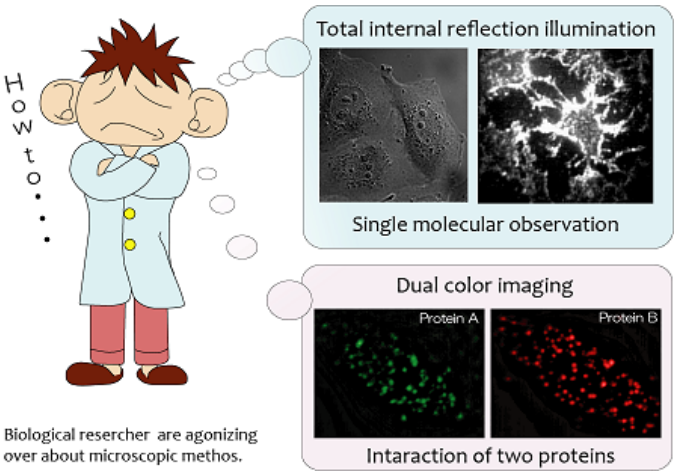
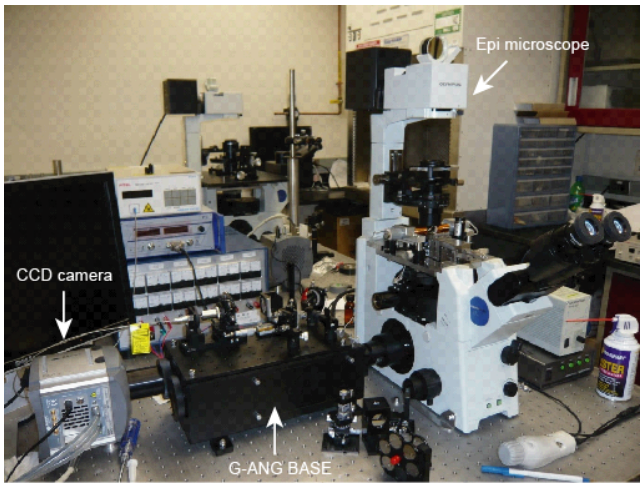
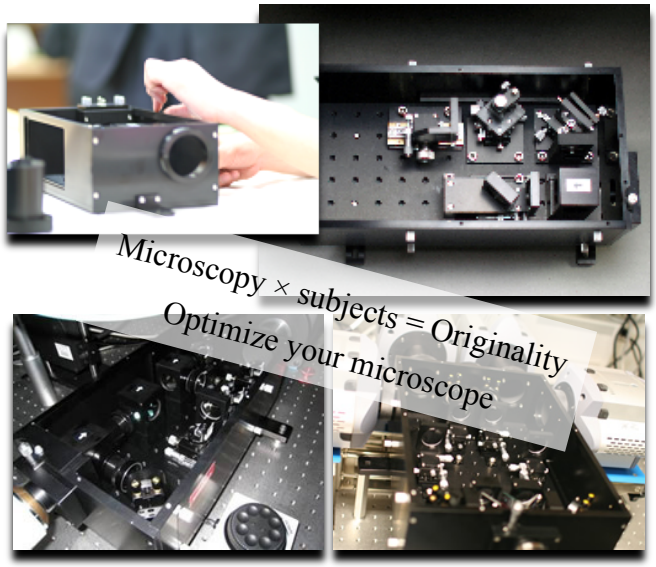
Persons who are in charge of a project have to choose the most appropriate microscope to fit their experiments, and in most cases, the use of multiple techniques is required. This causes time-consuming and making the system expensive for the biological researchers who are not familiar with optical microscopy. We developed G-ANG BASE to solve this problem. It allows them to easily adjust and modify their optical system depending on the subject of interest, thereby adding originality and variety to their approach.



The G-ANG BASE is the optical base for relay optics designed to facilitate free and easy customization of your own optical systems. It allows you to easily adjust and modify your optical system depending on the subject of interest, thereby adding originality and variety to your approach. The G-ANG BASE can relay images to a microscope and a camera with C-mount port (Fig 1). It is vibration resistant and prevents light leakage, and can be applied in single molecule nanometry. It provides 6 mm screw sockets in 25 mm intervals, allowing the construction of a variety of parts into a custom designed optical system. All these features makes the G-ANG BASE an ideal tool that supports research under constantly changing environments.



Many biological researchers developed their-own system in the G-ANG BASE, and obtained new findings. We promise you that the G-ANG BASE provides unlimited freedom in constructing an ideal optical system of your choice depending on your research of interest.



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