Supplementary Information

High-speed atomic force microscopy combined with inverted optical microscopy for studying cellular events

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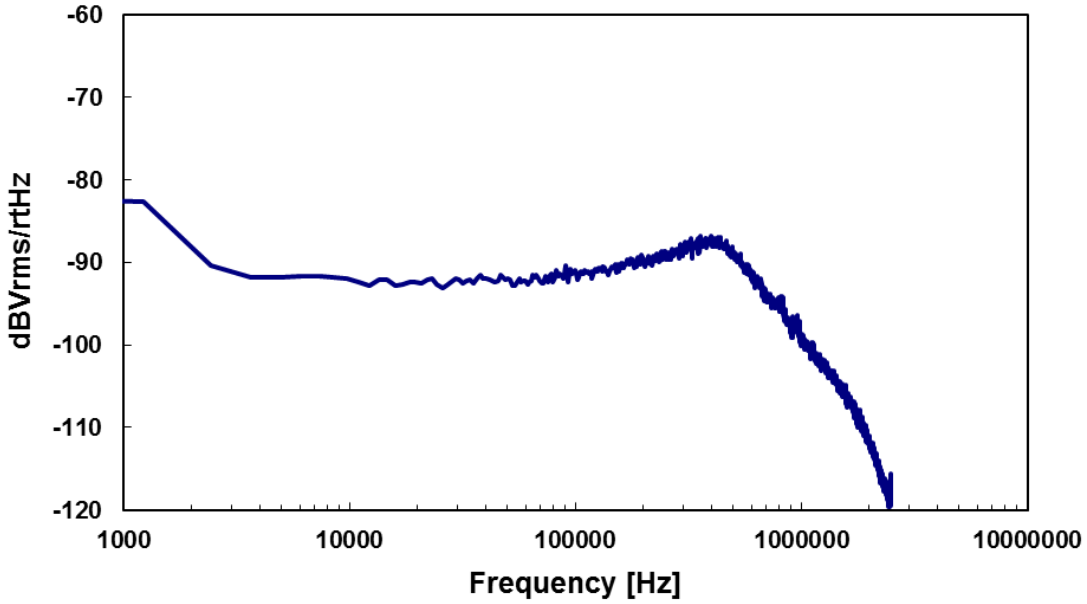
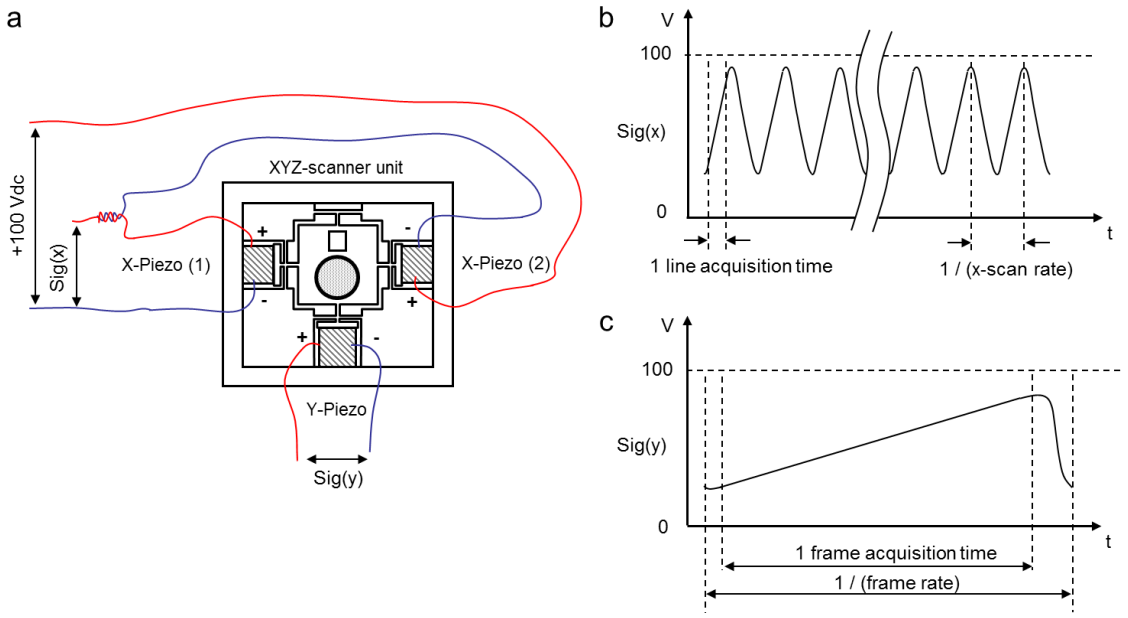
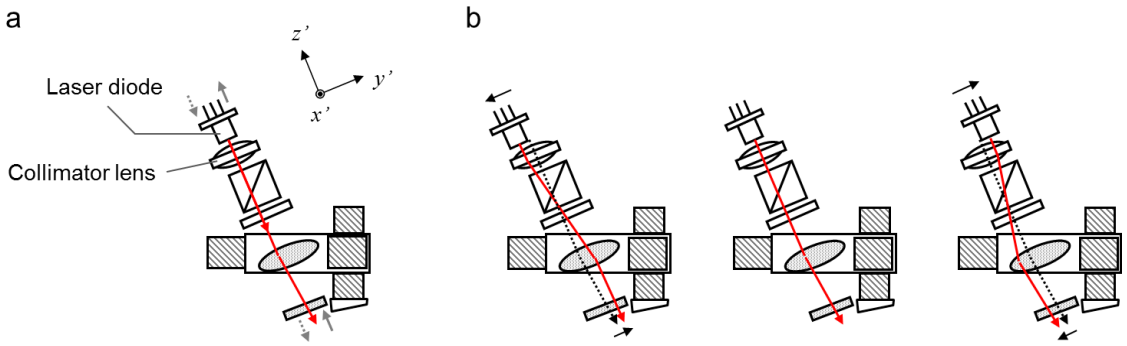


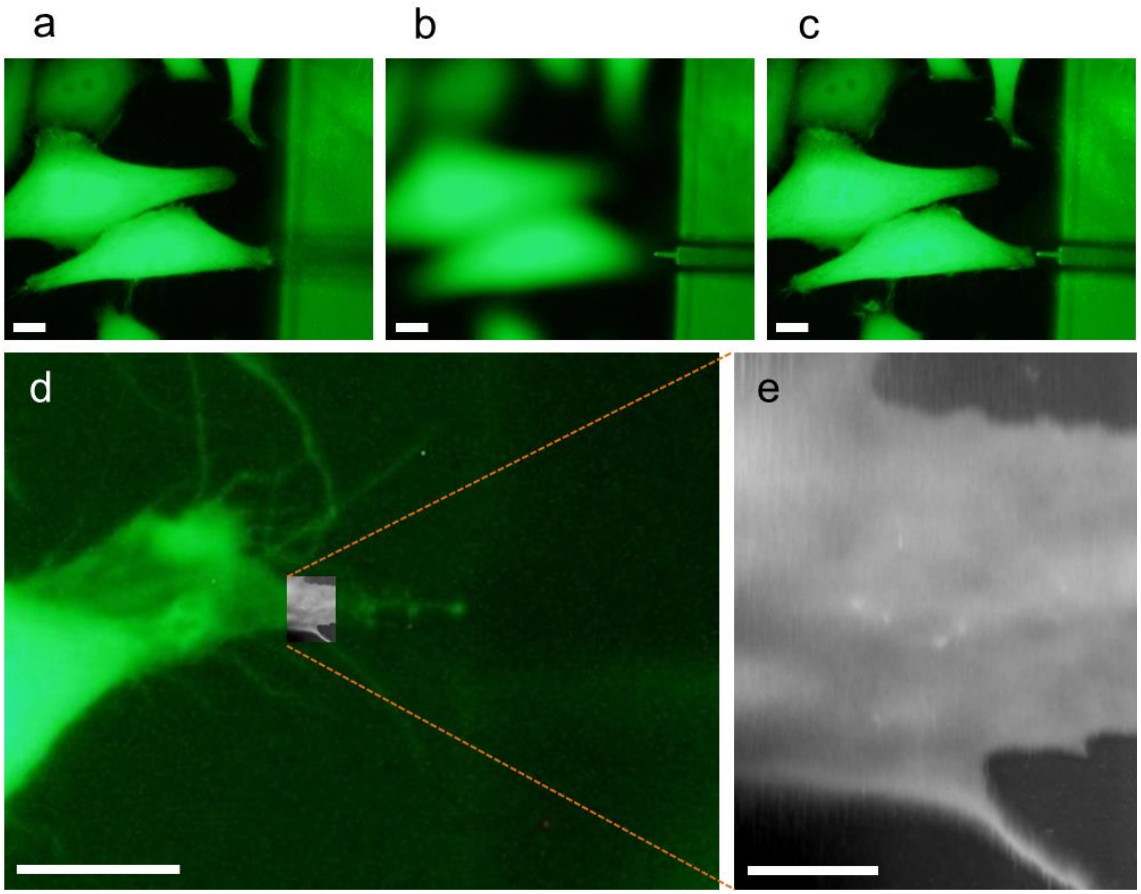
Supplementary Figure S1. Frequency spectrum of thermal fluctuations of a small cantilever (BL-AC10FS, Olympus, Tokyo, Japan) in water.



Supplementary Figure S2. A wiring diagram for the XY-piezo and scanning waveforms. (a) DC voltage of 100 V is applied to two serially connected X-piezos and the scanning signal for the *x* direction is fed in between the two piezos. (b, c) Scanning signals for *x* (b) and *y* (c) directions. Both scanning signals smoothly curve at the turn of the scanning to reduce the harmonic wave. To avoid distortions of the captured image, both *x* and *y* scans are overrun at approximately ±10% of the scan size, and data around the turn of the scanning signal are not recorded.



Supplementary Figure S3. Schematic illustrations of laser alignment. The laser focusing position relative to the cantilever is aligned by moving the position of the laser diode against the collimator lens. (a) Laser alignment in the *z'* direction. The laser focusing position moves in the same direction that the laser diode moves in the *z'* direction. (b) Laser alignment in the *x'* or *y'* direction. The laser focusing position moves in the direction opposite that of the laser diode in the *x'* or *y'* direction.



Supplementary Figure S4. Engagement of cantilever. (a, b) Optical/fluorescent images before engagement of a cantilever. (a) Fluorescence image of live HeLa/GFP cells, which stably expressed GFP (Cell Biolabs, Inc., San Diego, CA). (b) Cantilever positioned at approximately 10 μm above the cells in (a) was focused and observed using the white LED. (c) Fluorescence image of the HeLa cells after engagement of the cantilever. The relative position of the cantilever to the cells can be easily and precisely determined by combining the white LED and filtered excitation light. Scale bars: 10 μm. (d) AFM image of an area of interest overlaid on the fluorescent image. Scale bar: 10 μm. (e) Enlarged AFM image. Scale bar: 1 μm.

Supplementary Movie S1. High-speed AFM movie of a HeLa cell surface. Image size: 4480 nm × 3360 nm. Original scan rate: 0.2 fps. The movie is sped up 10x.

Supplementary Movie S2. High-speed AFM movie of a 3T3 fibroblast cell surface. Image size: 4000 nm × 3000 nm. Original scan rate: 0.1 fps. The movie is sped up 10x.