nature nanotechnology

SUPPLEMENTARY INFORMATION

DOI: 10.1038/NNANO.2011.226

Nanowire-based Single Cell Endoscopy

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I. Supplementary Methods

SnO_2 Nanowire Synthesis

SnO_2 nanowire waveguides were synthesized using a chemical vapor transport method previously described[31]. SnO powder was heated in a quartz tube reactor for 90 min at 1,100 °C under 350 Torr of flowing argon (50 standard cubic cm per min). Milligram quantities of nanowires were collected on an alumina boat near the center of the reactor and deposited onto clean substrates by dry transfer. The nanowires with diameter of 100~250 nm and length of 50~200 μ m were chosen for the nanowire endoscope fabrication.

Optical Fibre Etching.

Tapered optical fibre tips were fabricated by etching the fibre tips in the stack solution of hydrofluoric acid (HF, 49%) and 2,2,4-trimethylpentane (Sigma) for approximately 70 mins as previously described[31]. The fibre tips were then rinsed with water and ethanol repeatedly. The cone angles of tapered tips were adjusted by tuning the etching time.

QD Conjugation onto SnO_2 Nanowire.

SnO_2 nanowire was first aminated by soaking the nanowires in 2% 3-Aminopropyltriethxysilane (APTES, Sigma-Aldrich) ethanol solution overnight and then rinsing with ethanol several times. The aminated nanowire was then fabricated into aminated endoscope following the procedure described above. The aminated endoscope was then immersed in a 0.5 mg/mL phosphate buffered saline (PBS) solution of photocleavable crosslinker NHS-PC-LC-Biotin (Ambergen) for 1 hr and rinsed with PBS several times. The tip of the biotin-modified nanowire endoscope was carefully dipped into a droplet (2 μL) of PBS solution of streptavidin-modified QDs (Qdot® 655 streptavidin conjugate, Invitrogen) on a PDMS substrate. In order to confine QDs attachment to the tip of the nanowire, the position of the nanowire endoscope was controlled by the micromanipulator and the insertion process was monitored under

microscope. The tip was immersed in the droplet for ~30 mins before it was rinsed with PBS to remove noncovalently absorbed QDs.

II. Optimization of the Nanowire-Fibre Coupling.

The coupling loss, which originates mainly from scattering and mode mismatch, can be suppressed by adjusting the tapering angle, smoothness and the intensity profile of the tapered tip of the optical fibre. To minimize the scattering loss at the nanowire-fibre junction and optimize the coupling efficiency, a number of issues have to be addressed, including the fabrication method of the tapered optical fibre, its tapering angle and surface smoothness. By optimizing these parameters, light escaping from the junction was kept at minimal level.

Two different methods are commonly used to make sharp fibre tips: mechanically pulling the fibre while locally heating it with focused IR laser beam, and chemically etching the fibre with concentrated HF solution. Although the pulled fibre (Figure S2a) appears to have smooth surface that would minimize scattering loss from surface defects, the fibre core got stretched and thinned, which causes the significant leakage of the waveguided modes throughout the tapered region (up to 50 μm from the tip). Thus, little light propagated to the junction and was coupled into the nanowire. The etched fibres (Figure S2b) have relatively rough surface due to etch pits which would give rise to surface scattering and reduce effective area of overlapping between the nanowire and the fibre. Nevertheless, the chemical etching does not deform the fibre core, which only starts to be exposed and tapered ~3 μm from the tip. In this case, the light field is concentrated at the fibre tip where it is in physical contact with the nanowire. Thus, the guided optical modes are effectively coupled with those in the nanowire.

We next examined the influence of the tapering angle on the coupling efficiency. Figure S2b and c presented two nanowire endoscopes fabricated with etched fibres with the same level of roughness, but different tapering angle of 12º and 4º, respectively. The fibre

with small tapering angle (Figure S2c) has much lower coupling loss at the junction than the one with larger tapering angle. This difference originates from the effective coupling length within which the optical modes in the fibre and the nanowire are overlapped. The coupling length for 4º tapered fibres is almost 4 times as long as that for 12º tapered fibres, resulting in more efficient coupling.

For fibres with small coupling angles, surface roughness from etch pits on the optical fibre is the major source of scattering loss at the nanowire-fibre junction. The smoother the fibre surface is, but less the scattering loss would be (Figure S2c and d). Repeated rinsing with DI water and ethanol after the etching is an effective way to reduce such etching pits on the fibre surface.

We used etched optical fibres (both S405-HP and 630HP) that have a relatively smooth surface and 3-5º coupling angle (similar to Figure S2d) for fabricating all the nanowire probes used in our experiments to ensure good coupling between the nanowire and optical fibre, as demonstrated by the intensity profile in Figure 1f-g. On the contrary, a poorly coupled endoscope would have light leaking out all over the tapered optical fibre tip, resulting in a diffused and divergent output beam, which is the same as the emission profile of the tapered optical fibre tip itself (Figure S4).

Video S1 shows the nanowire endoscope holds up well mechanically and optically even when it was bent and buckled.

III. Cell Viability Test.

HeLa cells were seeded into 35 mm petri-dish with grids and cultured overnight (22,000 cells per dish) at 37^{o}C in the presence of 10% FBS. Nanowire endoscopes or tapered optical fibres used in the tests were mounted on a 3-axis micromanipulator to ensure precision control over the insertion position.

A. Cell Survival Rate.

For all the cell viability tests, single cells were randomly chosen from the grid petri-dish. The positions of each cell tested were documented and dark field images were taken on the cell and its surrounding area for references.

To compare the insertion induced cell fatality rate between conical optical fibre and the nanowire endoscope, a chemically etched tapered fibre tip with dimensions similar to the one shown in Figure S1, was slowly inserted into the cytoplasm of a randomly chosen cell from the grid Petri dish, remained inserted for 1 min and then retracted slowly (Table S1, Row 1). The cell viability tests for nanowire endoscope insertion were performed in the exact same way but for 3 different insertion durations: 1 min (Table S1, Row 2), 3 mins (Table S1, Row 3), and 5 mins (Table S1, Row 4).

For cell viability tests on payload delivery, an unmodified nanowire endoscope was slowly inserted into the cytoplasm, and the insertion region was immediately irradiated with the focused HeCd laser (325 nm, 0.4 mW/cm^2) for 1 min (Table S2, Row 2). The nanowire endoscope was slowly retracted from the cell right after the irradiation. Cell viability on the focused laser irradiation without nanowire insertion was also tested, with the same power density and beam size (Table S2, Row 1).

For cell viability tests on local illumination, an unmodified nanowire endoscope was slowly inserted into the cytoplasm (Table S2, Row 3) or placed about ~ 2 μm away from the cell membrane (Table S2, Row 4), and the tip illumination from the nanowire endoscope was immediately turned on for the duration of 1 min. The 442 nm blue HeCd laser was coupled to the nanowire endoscope through the optical fibre providing the tip illumination. The input laser power to the optical fibre was 0.4 mW and an average output laser power of 10 μW was measured from the tip of the nanowire endoscope. The nanowire endoscope was immediately removed after the 1 min irradiation.

After the procedure, the petri dish was incubated for 10 hrs at 37^oC in the presence of 10% FBS and the cell viability was evaluated using the Calcein live cell assay

(Invitrogen). Phase contrast or dark field images, and fluorescent images were taken on the each cell tested for correlation.

B. Cell Apoptosis Test.

For all the apoptosis tests, single cells were randomly chosen from the grid petri-dish. The positions of each cell tested were documented and dark field images were taken on the cell and its surrounding area for relocation. During the test, a nanowire endoscope was slowly inserted into the cytoplasm of a randomly chosen cell, remained inserted for 1 min or 15 mins and then retracted slowly. After the procedure, the petri-dish was incubated for 1 or 12 hrs at 37^{o}C in the presence of 10% FBS and the cell apoptosis behavior was evaluated using "Annexin V: FITC Apoptosis Detection Kit I" (BD Pharmingen^{TM}). Bright field and fluorescent images were taken on the each cell tested for correlation. In a positive control experiment (data not shown), apoptosis was intentionally induced by high power UV treatment for 5 mins, and positive staining for both the nucleus and the cell membrane was observed.

For the insertion duration of 1 min, 6 out of 6 cells tested showed no sign of apoptosis after 1 or 12 hours incubation. For the insertion duration of 15 mins, 2 out of 2 cells tested showed no sign of apoptosis after 1 and 12 hours of incubation. Characteristic apoptosis test result is given in Figure S6. After nanowire insertion, the cell under investigation did not undergo significant morphology changes characteristic to apoptosis, such as membrane blebbing and cell shrinkage, nor did it develop positive membrane and nucleus fluorescence after treatment with apoptosis staining kit. The apoptosis staining kit used in the experiment contains FITC Annexin V, which is widely used as a marker for the early stages of apoptosis, and propidium iodide (PI), a cell impermeant nucleus stain that is only uptaken by cells that have lost membrane integrity. Plasma membrane asymmetry is an important feature in the early stage of apoptosis. At this stage, cell membrane is not porous to allow the uptake of PI, but Phopholipid phophatidylserine (PS) in the plasma membrne is translocated from the inner to the outer leaflet in apoptotic cells, exposing PS to the external cellular environment. Annexin V has a high affinity for PS and thus able to identify apoptosis at an early stage. Cells that are already dead,

undergoing necrosis, or at late stages of apoptosis can also be stained with Annexin V. So apoptotic cells stains positive with FITC Annexin V, but negative with vital dye PI, whereas viable cells stained negative with both and demised and necrotic cells stain positive with both.

C. Insertion Induced Intracellular Mechanical Stress.

Hela cells were stained with calcium indicator Fluo-4 AM (Invitrogen) prior to the experiment. A tapered optical fibre tip was inserted into the cytoplasm of a cell, remained for 1 min and then retracted. The exact same procedure was also adopted for nanowire probe insertion. Cellular fluorescence level was monitored during the insertion with 442 nm excitation. The mechanical stimuli introduced by tapered optical fibre tip insertion result in an instant, drastic and global increase in the intracellular Ca^{2+} level, as shown in Figure S7a-b and Video S2a. The increase is also long lasting, with a typical recovery time of more than 10 mins. Although with a similar tip dimensions to tapered optical fibre tip, the nanowire endoscope induced a more retarded, mild, and local intracellular Ca^{2+} response, accompanied by fast recovery, as demonstrated in Figure S7c-d and Video S2b, indicating much less mechanical stress imparted on the cells during nanowire endoscope insertion, which can be associated with its much higher post-insertion cell viability rate.

D. Membrane Integrity Test.

Membrane integrity during nanowire endoscope insertion and retraction was also tested and compared with tapered optical fibre tips. Cells were stained with Calcein AM (Invitrogen) prior to the experiment, during which its fluorescence were monitored in real time. After a tapered optical fibre tip was inserted into the cell, the calcein fluorescence experienced a dramatic drop in merely 20 seconds (Figure S8a-b), indicating the fast release of dye molecules into the cell culture medium resulted from leakage created on the cell membrane by fibre tip insertion. On the contrary, nanowire endoscope did not pose such threat on plasma membrane integrity. Cell membrane remained sealed during the entire process of nanowire endoscope insertion, 1 min interrogation and retraction (Figure S8a-d).

IV. Payload delivery control experiments with photo-inactive nanowire endoscopes

Control experiments show that 3 min-insertion of photo-active endoscope without UV irradiation and 1 min-insertion of photo-inactive endoscope (conjugated via non-cleavable linkers) with UV irradiation did not result in QD release, demonstrating that the QD delivery was specific to the photo-activation (Figure S10 and S11).

Control experiments were also performed with two types of photo-inactive nanowire endoscopes: QD-conjugated nanowire endoscope via a disulfide bond linker that can be cleaved in the reducing environment of the cytoplasm, and QD-conjugated nanowire endoscope with non-cleavable linker that cannot be cleaved chemically or optically.

To fabricate the disulfide linker QD-conjugated nanowire endoscope, an aminated nanowire endoscope was first immersed in 0.5 mg/mL PBS solution of sulfosuccinimidyl 6-(3'-[2-pyridyldithio]-propionamido) hexanoate crosslinker (Sulfo-LC-SPDP, Thermo Scientific) for 1 hr and rinsed with PBS. The amine group on the surface of QD (Qdot® 655 ITK™ amino (PEG), Invitrogen) was then converted to sulfhydryl group using 2-Iminothiolane•HCl (Traut's Reagent, Thermo Scientific) and dispersed in PBS. The tip of the Sulfo-LC-SPDP-modified nanowire endoscope was then dipped into a small droplet (2μL) of the sulfhydryl-modified QDs solution (0.1 μM) and immersed for 30 min. The concentration of QD solution was optimized to coat the nanowire endoscope surface with a monolayer of QDs. To ensure the QD attachment only to the tip of the nanowire endoscope, the position of the nanowire endoscope was controlled by the micromanipulator and monitored under microscope. The derived QD-conjugated nanowire endoscope was then rinsed with PBS to remove noncovalently attached QDs.

To fabricate the non-cleavable linker QD-conjugated nanowire endoscope, the aminated nanowire endoscope was reacted in 0.5 mg/mL PBS solution of Sulfo-NHS-LC-Biotin

(Thermo Scientific) for 1 hr and rinsed with PBS several times. The tip of the biotin-modified nanowire endoscope was then dipped into a small droplet (2μL) of the streptavidin-modified QDs (Qdot® 655 streptavidin conjugate, Invitrogen) solution (0.1 μM) and immersed for 30 min. The concentration of QD solution was optimized to coat the nanowire endoscope surface with a monolayer of QDs. To ensure the QD attachment only to the tip of the nanowire endoscope, the position of the nanowire endoscope was controlled by the micromanipulator and monitored under microscope. The derived QD-conjugated nanowire endoscope was then rinsed with PBS to remove noncovalently attached QDs.

The passive delivery experiment was conducted by slowly inserting the disulfide linker QD conjugated nanowire endoscopes into the nucleus or cytoplasm with the micromanipulator for duration of 1 min and 20 mins (Figure S9) and then retracted from the cell. The control experiment with the non-cleavable linker QD-conjugated nanowire endoscopes was performed according to the same procedure for the duration of 1 min (Figure S11).

V. High Resolution Excitation of Intracellular Fluorescent Markers.

For the following experiments, HeLa cells were seeded into 35 mm petri-dish with grids and cultured overnight (22,000 cells per dish) at 37^{o}C in the presence of 10% FBS. Single cells were randomly chosen for the study. The nanowire endoscope was mounted on a 3-axis micromanipulator for precision control over the tip position.

A. Imaging of cytoplasm-stained Hela Cell

The cytoplasm of the Hela cells were stained with calcein AM prior to the experiments. The nanowire endoscope, which was coupled to a 442 nm blue laser, was inserted into the cytoplasm of a cell to optically excite the fluorescence of the calcein dye molecules located near the nanowire tip. Weak back light was left on to show the outline of the cell and the position of the nanowire. As shown in Figure S12b, the endoscope demonstrated pico liter illumination volume which gives rise to not only the possibility of high resolution imaging, but also low imaging background and high signal to noise ratio.

B. Imaging of mitochondria-stained Hela Cell

Prior to the experiments, the Hela cells were stained with MitoTracker Green (Invitrogen), a mitochondrial-selective fluorescent label. The fluorescence image was first taken under wide field illumination (Figure S13a), which showed the distribution of mitochondria in the cytoplasm. The image, however, suffered from low signal to noise ratio and poor resolution even under the high magnification objective lens (50x). When illuminated with the nanowire endoscope, the small illumination volume ensures localized excitation of the mitochondria marker, much quieter background and enhanced contrast. As demonstrated in Figure S13c, single mitochondrion within the close proximity of the probe tip was excited by the tip emission from the endoscope.

C. Imaging of membrane-stained Hela Cell

Prior to the experiments, Hela cells were stained with Alexa Fluor® 488 conjugate of wheat germ agglutinin (WGA, Invitrogen), a fluorescent label that bounds specifically to the sialic acid and N-acetylglucosaminyl residues on the cell membrane. Under wide field illumination (Figure 3d), the observed fluorescence marked the outline of the plasma membrane, with a bright rim from the side walls and a dimmer glow from the center. Under such global excitation condition typical for epi-fluorescence microscopy, both the top and bottom surfaces of the cell membrane are excited due to the large illumination depth. As a result, out of focus fluorescence would enter the focal plane of the objective and obscure the in-focus image details[2] and render it difficult to image the top and bottom surfaces of the cell membrane separately with good enough contrast and sharpness.

The nanowire endoscope, on the other hand, has much smaller illumination depth and is thus able to excite tiny areas on the cellular membrane with much lower background. Figure 3e-f demonstrated the spot illumination ability when the nanowire approached the cell membrane from the side. In Figure S14, we further demonstrated that it is possible to distinguish the fluorescence signals from the top and the bottom of the cell membrane with the endoscope. When the endoscope tip is inserted deep into the cell, as illustrated in Figure 14a, two spots on the cell membrane can be lit up. One is located at the bottom

surface of the cell membrane, which is close to the tip of the nanowire (dotted orange circle) and illuminated by the endoscope tip emission. The other spot is located at the top surface of the cell membrane, where the nanowire intersects with the top surface of the cell membrane (red dotted circle). In subwavelength waveguide such as the SnO_2 nanowire used for the endoscope, a considerable portion of the transmitted optical power (20% for a 200 nm SnO_2 nanowire waveguiding 442 nm laser in aqueous solution) is contained in the evanescent field bound to the nanowire surface[3], which will excite the fluorophores within close proximity (< 200 nm) to the nanowire-membrane interface. Since the endoscope was inserted into the cell at an access angle of ~20°, the two fluorescent spots from the top and bottom of the cell membrane respectively can be readily distinguished from the microscope view. As demonstrated in the live cell experiment (Figure S14b-c), two membrane fluorescent spots were found on the fluorescent image when an endoscope was inserted into the cytoplasm. The one on the left in Figure S14c corresponds to the tip excitation of the bottom membrane (dotted orange circle), while the spot on the right corresponds to the evanescent excitation of the surface membrane (dotted red circle). The two spots are ~5.2 μ m apart, indicating a vertical separation of ~1.9 μ m. Since the bottom membrane is in contact with the substrate, we can estimate the height of the cell membrane at the point of endoscope insertion, which was also ~1.9 μm.

In a simpler scenario, when the endoscope tip is merely touching the top cell membrane, instead of inserted into the cell, as illustrated in Figure S14d, the endoscope emission would only excite the fluorescence of the membrane at the spot where they are in contact. The bottom membrane is too far from the nanowire tip to be excited. Figure S14e shows the endoscope tip at the same position on the image plane as in Figure S14b, but are lifted so that the tip is touching the cell membrane instead of inserted. In this case, only one fluorescent spot is detected (Figure S14g, dotted red circle), which is located on the top surface of the cell membrane, right above the bottom membrane fluorescent spot (dotted orange circle) shown in Figure S14c. With the help of a nanowire endoscope, we can achieve the selective excitation of the top and bottom cell membranes at the same planar

position and distinguish their fluorescent signals under a conventional fluorescent microscope.

D. QD tracking with nanowire endoscope illumination

For subcellular QD tracking, cells were pre-treated with the aminated QDs (50 nM in cell medium) for 1 hr and rinsed several times with PBS. The unmodified SnO_2 nanowire endoscope was placed close to the QDs in the cytoplasm using the micromanipulator and the blue laser (442 nm) was waveguided through the nanowire endoscope. The movement of the QDs (clusters) in the subcellular region was locally monitored over time with the nanowire endoscope illumination (Figure S15). When the endoscope was placed close to the cytoplasm where multiple clusters of QDs were loaded, its tip illumination allowed the tracking of one of the QD clusters (yellow arrow) which was moving around in the subcellular region (~ 10 μm^2).

VI. pH Sensing Experiment.

The pH sensitive nanowire endoscope was fabricated by capping the tip of the nanowire probe with the copolymer of acrylamide and acryloylfluororescein through photopolymerization, following the previous reported procedure[1]. Fluorescein amine was converted into its amide derivative, FLAC, by reacting 2:1 stoichiometric quantities of acryloyl chloride with fluoresceinamine in dry acetone. The mixture was stirred for ~1 hr until most of the product precipitated. The product was collected by filtration and washed by acetone and CH_2Cl_2, and then air-dried. The absorption spectra of FLAC showed strong pH dependence (Figure 4e). The derived FLAC was dissolved in ethanol. The FLAC solution (15 mM, 4 ml) was then mixed with 0.1M pH 6.5 buffer solutions of acrylamide (6.5 M, 10 ml) and N, N-methylenebis(acrylamide) (0.15 M, 40 ml) to form the monomer stock solution.

The nanowire endoscope tips were first silanized with APTES (10%, pH 3.45) for about 1 hr and then dried in air for another hour. To minimize possible dye attachment to the

sidewall of the nanowire endoscope, only the tip region of the nanowire was immersed into APTES solution. After silanization, nanowire tips were sensitized in a 0.2 M benzophenone/cyclohexane solution for about 15 min.

The photopolymerization of the monomer was performed in a passive N_2 environment and at an elevated temperature. The reactor used for photopolymerization was a small glass bottle with three holes in its cover, through which the N_2 flushing tubes and the nanowire endoscope tip was inserted into the monomer solution. An oil bath was used to control the reaction temperature at 50 ºC. The polymer solution was bubbled with N_2 for about 20 mins before laser light was directed into the fibre tip and the N_2 atmosphere was maintained during the reaction. Laser light of 442 nm from a HeCd laser was coupled into the fibre and transmitted to the tip where the photopolymerization was initiated directly on the silanized surface. Reaction time of 5-10 min was needed for the polymerization process. A XYZ translation stage was used to control the position of the nanowire endoscope so that only the tip was in contact with all the solutions. After the polymerization, the nanowire endoscope was washed in water and ethanol in turn to remove unpolymerized dye. Figure 4 a-d show the dark field, waveguide and fluorescence images of a nanowire endoscope before and after the photopolymerization.

The nanowire endoscope was mounted on the micromanipulator and inserted into microdroplets of buffer solutions of different pH pre-patterned on a PDMS substrate. The 442 nm HeCd laser beam was coupled into the fibre as optical excitation to the fluorescent polymer. A 50x long working distance objective (Nikon) was used to collect the fluorescent signal from the tip of the nanowire, while the tip of the optical fibre was kept out of the field of view. The tip emission spectra of the nanowire endoscope showed strong pH dependence (Figure 4f). At high pH, polymer fluorescence was dominant by the 515 nm emission from the dianion of FLAC, while at low pH, emission from the monoanion appeared as a shoulder around 550nm. The intensity ratios at 515 nm and 610 nm were used to construct the calibration curve of the nanowire endoscope, which showed a sharp transition between pH 5-9 (Figure 4f).

VII. **Local Fluorescence Spectrum Collection.**

Figure S16 shows that the tapered tip of the optical fibre did not contribute to signal detection, because the QD fluorescence cannot be extracted through the fibre tip when it was beyond 25 μm from the target QD, whereas for our typical nanowire endoscopes, the protruding lengths of the nanowire from fibre tip are at least 50 μm.

References

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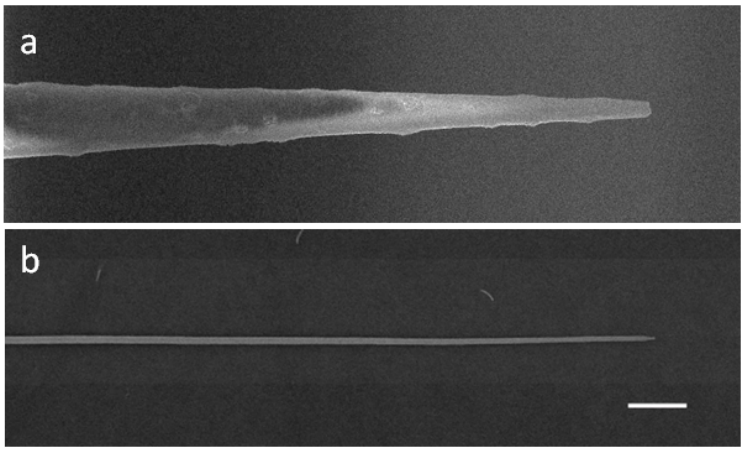

Figure S1 SEM images of the etched optical fibre and SnO_2 nanowires. a, SEM image of the fine tip of a single mode optical fibre chemically etched to a slowly tapered shape. The fibre tip has a tapering angle of 5º and end diameter of 300 nm. **b,** SEM image of a typical SnO_2 nanowire used for nanowire endoscope fabrication, with an average diameter of 100 nm. The nanowire is much smaller in dimension than etched optical fibre, thus less invasive to cell membranes. Scale bar: 1 μm.

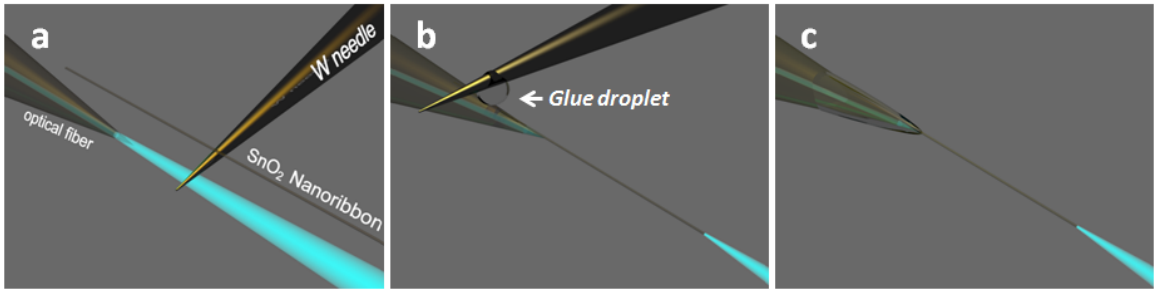

Figure S2 Procedure of nanowire endoscope fabrication. a, A SnO_2 nanowire is placed on the tapered tip of a single mode optical fibre with a tungsten needle mounted on a 3-axis micromanipulator. The blue laser coupled into the distal end of the fibre escapes at the tapered tip where the core of the optical fibre is exposed. **b,** A droplet of glue (Epoxy or Polydimethylsiloxane) was applied to the overlapping section between the nanowire and fibre. The blue laser is further coupled into the nanowire and re-emit at the tip of the nanowire instead. **C,** After the glue dried, the fibre and the nanowire were permanently bond together and the coupling was fixed.

Video S1 Mechanical and optical robustness of the nanowire probe under deformation, bending and buckling.

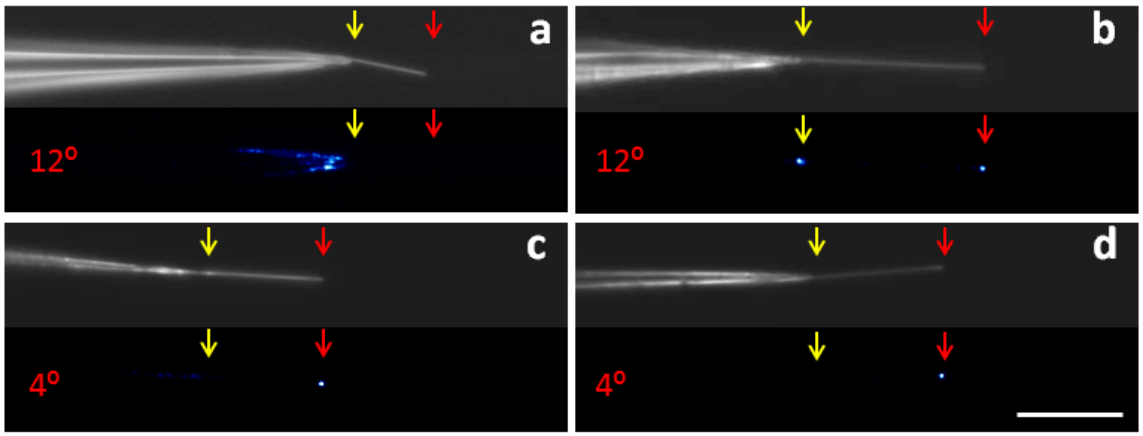

Figure S3 Nanowire endoscope coupling efficiency. a and b, Dark-field (upper) and waveguiding (lower) images of the nanowire endoscope fabricated from a pulled (a) and etched (b) single mode optical fibre with same tapering angle (12º). **c and d,** Dark-field (upper) and waveguiding (lower) images of the nanowire endoscope fabricated from an etched single mode optical fibre with rough (c) and smooth (d) surface and same tapering angle (4º). Yellow and red arrows point to the tip of the optical fibre and nanowire, respectively. Scare bar is 20 µm.

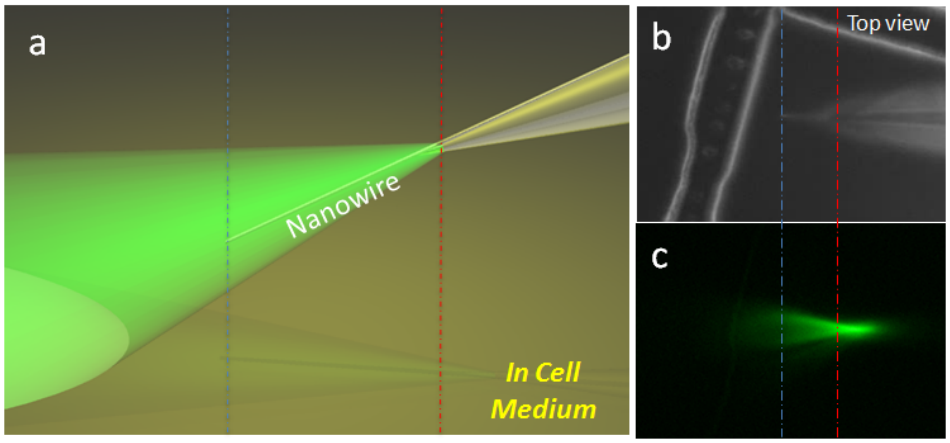

Figure S4 Illumination by a poorly coupled nanowire endoscope. a, Schematic of the nanowire endoscope with poor coupling efficiency illuminating fluorescent proteins in the cell medium by the blue light (442 nm) delivered through it. The emission was given out predominantly from the optical fibre tip and the illumination profile is in essential that of the tapered optical fibre itself without the nanowire. Blue and red dashed lines indicate nanowire and optical fibre tips, respectively. **b and c,** Dark-field image (top view) of the nanowire endoscope (tapered optical fibre) placed in the cell medium (**b**). Fluorescence image of the nanowire endoscope broadly illuminating fluorescent proteins in the cell medium by the blue light (**c**). A 442 nm long-pass filter was used for the fluorescence imaging.

	Insertion duration (min)	Cell viability rate	
Tapered optical fiber tips	1	**41%**	(9/22)
Nanowire probes	1	100%	(8/8)
	3	100%	(6/6)
	5	100%	(10/10)

Table S1 Cytotoxicity of the nanowire probe v.s. a tapered optical fibre tip. The cytotoxicity test compared cell viability rate 10 hours after a 1 minute tapered optical fibre tip insertion and 1, 3, and 5 minutes nanowire insertion. Tapered optical fibre tips were fabricated by wet chemical etching and have a tip diameter of 300-500 nm, as shown in the SEM image in Figure S1, whereas nanowires used have a cross section dimension of 150-300 nm. All data were acquired with nanowire endoscope inserted in the cytoplasm.

Laser wavelength (nm)	Power density of focused laser (mW/cm^2)	Laser input into optical fiber (mW)	Nanowire Insertion Time (min)	Laser Radiation Time (min)	Cell viability
325	0.4	-	-	1	5/5 (100%)
325	0.4*	-	1	1	10/10 (100%)
442	-	0.4**	1	1	5/5 (100%)
442	-	0.4	0***	1	5/6 (83.3%)

Table S2 Nanowire endoscope cytotoxicity for QD delivery and spot illumination. All data were acquired with nanowire endoscope inserted in or laser focused on the cytoplasm and the size of the nanowires used in this study are between 150-300nm in diameter.

* For all QD delivery experiments, laser power density of 0.4 mW/cm^2 and radiation time of 1 min were used as a standard operation condition.

** For all local illumination experiments, input laser power of 0.4 mW was used for the optical fibre. An average output laser power of 10 μW was measured from the nanowire end of the optical fibre.

*** In this case, the nanowire probe was placed in close proximity (~2μm), but not touching, the cell membrane (non-contact mode).

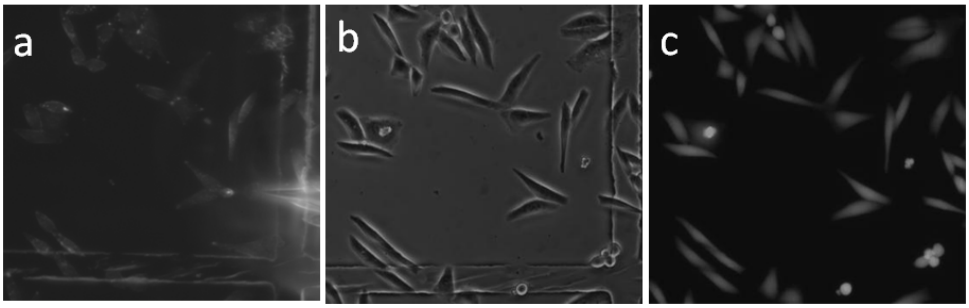

Figure S5 Cytotoxicity of the nanowire endoscope insertion and illumination in a single living HeLa cell. a, Dark-field image of the nanowire endoscope insertion and illumination. The nanowire endoscope is introduced into the cytoplasm illuminating the micro-scaled region for 1 min with the waveguided blue light (442 nm). **b and c,** Viability of the cell 10 hrs after the nanowire endoscope insertion and illumination. Phase contrast image of the cell treated with the nanowire endoscope (**b**). Fluorescence image of the cell treated with the nanowire endoscope after staining with live cell assay dye, Calcein (**c**).

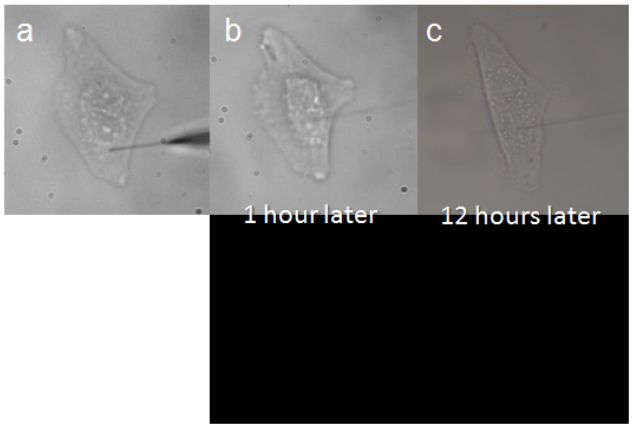

Figure S6 Apoptosis assay of a single cell after nanowire endoscope insertion. a, Bright-field image of the tested cell during the nanowire endoscope insertion. The nanowire endoscope is introduced into the cytoplasm for 15 mins. **b and c,** Bright field (upper panel) and fluorescent (lower panel) images of the same cell after 1 hour (**b**) and 12 hours (**c**) of post-insertion incubation. Cell did not show significant morphology change characteristic to apoptotic cells. The cell was stained with apoptosis assay kit prior to fluorescent imaging.

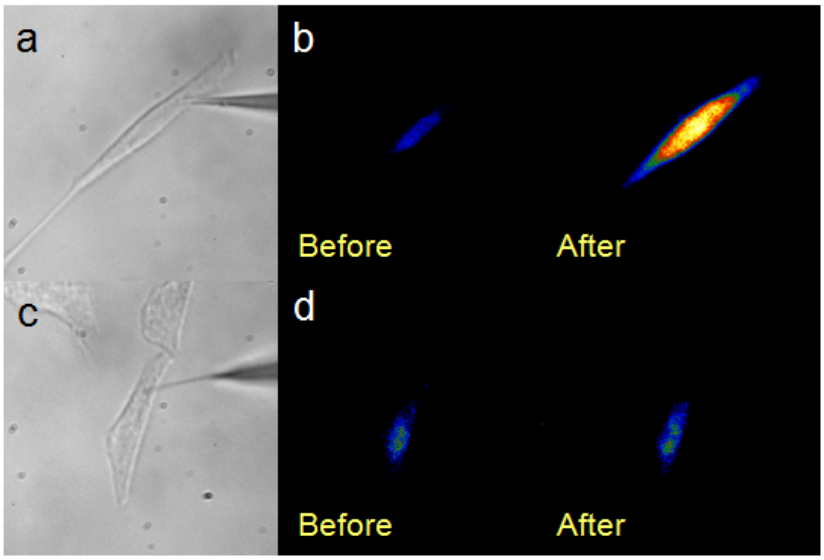

Figure S7 Mechanical insertion induced intracellular Ca^{2+} response: nanowire v.s. tapered optical fibre tip. a, Bright field image showing a conical glass tip inserted into a live Hela cell pre-loaded with calcium indicator fluo-4 AM. **b,** Pseudo-colour fluorescence images of the cell shown in **A** before (left) and after (right) 1 min of insertion by a tapered optical fibre tip. **c,** Bright field image showing a nanowire inserted into a live Hela cell pre-loaded with calcium indicator fluo-4 AM. **d,** Pseudo-colour fluorescence images of the cell shown in **c** before (left) and after (right) 1 min of nanowire insertion. Tapered optical fibre tips were fabricated by wet chemical etching and have a tip diameter of 300-500 nm, as shown in the SEM image in Figure S1, whereas nanowires used have a cross section dimension of 150-300 nm.

Video S2 Mechanical insertion induced intracellular Ca^{2+} response: nanowire v.s. tapered optical fibre tip. a, Video clip shows the intracellular Ca^{2+} level change during the insertion of a tapered optical fibre tip into a Hela cell preloaded with Calcium indicator fluo-4 AM. **b,** Video clip shows the intracellular Ca^{2+} level change during the insertion of a nanowire endoscope into a Hela cell preloaded with Calcium indicator fluo-4 AM. Dark field imaging condition was chosen for the videos while 442nm excitation was used to monitor fluo-4 fluorescence. The video speed was 2x the actual speed.

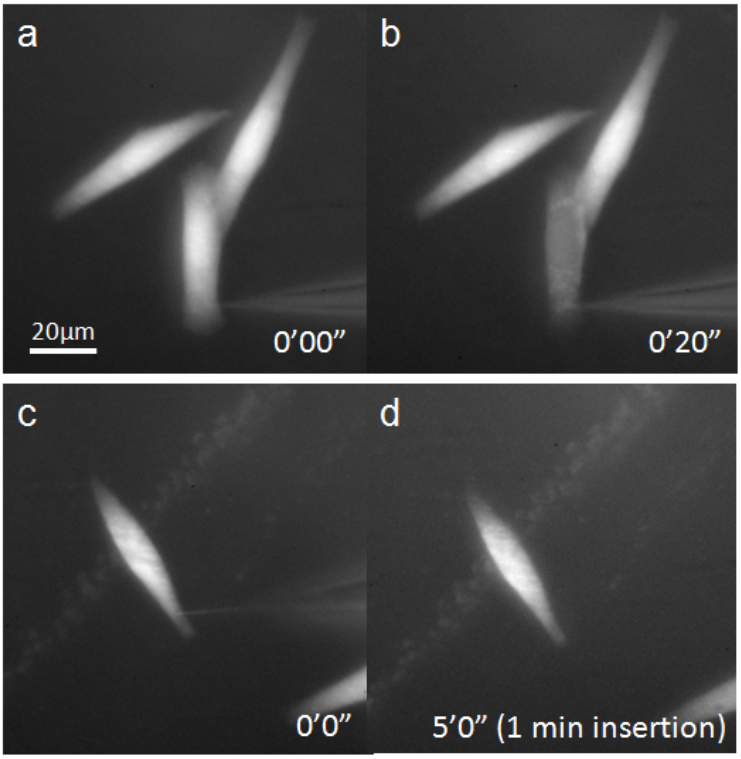

Figure S8 Cell membrane integrity during insertion: nanowire v.s. tapered optical fibre tip. a, Dark field/fluorescent images immediately taken after of a tapered optical fibre tip was inserted into the cytoplasm of a calcein AM stained Hela cell. **b,** Dark field/fluorescent images of the same cells 20 seconds after **a** was taken. **c,** Dark field/fluorescent images of a calcein AM stained Hela cell, taken immediately after a nanowire probe was inserted into the cytoplasm. **d,** Dark field/fluorescent images of the same cell shown in **c**after 1 min nanowire insertion followed by 4 mins of incubation. Tapered optical fibre tips were fabricated by wet chemical etching and have a tip diameter of 300-500 nm, as shown in the SEM image in Figure S1, whereas nanowires used have a cross section dimension of 150-300 nm.

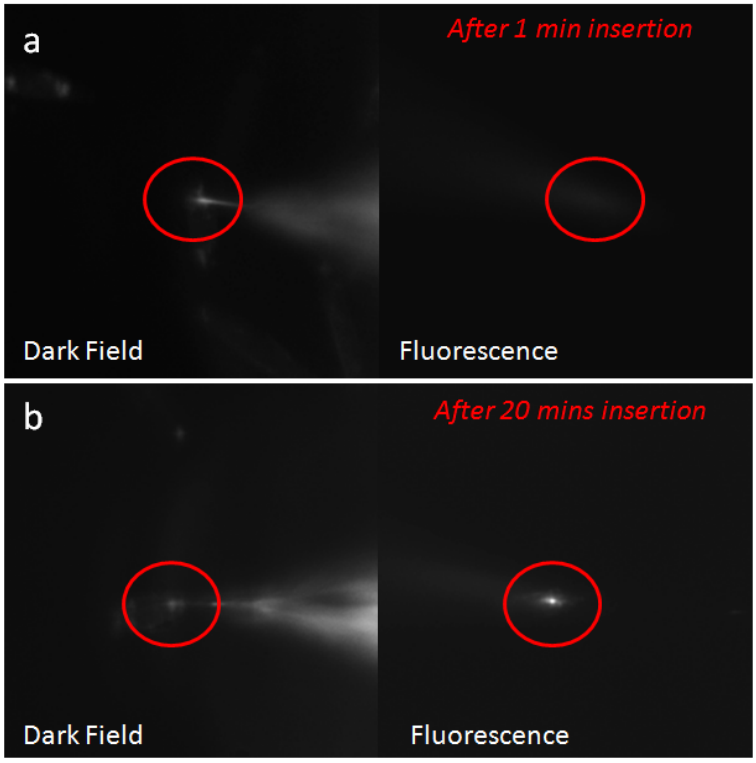

Figure S9 Passive delivery of quantum dots (QDs) into a single living HeLa cell with disulfide bond (S-S) linkers. a. Dark-field image showing the nanowire endoscope coated with QDs via S-S linker inserted into the cytoplasm of a living HeLa cell (left panel). Fluorescence image of the cell after probe insertion for duration of 1 min showing no QD signal (right panel). **b.** Dark-field image showing the nanowire endoscope coated with QDs through S-S bond linker inserted into the cytoplasm of a living HeLa cell (left panel). Fluorescence image of the cell after probe insertion for a duration of 20 mins showing an oval shaped QD fluorescence signal as indicated by the red arrow (right panel). Note that this passive delivery method requires relatively long insertion time (20 mins) and causes the non-specific release of QDs (from all parts of the probe immersed in cytoplasm). 442 nm focused laser excitation and a 532 nm long-pass filter was used for the QD fluorescence imaging.

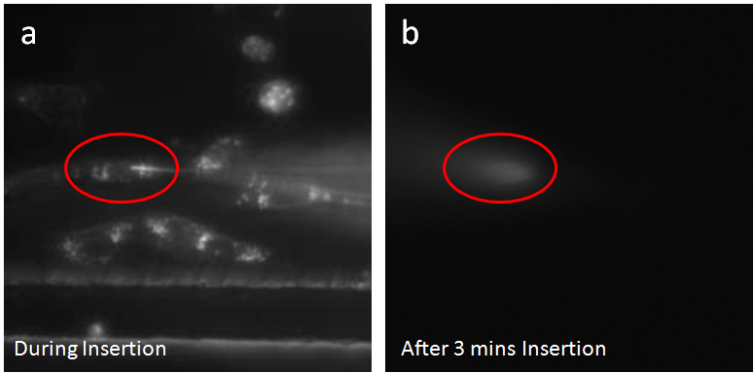

Figure S10 Quantum dot (QD) delivery into a single living HeLa cell without irradiation. a, Dark-field image showing the photoactivable QD-conjugated nanowire endoscope inserted into the cell. **b,** Dark-field image along with the excitation by the focused blue laser showing that no QDs were delivered into the cell after the insertion for 3 min without irradiation (photoactivation). 442 nm focused laser excitation and a 532 nm long-pass filter was used for the QD fluorescence imaging.

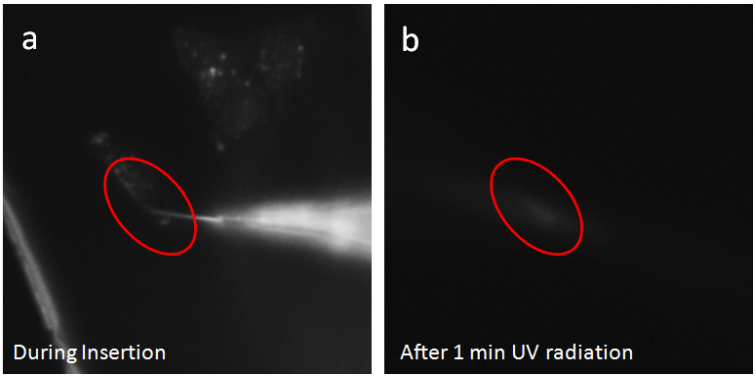

Figure S11 Quantum dot delivery into a HeLa cell with QD-conjugated nanowire endoscope via photo-inactive linkers. a, Dark-field image showing the non-photocleavable linker QD-conjugated nanowire endoscope inserted into the cell. **b,** Fluorescence image of the cell after UV radiation for 1 min, showing no QDs was present in the cell after the attempt. 442 nm focused laser excitation and a 532 nm long-pass filter was used for the QD fluorescence imaging.

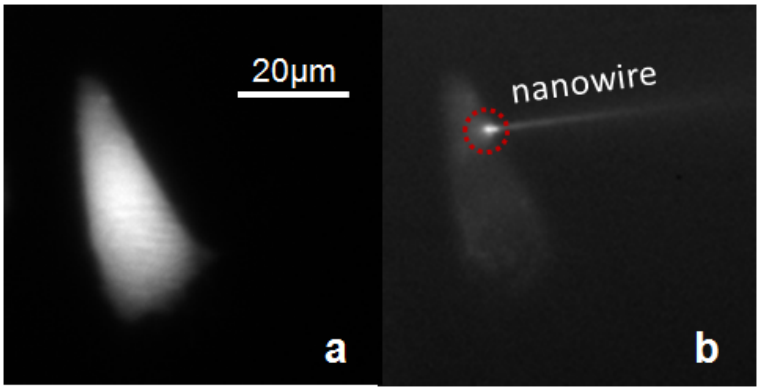

Figure S12 Excitation of calcein AM stained Hela cell through nanowire probe emission. a, Fluorescence image of a live Hela cell preloaded with calcein AM. Wide field illumination was used to excite the calcein fluorescence. **b,** Fluorescence/dark field image of the same Hela cell being illuminated by the waveguided laser (λ=442 nm) released from the tip of the nanowire probe showing a localized bright fluorescent spot, as marked by the dotted red circle.

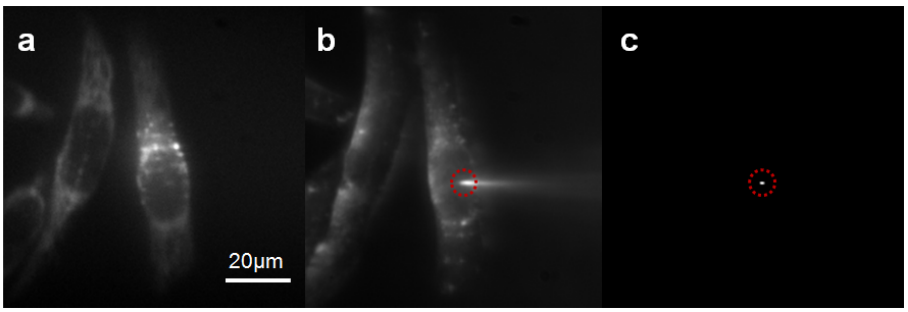

Figure S13 Excitation of mitochondria stain in a Hela cell through nanowire probe emission. a, Fluorescence image of live Hela cells stained MitoTracker Green, a fluorescent probe that localize to mitochondria. Wide field illumination of 442 nm was used to excite the dye fluorescence. **b and c,** Dark field **b** and Fluorescence **c** images showing one of the cells shown in **a** illuminated by the nanowire probe (λ= 442 nm). The laser light emitted from the tip of the probe (the dotted red circle in **b** excited the mitochondria located in its close proximity showing a single bright fluorescent spot, marked by the dotted red circle in **c**. The illuminated mitochondrion was sitting in the cytoplasm between the nucleus and the cell membranes.

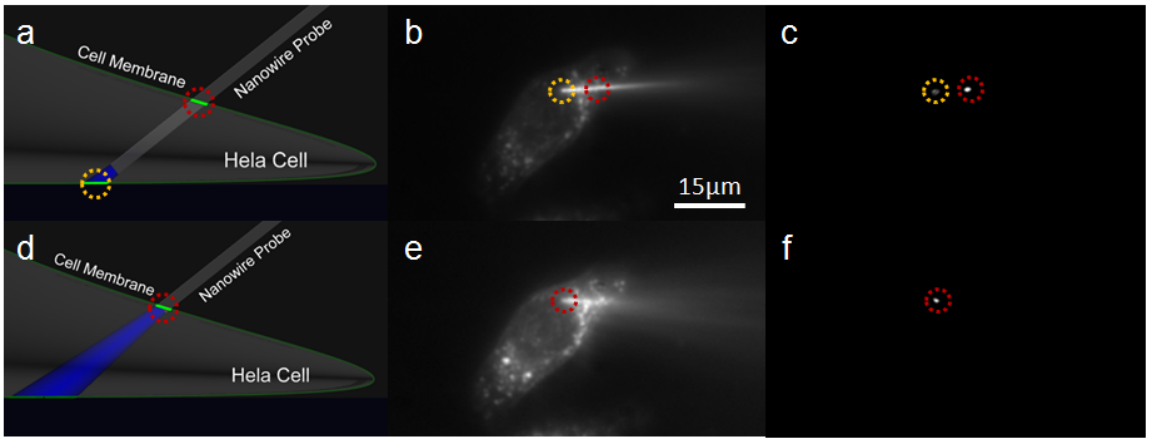

Figure S14 Excitation of cell membrane stain through nanowire probe emission. a, Schematics showing fluorescently labeled plasma membrane of a Hela cell illuminated with a nanowire probe inserted into the cell. The spot on the top surface of the membrane, which is in contact with the nanowire (red circle), is excited by the evanescent field of the propagating light on the surface of the nanowire, corresponding to the bright spot (red circle) shown in **c**. The spot on the bottom surface of the membrane, which is close to the tip of the nanowire (orange circle), is excited by the laser light emitted from the tip of the nanowire probe, corresponding to the dimmer spot (orange circle) shown in **c**. **b and c,** Dark field **b** and fluorescence **c** images showing a live Hela cell being illuminated by the nanowire probe (λ= 442 nm) inserted into the cell. Excitation wavelength is 442 nm and a 532 nm long pass filter was used. **d,** Schematics showing fluorescently labeled plasma membrane of a Hela cell illuminated with a nanowire probe when the tip is touching the top surface of the cell membrane. The spot on the membrane in contact with the nanowire tip (red circle) is excited by the laser light emitted from the tip of the nanowire probe, corresponding to the bright spot (red circle) shown in **f**. **e and f,** Dark field **e** and Fluorescence **f** images showing a Hela cell, the membrane of which was stained with Alexa Fluor® 488 conjugate of WGA, being illuminated by the nanowire endoscope. The nanowire tip was in direct contact to the top surface of the plasma membrane.

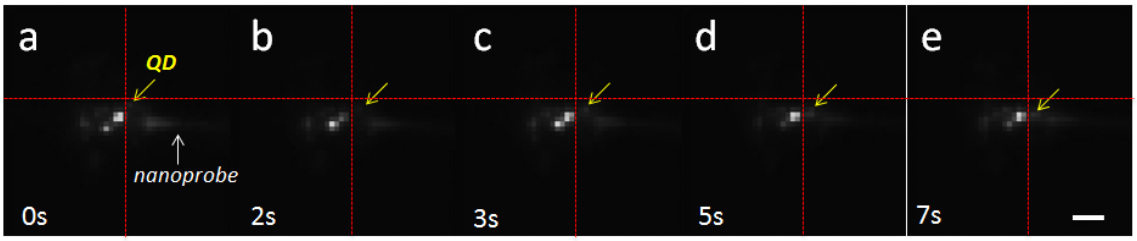

Figure S15 Subcellular tracking of quantum dots (QDs) movement in a single living cell. a-d, Fluorescence images showing the tracking of QD movement in the subcellular region during the time period of 0-7 s using the nanowire endoscope illumination. Yellow arrows mark the current position of the QD in each image and the crosses of the dotted lines mark the initial position of the QD at t=0s. Cells were pre-treated with the aminated QDs for 1 hr before the nanowire endoscope illumination. A 532 nm long-pass filter was used to screen the excitation laser for the fluorescent imaging. Scale bar is 5 μm.

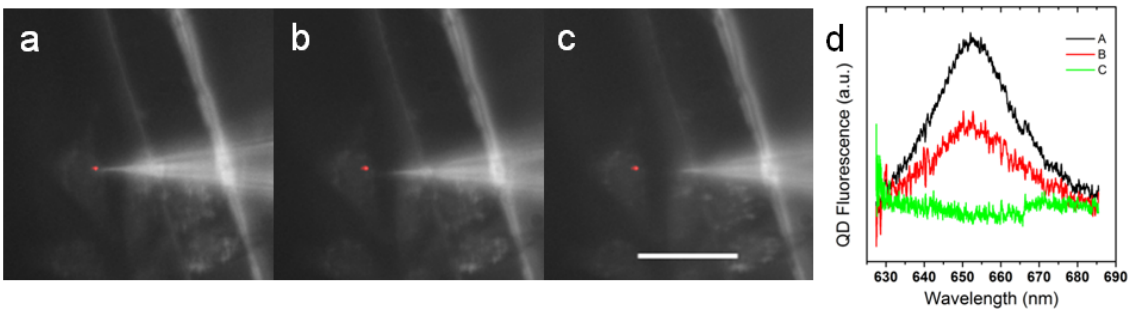

Figure S16 Quantum dot fluorescence collection through a tapered optical fibre tip. **a,** Dark-field image superimposed with the QD fluorescence image of QD-loaded live HeLa cells. A tapered optical fibre (3º taper angle) is placed close to QDs in the cytoplasm. **b-c,** Dark-field/fluorescence image showing the optical fibre retracted by ~ 12 and ~22 μm from the QD. **d,** Spectra of QD fluorescence collected from the tip of optical fibre for A (black curve), B (red curve) and C (green curve), respectively. Signal died out as the fibre is beyond 22 μm in separation with the QD. Scale bar is 50 μm.