This document provides supplementary information to “Extended field-of-view single-molecule imaging by highly inclined swept illumination,” https://doi.org/10.1364/optica.5.001063. It provides nine supplementary figures and one supplementary table.
Fig. S2. DAQ timing diagram. (a,b) A trigger signal from a function generator starts the galvo mirror and sCMOS acquisition. (c) Details of light sheet rolling shutter mode control. Tint is the integration time of each pixel line, TD is the delay time of consecutive pixels, Weff is the effective acquisition width. In our experiments, we used Tint = 60 ms, TD = 0.36 ms and Weff = 166.

Fig. S3. Effective beam width of tile illumination. (top) Fluorescence images of 20 nm beads in 3D hydrogel using a compression ratio of 5 and 8 with a tile length of 80 μm or 130 μm, respectively. Scale bar, 20 μm. (bottom) Standard deviation projection along y direction for each image. The illumination widths of r = 5 and r = 8 for ~80 μm tile are ~10 μm and ~14 μm, and those of r = 5 and r = 8 for ~130 μm tile are ~14 μm and ~24 μm, indicated by the double sided red arrows.
Fig. S4. Images of 20 nm beads in hydrogel and signal to background ratio (SBR) using different illumination methods at z = 5 μm. Scale bar, 5 μm.

Fig. S5. Fluorescence background corrected images of single Atto647N DNA in 3D hydrogel by epi, large area HILO, small area HILO, tile and HIST illumination. The images were taken 5 μm above the surface. Scale bar, 20 μm and 2 μm (inset).
Fig. S6. Full FOV imaging of HIST microscopy with (left) or without (right) a second galvo mirror for correcting different incidence angles during sweeping. Images were taken 5 μm above the surface, frame integration time 500 ms, averaged illumination power ~50 W/cm². Scale bar, 20 μm.

Fig. S7. Photobleaching curves for 130 x 130 x 5 μm³ volume of Atto647N DNA in a hydrogel sample by epi and HIST illumination. The decay rates are $6.5 \times 10^{-3}$ s⁻¹ and $5.4 \times 10^{-3}$ s⁻¹, respectively. Red curves are single exponential fits.
Fig. S8. Images of 4 FISH probes in A549 cells and SBR comparison with Epi, large area HILO, small area HILO, TILE and HIST illumination. The images were maximum intensity projected from an imaging depth of 5 μm to the surface. Scale bar, 20 μm.

Fig. S9. Photobleaching step distribution (a) and SBR distribution (b) for 4 FISH probes in A549 cells. The blue and red curves in (b) are Gaussian fitting for the distributions. 211 mRNA spots were used.
| Name | Sequence | Experiment |
|------|----------|------------|
| probe1 | 5'- Acryd/GCCTCGCTGGGGTGGGA/3ATTO647NN/-3' | Single probe hydrogel |
| P1 | /5AmMC6/CCC AGG TAG AAC CGA AAG AA | |
| P2 | /5AmMC6/CTA CCG TGA AGT TCA CCA TG | EEF2 A549 cells |
| P3 | /5AmMC6/CAG ACA TGT TGC GGA TGT TG | |
| p4 | /5AmMC6/GTA TCA GTG AAG CGT GTC TC | |
| p5 | /5AmMC6/GTT GAC TTG ATG GTG ATG CA | |
| P6 | /5AmMC6/GCG GTG AGA AGA GGG AGA TG | |
| P7 | /5AmMC6/ATG AGG TTG ATG AGG AAG CC | |
| P8 | /5AmMC6/TCC GAG GAG AAG TCG ACA TG | |
| P9 | /5AmMC6/CTT GTT CAT CAT CAG CAC AG | |
| P10 | /5AmMC6/AGG ATG GGG TCC AGG ATC AG | |
| P11 | /5AmMC6/GTA GGT GGA GAT GAT GAC GT | |
| P12 | /5AmMC6/GCA GGA CAG GAT CGA TCA TG | |
| P13 | /5AmMC6/AAA CTG CTT CAG GGT GAA GG | |
| P14 | /5AmMC6/AAC TTG GCC ACA TAC ATC TC | |
| P15 | /5AmMC6/TGG CTT GAG TGCT CGA TCT TG | |
| P16 | /5AmMC6/AGA ATG GGG TCC AGG ATC AG | |
| P17 | /5AmMC6/CAT GAT CGC ATC AAA CAC CT | |
| P18 | /5AmMC6/TGG ATG GTG ATC ATC TGC AA | |
| P19 | /5AmMC6/TAG AAC GTA CCT TTG TCG GA | |
| P20 | /5AmMC6/CGG AGA AGA CTC GCA TAG AG | |
| P21 | /5AmMC6/GTT ATT GCC TCC GGA TGC GG | |
| P22 | /5AmMC6/GCC CAT CAT CAA GAT TCT TG | |
| P23 | /5AmMC6/AAT GTT CCC ACA AGG GAC AT | |
| P24 | /5AmMC6/TGA ACT TCA TCA CCC GCA TG | |
| P25 | /5AmMC6/ACT CGT ACA ACA GGG CTG AC | |
| P26 | /5AmMC6/CAG TGA TGC ACT GCA TCA TG | |
| P27 | /5AmMC6/ACG TTC GAC TCT TCA CTG AC | |
| P28 | /5AmMC6/TTT TTT GAG GAC TTT GAG AG | |
| P29 | /5AmMC6/CAA AGC ACC AGA TCT TGC GG | |
| P30 | /5AmMC6/TGA TGT CGG TGA GGG TGT TG | |
| P31 | /5AmMC6/CTG TCT CTT GCA GTG AGT CT | |
| P32 | /5AmMC6/GTC AGA ACA GTC GCA TAG AG | |
| M1 | /5AmMC6/GCT GTG AGA AGA GGG AGA TG | EEF2 mouse brain |
| M2 | /5AmMC6/ATG AGG TTG ATG AGG AAG CC | |
| M3 | /5AmMC6/CTG TCT CAG GGT GAA GG | |
| M4 | /5AmMC6/AAC TTT GCC ACA TAC ATC TC | |
| M5 | /5AmMC6/CAA AGC ACC AGA TCT TTG CG | |