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Title: Microfluidics-based automatic immunofluorescence staining for single-molecule localization microscopy

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Supplementary Material

Microfluidics-based automatic immunofluorescence staining for single-molecule localization microscopy

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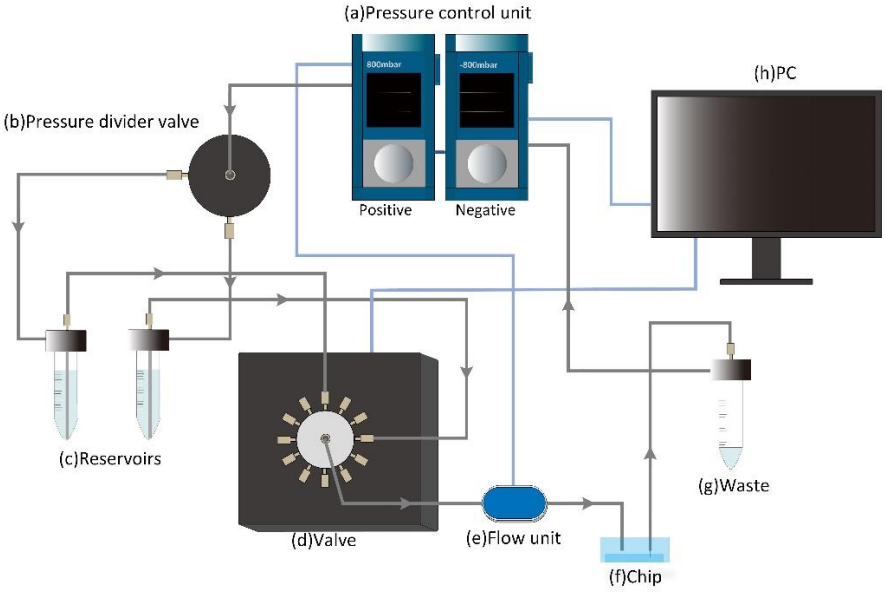

Fig. S1 Schematic illustration of the SRIF-fluidics system. (a)Pressure control unit (Flow EZ, Fluigent): supply positive pressure and negative pressure separately. (b)Pressure divider valve: divide pressure to relative reservoirs. (c)Reservoirs: store different reagents. (d)Valve (MUX DISTRIB, Elveflow): switch to supply correct reagent in right time. (e)Flow unit: monitor real-time flow rate (f)Chip: the chamber for Immunofluorescence. (e)Waste: store used reagents. (h)PC: control the whole experimental procedure.

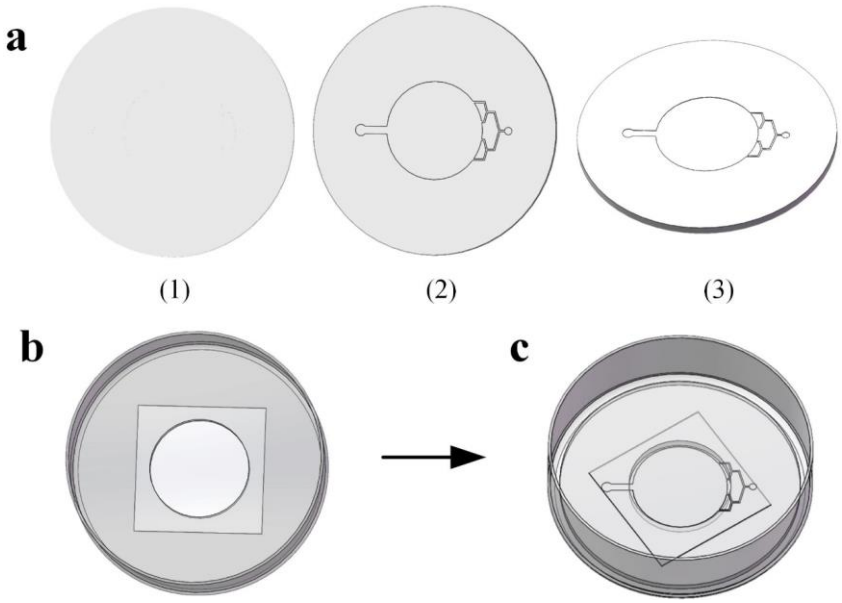

Fig. S2 Schematic illustration of microchip structure. (a)The microchip which show it's front side view (1), back side view (2) and side view (3). (b) The cell culture dish. (c)The chip section consisting of the chip and the cell culture dish.

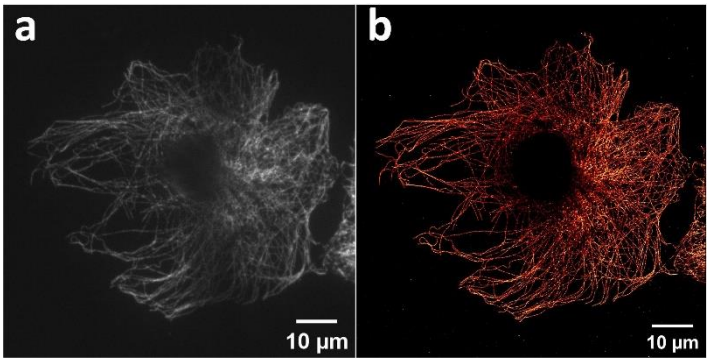

Fig. S3 Results of one incubation protocol with 800-fold antibody dilution. (a) Widefield fluorescence image of microtubule of cos-7 cells. (b) Super-resolution image of the microtubule of cos-7 cells shown in (a).

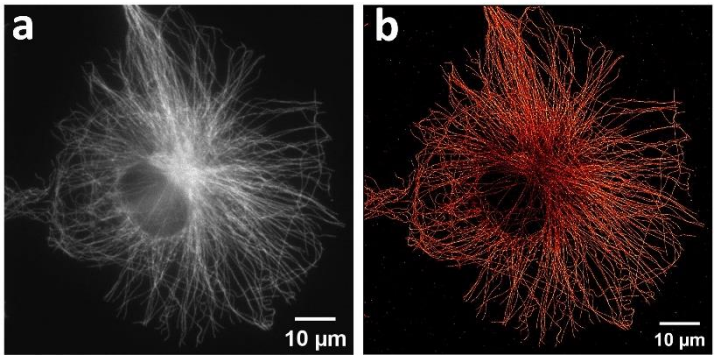

Fig. S4 Results of SG protocol with 800-fold antibody dilution. (a) Widefield fluorescence image of microtubule of cos-7 cells. (b) Super-resolution image of the microtubule of COS-7 cells shown in (a).

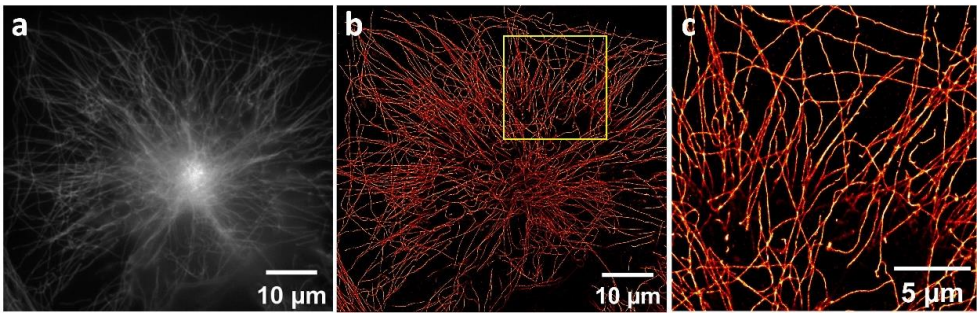

Fig. S5 The manual protocol results after replacing the blocking solution. (a) Widefield fluorescence image of microtubule of COS-7 cells. (b) Super-resolution image of microtubule of COS-7 cells from (a). (c) Enlarged view of the yellow boxed area in (b).

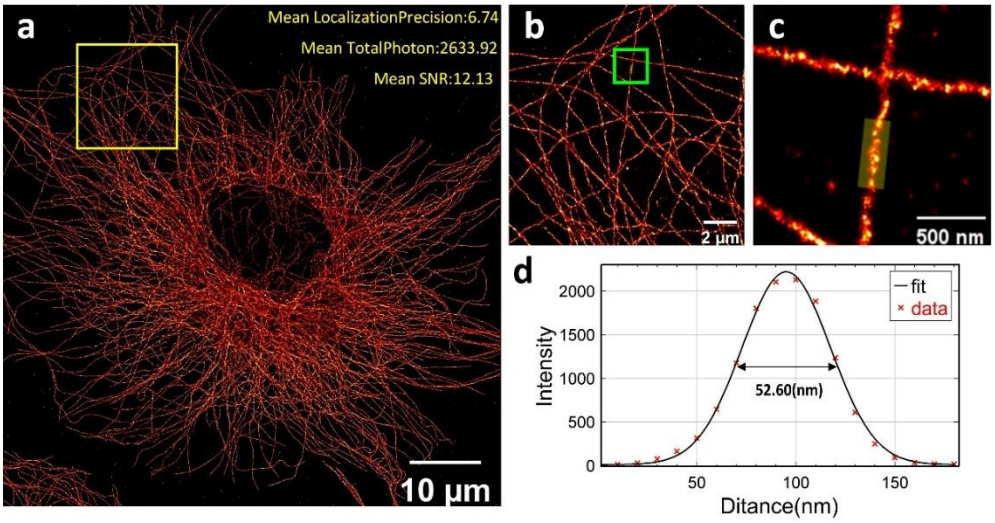

Fig. S6 The IF staining results under a half-time SG protocol. (a) Super-resolution image of single-cell

microtubules. (b) Enlarged view of the yellow boxed area in (a). (c) Enlarged view of the yellow transparent region highlighting the microtubule used for FWHM calculation. (d) The fitted distribution and FWHM value of the microtubule selected in (c).

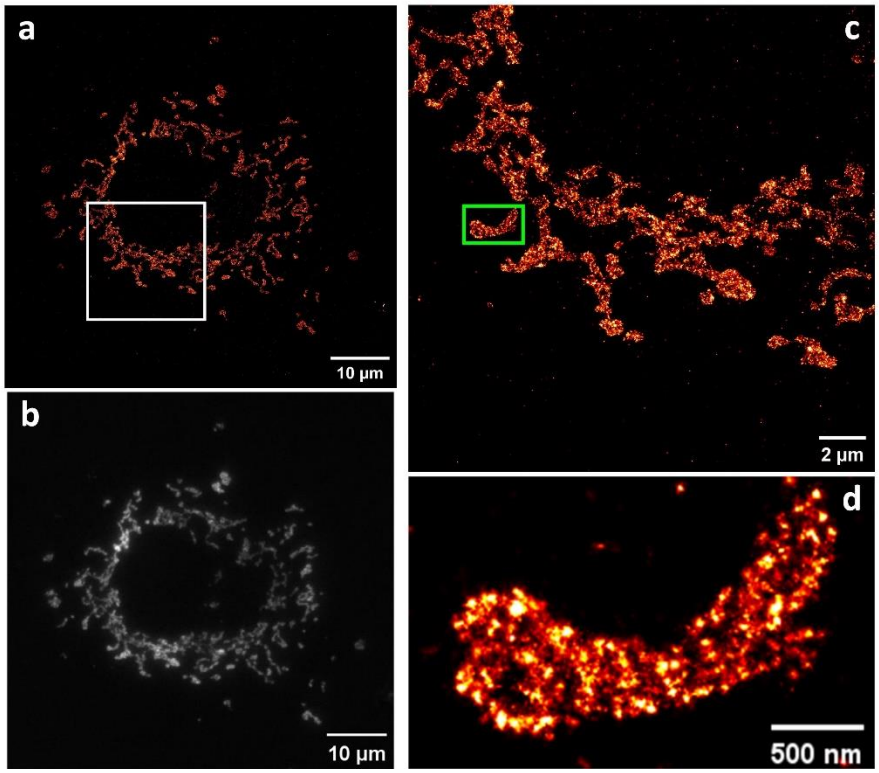

Fig. S7 Results of SRIF-fluidics -based CF protocol of mitochondria of COS-7 cells. (a) Super-resolution image of mitochondria. (b) Widefield fluorescence image of mitochondria. (c) Enlarged view of the white boxed area in (b). (d) Enlarged view of the green boxed area in (c).

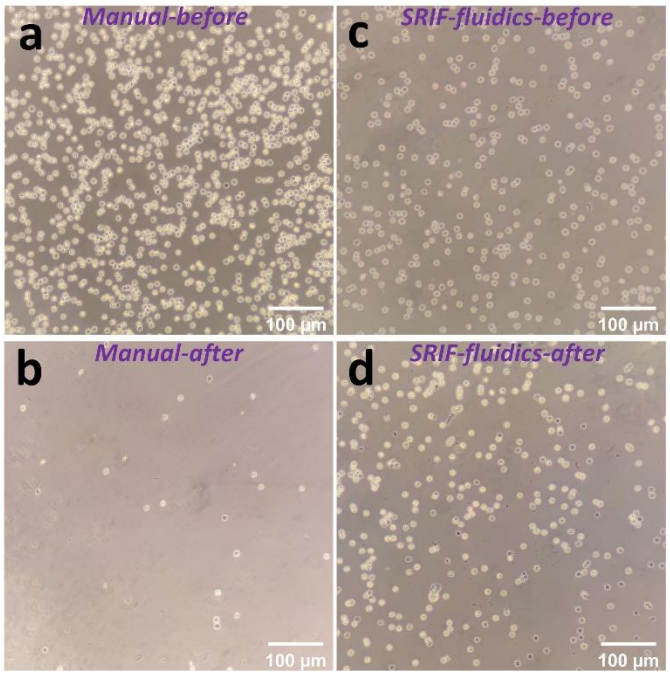

Fig. S8 Comparison of manual and SRIF-fluidics-based CF protocols for erythrocytes. (a) Seeded erythrocytes before IF staining using manual protocol. (b) Erythrocytes remaining after manual IF staining. (c) Seeded erythrocytes before IF staining using SRIF-fluidics-based CF protocol. (d) Erythrocytes remaining after SRIF-fluidics IF staining.

Supplemental Experimental Methods

Materials and Reagents.

Glass-bottom culture dish (MetTak, P35G-1.5-14-C), PFA tube (IDEX,0106-0514-00), SYLGARD 184 Slicone Elastomer Kit (Dow, 1673921), Fetal Bovine Serum (Gibco, 10099141), DMEM (Gibco, C11995500BT), Trypsin-EDTA (Gibco, 2520056), PBS (Amerko, R020859), Penicilin-Streptomycin (Gibco, 15140122), Anti-α-Tubulin antibody (Sigma-Aldrich, T5168), Anti-Tomm20 antibody (Sigma-Aldrich, HPA011562), Anti-EPB41 antibody (Sigma-Aldrich, HPA028414), Alexa Fluor 647-conjugated secondary antibody (Invitrogen, A-21235 and A-21245), DyLight 633-conjugated secondary antibody (Invitrogen, 35512)，CF680 -conjugated secondary antibody (Biotium, 20820), 8% Paraformaldehyde aqueous solution (Electron Microscopy Sciences, 157-8), 8% Glutaraldehyde EM Grade (Electron Microscopy Sciences, 16020), Triton X-100 (Sigma-Aldrich, X100-100ML), Bovine Serum Albumin (Jackson ImmunoResearch, 001-000-161), Tris (Sigma-Aldrich, T1503), Glucose (Sigma-Aldric, G8270), NaCl (Sinoreagent, 10019318), Glucose oxidase (Sigma-Aldrich, G2133), Catalase (Sigma-Aldrich, C1345), Cysteamine (Sigma-Aldrich, 30070), Poly-Lys (Sigma-Aldrich, P1274).

Cell culture

COS-7 cells (ATCC, CRL-1651, Bohui Biological Technology [Guangzhou] Co., Ltd) were

cultured in a common condition of 5% CO2 at 37°C with complete DMEM. For cell seeding, after the COS-7 cells grow to 80% ~ 90% fluency, digested to seed in a glass-bottom culture dish. Until cells have grown to a suitable density (firmly adhered, cell edges are clear and non-overlapping), then could be used for immunostaining.

Erythrocytes

The culture dish should be coated with Poly-L-lysine hydrobromide (Poly-Lys). Added 400 μL of 0.1 mg/mL Poly-Lys solution to the dish, stood for 3 hours, sucked up the upper solution, and put the dish into a 37°C oven to dry overnight. Take 4 μL of finger blood in 6 mL PBS-GB (10 mM glucose, 5 mg/mL PBS solution of BSA) solution. Then centrifuged at 1200 rpm for 5 minutes. Discarded the supernatant and diluted it to appropriate concentrations. Then seeded erythrocytes in Poly-Lys treated Petri dish. Incubated for 30 minutes at room temperature and rinsed once with PBS-GB solution to remove unattached erythrocytes, then could be used for immunostaining.

dSTORM super-resolution imaging

The SMLM system was home-built. It was based on an Olympus IX83 microscope equipped with Olympus 60X/NA1.50 objective lens (UPL APO 60X NA 1.5, Olympus) and sCMOS camera (Dhyana 400BSI V2, Tucsen). The final pixel size of the system was 108 nm. For illumination, the 405 nm laser (LWVL405-200mW, Laserwave) was coupled with the 640 nm laser (LWRL640-3W, LaserWave) into self-designed multi-mode optical fibers. The laser should be collimated and focused on the back focal plane of the objective lens. The angle of incidence of the light could be adjusted by moving the electric translation stage (M1T-Z8, Thorlabs) to switch the illumination mode (Epi illumination or TIRF illumination). The system could be used for fluorescence imaging and SMLM imaging.

SMLM imaging was performed on the above-mentioned system. Labeled samples were immersed in imaging buffer (50 mM Tris, pH 8.5, 10 mM NaCl, 10% glucose, 100 mM MEA, 500 μg/mL glucose oxidase, 40 μg/mL catalase). And lens oil was added dropwise to the objective lens. Then placed the dish on the stage and pressed it tightly with the tablet clamp. When imaging, the 640 nm laser was used for AF647. A low-power laser was used for fluorescence observation. After a suitable imaging area was found in Epi illumination, then switch to TIRF illumination. And the laser power was increased to 5.1 kW cm^{-2}. When a proper molecular density was achieved, images should be collected. Typically 10000 frames were recorded per acquisition with an exposure time of 10 ms.

Muti-color super-resolution imaging

Muti-color imaging is also based on our SMLM system. When imaging, the camera was replaced with colorimetry camera (Retina 200DSC, Tucsen Photonics). Muti-color samples were immersed in imaging buffer (50 mM Tris, pH 7.5, 10 mM NaCl, 10% glucose, 100 mM MEA, 500 μg/mL glucose oxidase, 40 μg/mL catalase). And lens oil was added dropwise to the objective lens. Then placed the dish on the stage and pressed it tightly with the tablet clamp. When imaging, the 640 nm laser was used for AF647. A low-power laser was used for fluorescence observation. After a suitable imaging area was found in Epi illumination, then switch to TIRF illumination. The laser power was increased to 5.1 kW cm^{-2}. When a proper molecular density was achieved, images should be collected. To activate molecules, the 405 nm laser power was turned up appropriately after the density decreases. Typically, 40000 frames were recorded per acquisition, with an exposure time of 30 ms.

Data processing

Data processing was carried out using the ImageJ plugin QC-STORM developed by our group. The raw data were opened by the QC-STORM plugin. The parameters such as camera parameters, pixel size, sub-area size, etc. were set based on the imaging system. Then the single molecule positioning and rendering were automatically performed. The plugin can also be used to perform drift correction based on cross-correlation on the positioning data.

Data analysis

To calculate FWHM resolution for microtubule super-resolution image, an ImageJ macro "FWHM_Line.ijm" was used. For the first use, the macro needs to be installed into ImageJ and a 500 nm wide scale is scribed along the microtubule cross-section to obtain the fitted distribution of the cross-section and the corresponding FWHM value. The macro can be downloaded from this website: http://imagej.1557.x6.nabble.com/attachment/5004792/0/FWHM_Line.ijm.