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Multiple single-molecule tracking in nanoscale based on spectral discrimination

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1. Introduction

Proteins work as nanomachines in living organisms by assembling into a complex of one type of protein and/or its associated proteins. To unveil the dynamic behavior and functions of individual proteins on such a small scale, single-molecule measurement techniques have advanced tremendously in the past two decades, including total internal reflection fluorescence (TIRF) microscopy, and optical trapping forcemetry. However, almost of these studies focused on only the single molecule. To further understand the physicochemical mechanism of protein functioning, attention is now shifting upward through the hierarchy, from individual elements to the cooperative behavior of their supramolecular system [1]. It is necessary to develop the ability to resolve individual molecules simultaneously within a working complex system at nanometer scale.

In this presentation, we introduce a simple and versatile scheme to simultaneously observe multiple individual proteins moving on the nanometer scale, which is based on imaging spectroscopy of semiconductor quantum dot (QD) probes of various colors [2].

2. Scheme for multiple single-molecule imaging

The measurement scheme was specially designed for measurement of linear motor proteins. The key concept is that the displacement of motor proteins in only one dimension (y in this paper) along a filament axis is measured, and its orthogonal dimension (x) is dedicated to spectral discrimination, which allows one-dimensional tracking of multiple proteins labeled with distinct fluorescent probes.

Our measurement scheme uses an imaging polychromator at the detector position of a conventional TIRF microscope. The entrance slit of the polychromator is placed on the image plane conjugate to the sample plane. Target proteins are labeled with QDs of various distinct emission wavelengths. The orientation of filaments is controlled to be parallel to the entrance slit. A target filament is positioned on the conjugate position of the slit by adjusting the position of the sample stage so that the fluorescence from QDs close to the filament can be transmitted through the slit. The image on the slit is then dispersed in the x direction by a diffraction grating in the imaging polychromator, and the first diffraction image is focused on the surface of a camera detector. As the y coordinate at the detector corresponds to the counterpart coordinate at the sample, one can estimate the y coordinate of fluorescence spots in the spectral dispersion image. Thus, the spectroscopic detection distinguishes multiple proteins along the filament, even if they are close to each other within a sub-diffraction-limit scale. One can achieve simultaneous tracking of individual single molecules with a speed as high as that of conventional single-molecule tracking techniques [3].

3. Tracking of multiple myosin molecules

For demonstration of the multiple molecule tracking, we used myosin-V (M5) as a model system, which is a vesicle transporter, forms homodimers and realizes walking-like behavior. Figure 1A shows the spatiotemporal variation of the two fluorescence spots originating from the two QDs on the two heads of a single M5. The centroid positions were estimated by curve-fitting of elliptical Gaussian functions at each frame. Temporal profiles of the displacement of the QDs are shown in Fig. 1B, where a stepwise hand-over-hand profile is clearly observed. The distance of both heads was ~36 nm during the walk, which is consistent with previous studies of M5 using conventional single-molecule imaging with the dual view system [4]. We achieved localization precision of 4 nm at 30 ms temporal resolution, which is sufficient for biophysical application.

In the presentation, we will show other experimental results of tracking of more than two molecules, and also introduce how to extend this measurement scheme to two-dimensional measurement.

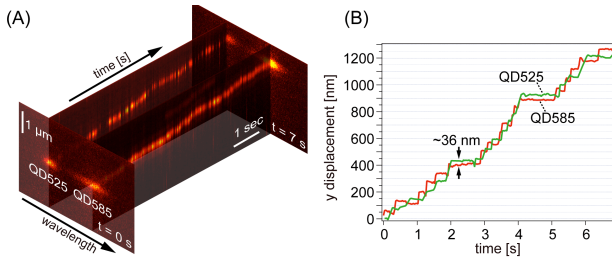

Fig. 1: Tracking of two heads of M5 walking along an actin filament. (A) Diffraction images at 0 and 7 s and kymographs of the two columns at the spectral peak wavelengths (525 and 585 nm) of the two QDs attached on the heads. (B) Temporal profile of the displacement of the two heads of M5.

References

[1] L. Hilbert, et al., Biophys. J. **105** (2013) 1466–1474.
[2] T. Kakizuka, et al., submitted.
[3] D. M. Warshaw, et al., Biophys. J. **88** (2005) L30–L32.
[4] K. Ikezaki, et al., Small **8** (2012) 3035–3040.