Supporting Information

Parallel Three-Dimensional Tracking of Quantum Rods Using Polarization-Sensitive Spectroscopic Photon Localization Microscopy

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1. Detailed description of the optical setup

The experimental setup of 3D-Polar-SPLM is illustrated in Fig. S1. The excitation light source was a 445 nm continuous-wave laser with a 500 mW maximum output. After passing through a laser clean-up filter (LF) and a spatial filter (SF), the intensity of the excitation beam was adjusted by an achromatic half-wave plate (HWP, AHWP05M-600, Thorlabs) and a Glan-Taylor polarizer (GTP, GT10, Thorlabs). The excitation beam was directed to an inverted microscope (Eclipse Ti-U with perfect-focus system, Nikon) by a dichroic mirror (DM, FF458-Di02, Semrock). We used a 100x objective lens with a numerical aperture (NA) of 1.49 (Nikon CFI apochromat TIRF). A 458-nm longpass filter (LPF, BLP01-458R-25, Semrock) was placed at the emission port to reject the excitation beam. The desired fluorescent emission was redirected by a reflecting mirror (RM), which formed a magnified real image by a tube lens (TL). At the projected imaging plane, an adjustable rectangular aperture was placed to define the optical field-of-view.

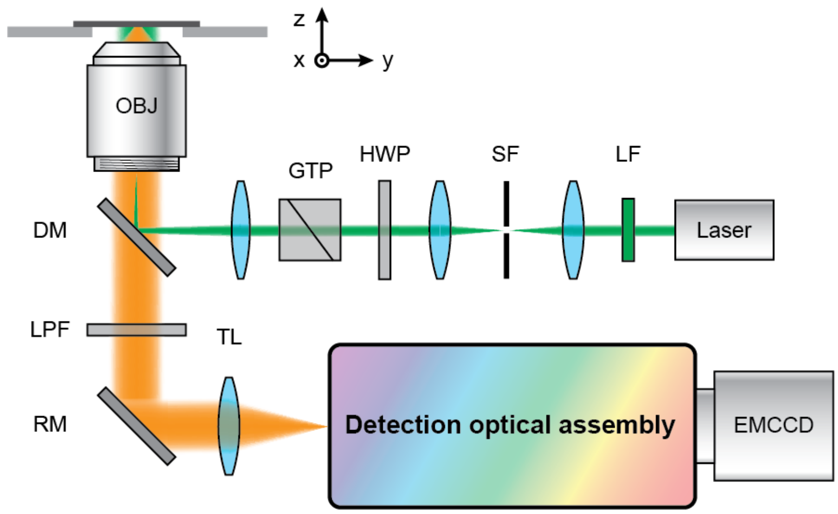

Fig. S1. Schematic of the experimental setup. LF: laser clean-up filter; SF: spatial filter; HWP: achromatic half-wave plate; GTP: Glan-Taylor polarizer; DM: dichroic mirror; OBJ: objective lens; LPF: longpass filter; RM: reflecting mirror; TL: tube lens. EMCCD: electron multiplying charge coupled device.

2. Emission polarization dependence of quantum rods

To verify the emission polarization dependence of QRs, we replaced the detection optical assembly with a linear polarizer to measure the fluorescence intensity with respect to the rotating angle θ of the linear polarizer. As shown in Fig. S2, by analyzing 10 randomly distributed QRs, their normalized intensity closely follows a $\cos^2(\theta)$ dependence on the polarization angle. In this study, the out-of-plane tilt angles of the deposited QRs were close to zero; however, the influence of a high-NA collection may contribute to the observed deviation from the theoretical curve[1].

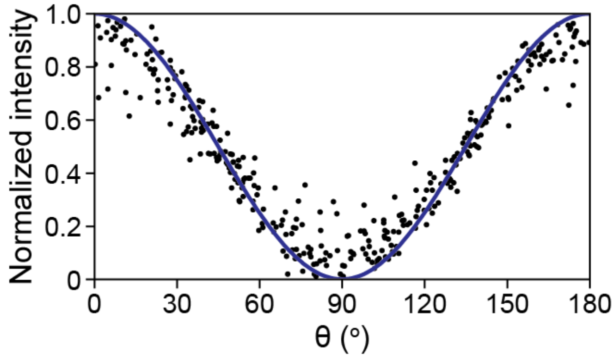

Fig. S2. Emission polarization dependence of quantum rods.

3. Measure the in-plane polarization angle with a high-numerical-aperture objective

When considering the influence of a high-NA collection, a correction should be applied to the in-plane polarization angle ρ based on the theoretical model[2]:

$$P_{corrected} = \frac{C\sin^2(\theta)\cos(2\rho)}{A + B\sin^2(\theta)}.$$

Where θ is the out-of-plane title angle and

$$A = \frac{1}{6} - \frac{1}{4}\cos\alpha + \frac{1}{12}\cos^3\alpha,$$
$$B = \frac{1}{8}\cos\alpha - \frac{1}{8}\cos^3\alpha,$$
$$C = \frac{7}{48} - \frac{1}{16}\cos\alpha - \frac{1}{16}\cos^2\alpha - \frac{1}{48}\cos^3\alpha.$$

The maximum of the collecting angle α is determined by the objective's numerical aperture (NA) and the refractive index of the medium (n) as

$$\alpha = \sin^{-1}(NA/n).$$

When θ = 0, NA = 1.49 and n = 1.54, we have

$$P_{corrected} = 0.935\times\cos(2\rho).$$

This shows the measurements only deviates slightly from the theoretical model, which is consistent with the results shown in Fig. S2.

4. Fabrication of polymeric grating pattern

Polymeric grating pattern was fabricated using the nanotransfer printing process. The fabrication process starts with pre-fabricated silicon mold containing periodic one-dimensional gratings with the periodicity of 300 nm. UV-curable PMMS poly[(mercaptopropyl)methulsiloxane] based photopolymer was poured onto the silicon mold and vacuum loading was used for degassing and improving the mold filling process. The polymeric pattern was cured into the solid elastomer by exposing to the ultra-violate light for 5 minutes and can then be easily peeled off from the silicon mold.

Reference:

1. Cruz, C. A. V.; Shaban, H. A.; Kress, A.; Bertaux, N.; Monneret, S.; Mavrakis, M.; Savatier, J.; Brasselet, S., Quantitative nanoscale imaging of orientational order in biological filaments by polarized superresolution microscopy. *P Natl Acad Sci USA* **2016,** *113* (7), E820-E828.
2. Wei, C. Y. J.; Kim, Y. H.; Darst, R. K.; Rossky, P. J.; Vanden Bout, D. A., Origins of nonexponential decay in single molecule measurements of rotational dynamics. *Phys Rev Lett* **2005,** *95* (17).