





Supplementary information, Figure S1 (A) The reconstruction results and running time analysis. (a) A 200-frame, superimposed, diffraction-limited image (38×50 pixels, 1 pixel = 100 nm). (b-d) Reconstruction results using 3B (b), Quick-3B (c) and SIMBA (d). (e) Comparison of the running time with the above methods. The running times were 19.02 h, 1.13 h and 11 min, respectively. The speedup ratios of Quick-3B and SIMBA were 16.8 and 103.7, respectively. The scale bars are 200 nm. (B) Correlative analysis of the SIMBA and the PALM imaging of the whole cell actin

networks. (a-c) A whole cell imaging showing PALM analysis (a), SIMBA analysis (b) and overlay of a and b (c). (d, e) The magnified cyan box in a and b respectively. (f) The magnified yellow box in b. (g, h) The profile of cyan line in d, e. (i) A 49-nm resolution was achieved with the yellow line in f. The scale bars are 2 μm (a-c), 500 nm (d, e) and 200 nm (f). (C) SIMBA analysis of the actin network in a living U2OS cell. (a) Diffraction-limited image of mEos3.2-lifeact created by summing the first 100 TIRF frames. (b) The reconstructed SIMBA SR image from the same 100 frames. (c) Dynamics and reformation of actin structures at different time points. The scale bars are 5 μm (a, b), 1 μm (c). (D) Comparison between live-cell SIMBA and PALM of clathrin coated pits in Hela cells. (a) A 100-frame, superimposed, diffraction-limited image of clathrin coated pits. (b) PALM image reconstructed from 100 frames (acquisition time 2 s). (c) PALM image reconstructed from 5,000 frames (acquisition time 100 s). (d) SIMBA result reconstructed from 100 frames (acquisition time 2 s). The scale bar is 1 μm . (E) SIMBA analysis of the Mito and ER structures in COS7 cells. (a) Diffraction-limited image of mEos3.2-Mito created by summing the first 200 TIRF images. (b) The reconstructed SIMBA SR image from the same 200 frames. (c) Diffraction-limited image of ER-Tracker Red labeled ER created by summing the first 200 TIRF images. (d) The reconstructed SIMBA SR image from the same 200 frames. The scale bars are 2 μm .