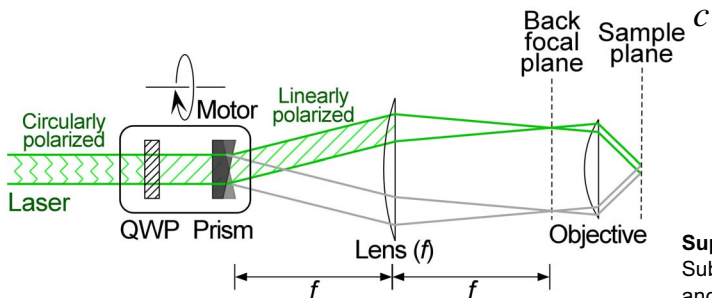


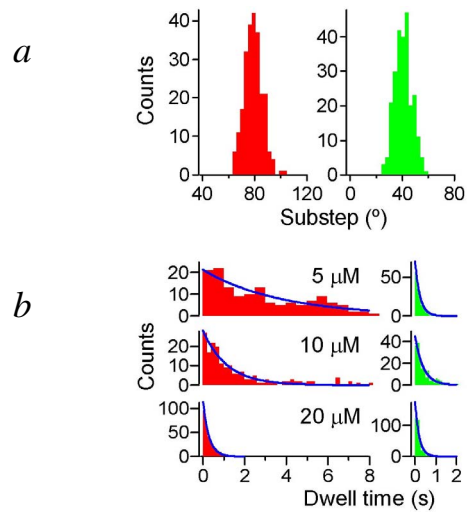
Supplementary Figure 1

Schematic diagram of the microscope system. Optical paths for different wavelengths are distinguished by color. DM, dichroic mirrors; F, filters (transmission range in parentheses); M, mirrors. Bright-field and fluorescence images were separated by a beam splitter at 620 nm, and captured by two cameras (CCD-300; Dage-MTI), the one for fluorescence after an image intensifier (VS4-1845; Video Scope). The two cameras were operated synchronously and combined images were videotaped.



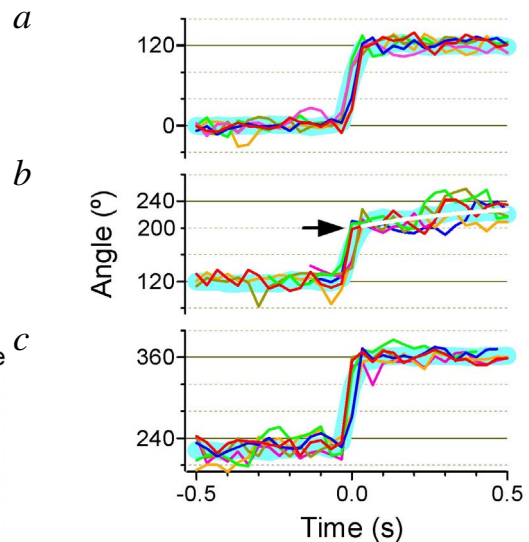
Supplementary Figure 2

Schematic illustration describing the optical configuration to realize the illumination shown in Figure 1b in text. A quarter-wave plate (QWP), which transforms the circularly polarized laser beam into a linearly polarized beam, is rotated together with a beam-deflecting prism in a hollow stepping motor. We placed a lens (focal length $f = 200$ mm) at distance f from the back focal plane of the objective, and a custom-made prism at distance f from the lens. The beam, which was refracted by the prism rotated at a constant speed together with a quarter-wave plate, was collimated and focused at the back focal plane.



Supplementary Figure 3

Analyses of substeps in rotation by Cy3-ATP. **a**, Distribution of substep sizes. We selected by eye those angle-displacements that were completed within one video-frame and accompanied by dwells of more than two video-frames both before and after the displacements. The dwelling angles before and after each displacement were averaged, and the difference was taken as a substep angle. **b**, Histograms of dwell times before an 80° substep (' 0° dwell', red) and dwell times after an 80° substep (' 80° dwell', green) at indicated Cy3-ATP concentrations. The average dwell times τ were estimated by fit with $P(t) = P_0 \exp(-t/\tau)$ (blue lines). Figure 4d in text is the summary of these data.



Supplementary Figure 4

Substepping behaviors in the mixture of Cy3-ATP and ATP. The angles in the ordinate are measured from ' 0° ', defined as the angle immediately before binding of Cy3-ATP (see Fig. 2b in text). **a**, Stepping from 0° to 120° ; **b**, from 120° to $\sim 240^\circ$; **c**, from $\sim 240^\circ$ to 360° (0°). Six examples of step records (thin lines) and averages of 27 steps (thick cyan lines) in the mixture of 50 nM Cy3-ATP and 550 nM unlabeled ATP. Those data that showed dwells of more than two video-frames at all four angles (0° , 120° , 240° and 360°) were chosen and analyzed. After positioning individual steps at time 0, the preceding and subsequent steps and records beyond those were removed, and one further frame was trimmed off from each end. Before averaging, step records shorter than the -0.5 s to 0.5 s interval were extrapolated with a horizontal line(s) with the value of the end point. Then all steps were averaged. In **b**, the white line shows $-40^\circ \times \exp(-t/0.4 \text{ s}) + 240^\circ$. The time constant, 0.4 s, was in good agreement with the average 80° dwell of 0.3 s in the rotation by pure Cy3-ATP (see Fig. 4d in text).