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Supplementary information

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Supplementary Information

Constructing a cost-efficient, high-throughput and high-quality single molecule localization microscope for super resolution imaging

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Supplementary Figure 1

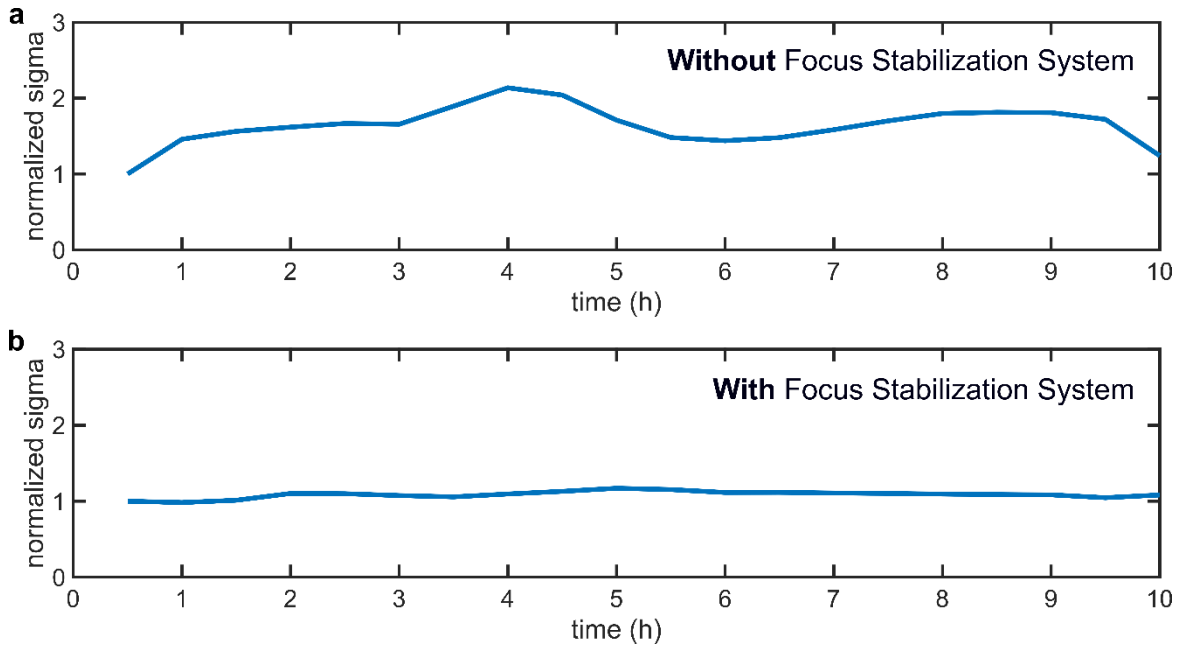

Figure s1 Characterization of the focus stabilization system. Fluorescent beads imaged over 10 hours without **(a)** and with **(b)** the focus stabilization system (i.e. autofocus system). The standard deviation (sigma) in the point spread function was measured using the ImageJ plugin ThunderSTORM and normalized to the first measurement. Standard deviation in the sigma measurements for **(a)** 249.9 nm and **(b)** 7.6 nm. For direct 3D measurements of the axial position, users are referred to[1].

Supplementary Figure 2

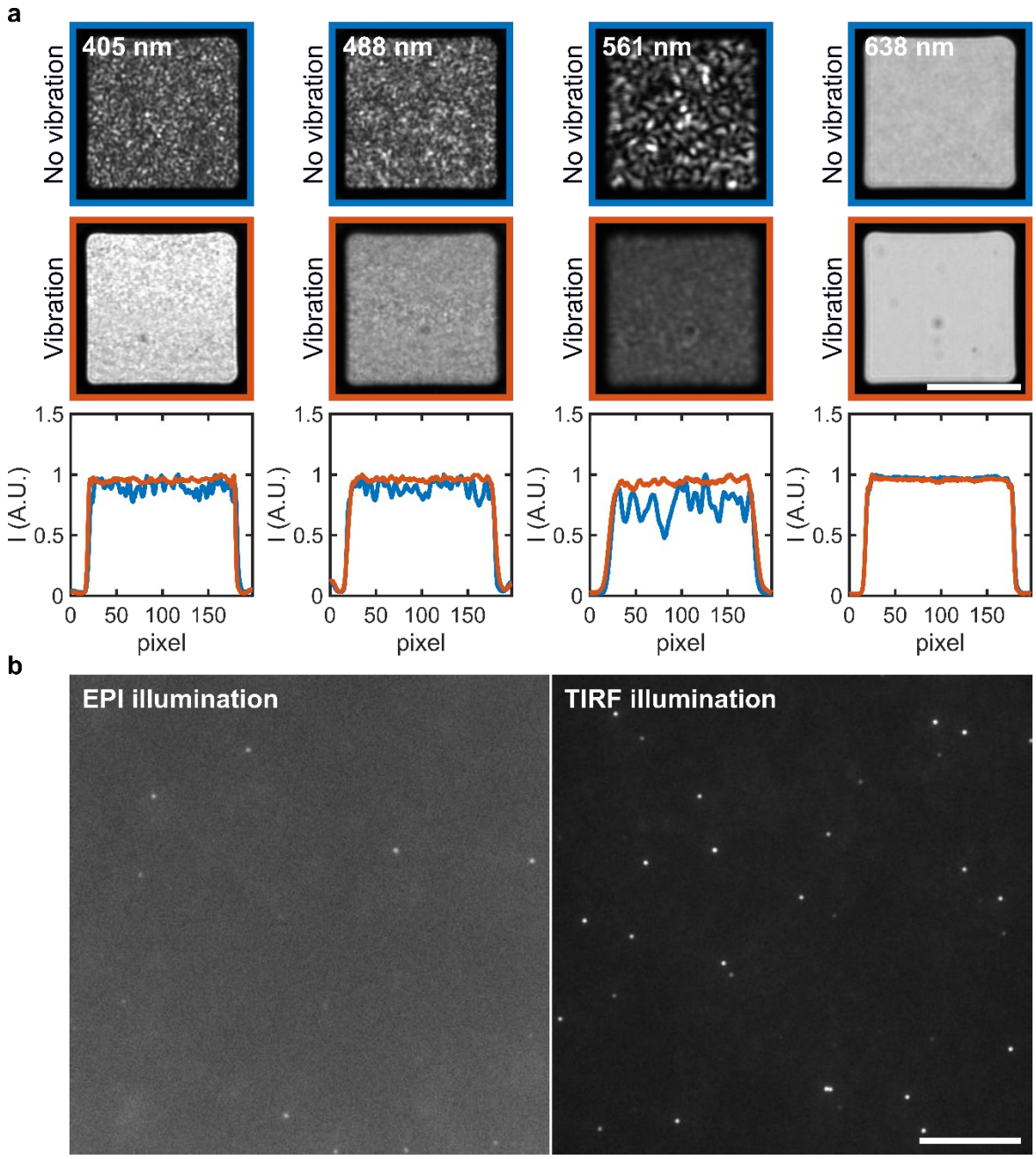

Figure s2 Characterization of the fibre-based illumination module. (a) Flat-field, square profile of the excitation sources at the output of the small, square-core multi-mode fibre (item 1) without (in blue) and with (in orange) agitation using a vibration motor (item 61) as measured on a CMOS camera (Thorlabs, cat. no. DCC1545M-GL). Scale bar, 500 µm. Intensity of the plotted profiles is normalized between 0 and 1 for clarity. **(b)** DNA-PAINT sample of 20 nm nanorulers imaged using NanoPro with an excitation of 561 nm matching the spectrum of the Cy3b conjugated DNA imager strands using EPI and TIRF illuminations. Full reconstructed image and information on localization precision can be found in **figure 3**. Scale bar, 10 µm.

Supplementary note 1

Imaging conditions

Different number of frames and exposure times were set for the different imaging experiments reported here: 20000 frames at 30 ms for dSTORM, 10000 frames at 150 ms for DNA-PAINT imaging of the nanorulers, 65535 frames at 150 ms for Exchange-PAINT imaging of the HeLa cells, and 4000 frames at 150 ms for AD-PAINT imaging of recombinant aggregates.

Preparation of oligonucleotides

All lyophilised oligonucleotides (Table s1) were purchased from ATDBio (Southampton, UK). IS1-cy3B and IS2-cy3B were synthesised on a 1.0 μmol scale, and purified by double HPLC. All other oligonucleotides were synthesised on the same scale but purified by HPLC. They were further dissolved in 18.2 MΩ cm water, filtered by 0.02 μm filter (VWR, Cat. No. 516-1501). Their concentrations (50-1000 μM) were confirmed by A_{260}, and they were aliquoted and stored at -20 °C.

Code	Sequence (5' – 3')
DBCO-DS1	DBCO TEG*-TTATACATCTATTTTTTTTTTTTTTTTTTT
DBCO-DS2	DBCO TEG*-TTATCTACATATTTTTTTTTTTTTTTTTTT
IS1-cy3B	CTAGATGTAT-cy3B
IS2-cy3B	TATGTAGATC-cy3B
Aptamer-DS1	GCCTGTGGTGTTGGGGCGGGTGCGTTATACATCTA

* DBCO TEG – dibenzocyclooctyne tetraethylene glycol

Table s1 List of oligonucleotides.

Antibody labelling for Exchange-PAINT imaging of the HeLa cells

DBCO-DS1 and DBCO-DS2 were selectively conjugated on the carbohydrates of the F_C region on a goat anti-mouse secondary antibody (Invitrogen, Cat. No. 31160, Lot No. UD2762551), and mouse anti-rabbit secondary antibody (Invitrogen, Cat. No. 31213, Lot No. UE2766762) respectively. To achieve this, the antibodies were first functionalised with SiteClick™ Antibody Azido Modification Kits (Invitrogen, Cat. No. S20026) according to the manufacturer's instructions. In brief, the antibodies (250 μg of each antibody) were concentrated and buffer-exchanged in the provided antibody preparation buffer to yield 3.1 mg/mL (goat anti-mouse IgG) and 3.4 mg/mL (mouse anti-rabbit IgG). They were then incubated overnight with β-galactosidase at 37°C, followed by overnight coupling to UDP-GalNAz using β-1,4-galactosyltransferase (GalT) on the following day at 30°C. The reaction mixtures were then purified by Amicon spin filters (50 kDa MWCO). The concentrations of the azido-modified antibodies were determined by A_{280} (1.7 mg/mL for goat anti-mouse IgG and 1.9 mg/mL for mouse anti-rabbit IgG). DBCO-DS1 and DBCO-DS2, in ten molar equivalents, were introduced in azido-modified goat anti-mouse IgG and azido-modified mouse anti-rabbit IgG respectively for copper-free strain-promoted click reaction in 1×PBS. The reaction mixtures were incubated overnight at 37°C, after which the excess oligonucleotides were removed using Amicon spin filters (100 kDa MWCO) and the concentrations of antibodies and degrees of labelling (2.1 docking strands per goat anti-mouse IgG and 3.2 docking strands per mouse anti-rabbit IgG) were determined by A_{260}/A_{280}. The purities and labelling efficiencies were further confirmed with SDS-PAGE under reducing conditions (**figure s3**).

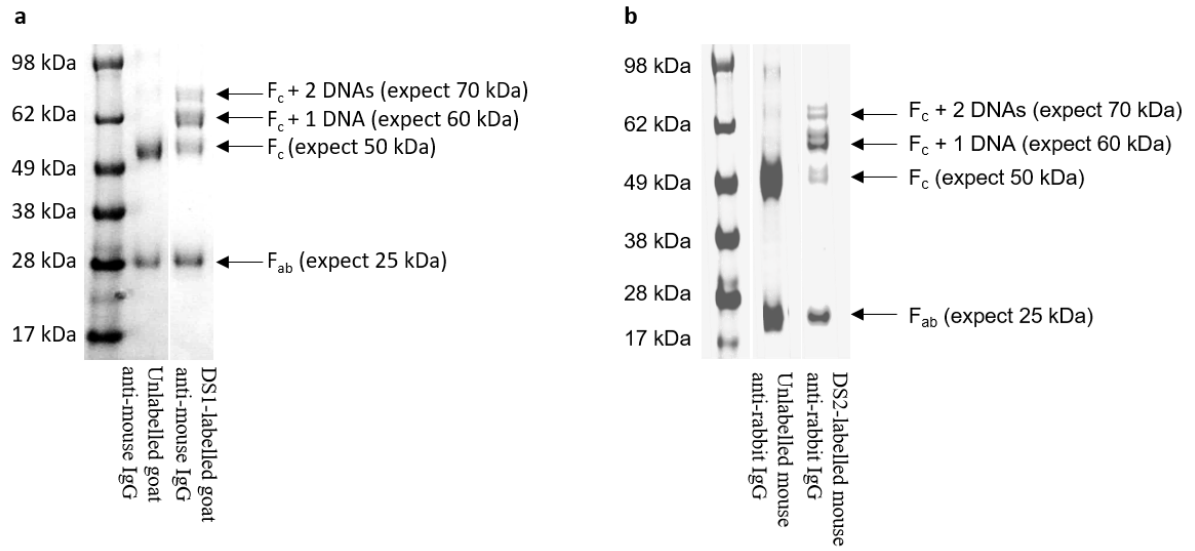

Figure s3 Characterisation of antibody labelling. (a) DS1-labelled goat anti-mouse IgG and **(b)** DS2-labelled mouse anti-rabbit IgG by SDS-PAGE under reducing conditions.

HeLa cells sample preparation

HeLa cells were maintained in complete DMEM supplemented with 10% FCS, 100 U/mL penicillin, 100 µg/mL streptomycin and incubated at 37°C and 5% CO_2. The cells were plated in µ-slide eight-well glass-bottom chambers (ibidi, Cat. No. 80827) and allowed to adhere overnight in complete medium. On the next day, each well was washed three times with PBS (Merck, Cat. No. D8537), fixed in a mixture of 3% paraformaldehyde (Thermo Scientific, Cat. No. 28908) and 0.1% glutaraldehyde in PBS for 10 min, washed three times with PBS, incubated with ~1 mg/mL $NaBH_4$ for 7 min, washed three times with PBS, and permeabilized using 0.25% (v/v) Triton X-100 in PBS for 10 min. The cells were then blocked for 30 min using blocking buffer, i.e. 3% BSA (Thermo Scientific, Cat. No. B14) in PBS for dSTROM and 3% BSA and 1 mg/mL salmon sperm (Invitrogen, Cat. No. AM9680) in PBS for Exchange-PAINT. The microtubules were immunostained overnight using a combination of two mouse anti-tubulin antibodies (Merck, Cat. No. T5168 and T6199, Lot No. 0000105-483 and 0000101-493) diluted 1:300 in blocking buffer. Meanwhile, Clathrin was immunostained overnight using a rabbit anti-Clathrin antibody (Abcam, Cat. No. ab2167, Lot No. GR3407177-1) diluted 1:200 in blocking buffer. On the next day, the cells were rinsed three times with PBS, and subsequently incubated with an Alexa Fluor 647-labeled goat anti-mouse IgG (H+L) (Invitrogen, Cat. No. A-21236, Lot No. 2300995) diluted 1:500 in the blocking buffer for dSTORM to stain the microtubules, or Alexa Fluor 647-labeled goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody (Invitrogen, Cat. No. A-21245, Lot No. 2299231) diluted 1:500 in the blocking buffer for dSTORM to stain Clathrin. Alternatively, the cells were labelled in a first step with a DS1-labelled goat anti-mouse antibody followed by a DS2-labelled mouse anti-rabbit at a concentration of 2 µg/mL for 30 min at room temperature for Exchange-PAINT imaging experiments. TetraSpeck™ microspheres, 0.1 µm, fluorescent blue/green/orange/dark red (ThermoFisher, T7279) were finally added at a dilution of 1:2000 as fiducial markers for drift correction. Finally, samples were rinsed three times with PBS before adding the imaging solution.

Imaging solution for dSTORM

The imaging buffer for dSTORM was prepared as reported in[2]. Briefly, the stock solutions were prepared as follows:

- 0.1 M of Tris supplemented with 20 mM NaCl, pH 8, filtered by 0.02 µm filter (VWR, Cat. No. 516-1501), stored at 4°C (2x imaging solution of dSTORM)
- 25% glucose, stored at 4°C (2.5x imaging solution of dSTORM)
- 1 M of cysteamine (Merck, Cat. No. 30070) in 0.36 M HCl, stored at 4°C for no more than one week (20x imaging solution of dSTORM)
- GOD buffer (24 mM PIPES, 4 mM MgCl2, 2 mM EGTA) at pH 6.8 and filtered by 0.02 µm filter, stored at 4°C
- 20 mg/mL glucose oxidase from Aspergillus Niger (Merck, Cat. No. G2133) in GOD buffer, centrifuge filtered with 0.22 µm filter (Merck, Cat. No. UFC30GV0S), flash frozen in liquid nitrogen, stored in -80°C (40x imaging solution of dSTORM)

- 5 mg/mL catalase (Merck, Cat. No. C40) in GOD buffer, centrifuge filtered with 0.22 µm filter (Merck, Cat. No. UFC30GV0S), flash-frozen in liquid nitrogen, stored in -80°C (125x imaging solution of dSTORM)

The working solution for dSTORM imaging contains 40 µg/mL catalase, 0.5 mg/mL glucose oxidase, 10% glucose and 50 mM cysteamine in 50 mM of Tris supplemented with 10 mM NaCl at pH 8. This solution was prepared freshly and used immediately for imaging.

Imaging solutions for Exchange-PAINT for HeLa cells

Imaging strands, IS1-cy3B and IS2-cy3B, were diluted to 4 nM separately in PBS supplemented with 0.5 M NaCl. Cells were carefully rinsed with PBS supplemented with 0.5 M NaCl for three times at 10-minutes' intervals. Replacement of imaging solution was visually confirmed after each rinsing step.

Preparation of recombinant α-Synuclein aggregates

Wild type α-Synuclein was expressed, purified in *E. coli* and stored at -80°C as reported[3]. The pre-aggregation seeds in the solution were removed by centrifugation at 91000g at 4°C for 1 h by an ultracentrifuge (Optima TLX Ultracentrifuge, Beckman). The monomer concentration of the supernatant was then determined by A_{280} (ε_{280} = 5960 M^{-1} cm^{-1}). The supernatant was then diluted to 70 µM in 1xPBS supplemented with 0.01% NaN_3 (Merck, Cat. No. 71290) and incubated at 37°C in a shaking incubator (ES-20, Grant-Bio) at 200 rpm for 61 days.

Annealing of the aptamer-DS1

The stock solution of aptamer-DS1 (1000 µM) was diluted to 100 µM with lithium cacodylate buffer, which contains 1 M of KCl (Breckland, EC No. 231-211-8, Stock Code: 0001276), 0.1 M of cacodylic acid (Merck, Cat. No. C0125) and 0.1 M of lithium hydroxide (Merck, Cat. No. 909025) at pH 7.3. The aptamer-DS1 solution at 100 µM was heated to 95 °C for 10 min, and then allowed to cool down slowly overnight to room temperature.

AD-PAINT

AD-PAINT was performed with modified protocol[4]. Briefly, a glass coverslip (VWR, Cat. No. MENZBC026076AC40) was cleaned with argon plasma (Harrick Plasma, Cat. No. PDC-002) for 1 h. A 50-well PDMS gasket (Merck, Cat. No. GBL103250) was cut into two-halves, and attached to the cleaned coverslip. The slide was then treated with 0.02 µm-filtered (VWR, Cat. No. 516-1501) 1% Tween (Fisher Scientific, Cat. No. BP337-100, Lot No. 179118) and PBS (ThermoFisher, Cat. No. 10010023) for 1 h. This was rinsed three times with 1x PBS (0.02 µm-filtered). Two-months alpha-synuclein diluted with PBS was added at a series of concentrations onto the slide and incubated for 15 min. After incubation at room temperature, the sample was removed, and the wells were filled with the imaging solution, i.e. 1 nM of IS1-cy3B and 100 nM of Aptamer-DS1 in PBS. To avoid evaporation over prolonged imaging, another clean coverslip was layered on top of the PDMS gasket.

Image reconstruction

All images were reconstructed using ThunderSTORM[5] as described in steps 276 to 279 of the protocol. Drift in images of samples containing fiducial markers was corrected using the positions of the fiducial markers instead of cross-correlation. Were multi-target images had to be aligned, this was roughly done through visual inspection.

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