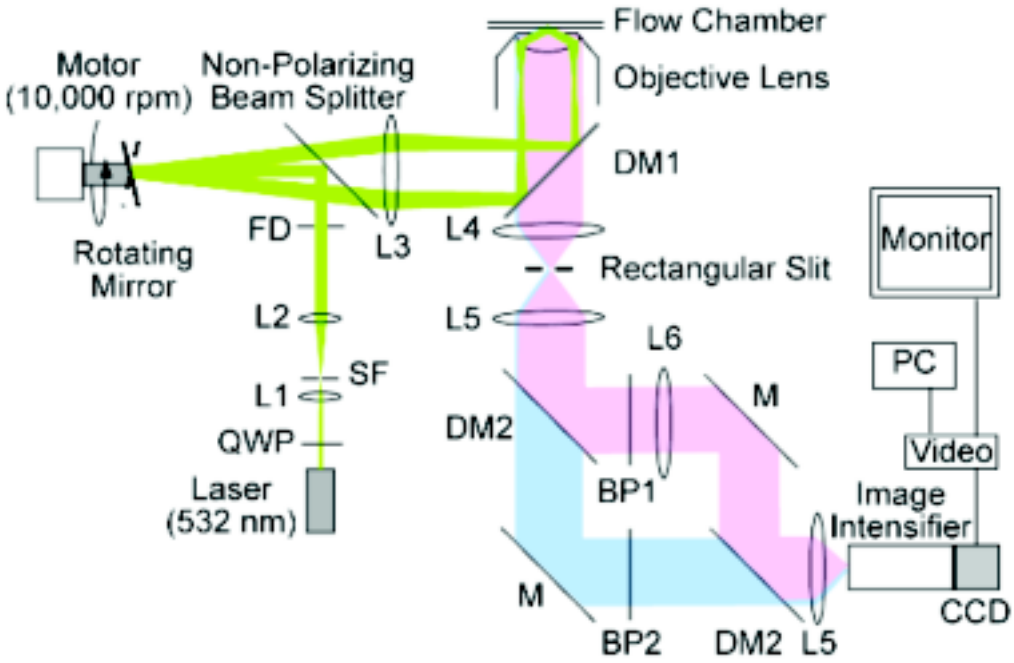

Instrumental set up for measurement of single molecule FRET (supplementary Figure 2).

A 532 nm wavelength laser beam (MG-532C-500, Spectra Physics, schematically shown in green) was expanded by two lenses (L1 and L2, f = 17 mm and 80 mm, respectively), and reflected by non-polarizing beam splitter toward a mirror attached to a spindle motor (SM5018-002, Softronics, Japan) rotating at the speed of 10,000 revolutions per minute (rpm). The laser light reflected by the rotating mirror was collimated and focused by a lens (L3, f = 350 mm) at the back focal plane of the objective lens (PlanApo60x, numerical aperture 1.45, Olympus), and non-polarized evanescent field was formed on the surface of the coverslip. Laser power was adjusted to 0.2-0.4 mW at the back focal plane of the objective lens, and the area of the illumination at the sample plane was ≈600 μm^2. Fluorescence images of donor and acceptor (shown in red and blue) were collimated by a lens (L5, f = 150 mm), divided and combined by dichroic mirrors (DM2, 620DCXR, Chroma), and projected onto an intensified (VS4-1845, Videoscope) charge-coupled device (CCD) camera (CCD-300T-RC, DAGE MTI) by another L5. Bandpass filters (BP1 and BP2, HQ585/80 and HQ700/90m, Chroma, respectively) were used to reduce the cross talk (<3%) between donor and acceptor fluorescence. A lens (L6, r = 20,000 mm) was

inserted at after BP1 to correct for chromatic aberration between Cy3 and Cy5 fluorescence. Images recorded by a digital video recorder (DSR-20, Sony) were captured in a personal computer (LB30A-D2, Library, Japan) and analyzed off-line with custom software (Library). QWP, quarter-wave plate; SF, spatial filter; FD, field diaphragm; DM1, dichroic mirror (550DCXR, Chroma); L4, second objective lens inside microscope (f = 180 mm); M, mirror.