Electronic Supplementary Material (ESI) for Chemical Communications.

A Precise Pointing Nanopipette for Single-cell Imaging via Electroosmotic Injection

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Experimental Section

1. Reagents

All reagents were of analytical grade and used as received without any further purification. All other materials are of analytical grade, and solvents were purified by standard procedures. All solutions were prepared by Milli-Q ultrapure water with resistance of 18.2 M Ω cm at 25 °C (EMD Millipore, TONDINO, Shanghai). MCF7 cells were from MoXi Biotech. Co. Ltd. (Shanghai, China). Dibasic Sodium Phosphate and Sodium Dihydrogen Phosphate were purchased from Aladdin, which were used for preparing buffer solution (pH = 7.4), Rhodamine B was obtained from Aladdin as fluorescence molecules, Rhodamine B (0.0096 g, 20 mmol) was prepared by PBS (100 mmol). Quartz capillaries (O.D.: 1.0 mm, I.D.: 0.7 mm; 7.5 cm length) were purchased from Sutter Instrument. The quartz capillaries were cleaned first by ethanol, and then washed successively in acetone and pure water with at least 30 min sonication. Lastly they were dried with nitrogen gas. Blue tack was obtained from the Chinese agency of Photo Tack.

2. Apparatus

2.1 P-2000 Laser Puller

Nanopipettes were fabricated from quartz capillaries (with outside diameter of 1.00mm and inside diameter of 0.70 mm bought (Sutter Instrument, Novato, CA) by using a P-2000 laser puller (Sutter Instrument, Novato, CA) according to relevant references [1]. A single pull cycle was used to fabricate nanopipettes. Pull parameters were heat=660, fil=3, vel=30, del=170 and pul=205. The mean diameter of the pore of the nanopipette is about 100 nm.

2.2 Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) observations of the nanopipettes were performed with a field-emission scanning electron microscope (Ultra 55, Carl Zeiss Ltd., Germany).

2.3 Adjustment and Controlling System

Optical dark-field spectrum measurements were recorded using a Nikon eclipse Ti-U inverted microscope equipped with a dark-field condenser (0.8 < NA < 0.95) and a 40 × objective lens (NA = 0.8), which was used to obtain the spectra in a technological channel. Illumination was provided by a 100 W halogen lamp which was used to generate the local plasmon resonance scattering light. A true-color digital camera (Nikon, DS-fi, Japan) was used to record the field of the microscope for coregistration with the monochromator. The dark-field condenser (0.8 < NA < 0.95, Nikon, Japan) with a 100 W halogen tungsten lamp was used for obtain the scattering light. The scattering light was focused onto the entrance port of a monochromator (Isoplane SCT 320) that was equipped with a grating (grating density: 300 lines/ mm; blazed wavelength: 500 nm) to disperse the scattering light. Then, the scattering light was recorded by a 400 × 1600 pixel cooled spectrograph CCD camera (ProEM+: 1600eXcelon3, Princeton Instruments, USA). The nanopipette is fixed to a microscope by a holder (Axon Instruments, Union City, CA). The holder is connected to an Axopatch 700B low-noise amplifier (Molecular Devices, Sunnyvale, CA) for current measurement, a MP-285 micromanipulator (Sutter Instrument, Novato, CA) for coarse control of the nanopipette positioning in the X, Y, and Z directions, a Nanocube piezo actuator (Physik Instrument, Irvine, CA) for fine control in the X, Y, and Z directions, and a PCIe- 7851R Field Programmable Gate Array (FPGA) (National Instruments) for hardware control of the system. The system is operated using custom coded software written in LabVIEW.

3. Cell Culture

Cells were cultivated and maintained with normal 1640 cell media, 10% Fetal Bovine Serum and antibiotics.

4. Filling of the Nanopipettes

The nanopipettes were backfilled by using a microloader (Eppendorf). First, about 10 μl of the solution was drawn into the microloader using a commercially available pipette, and the microloader was inserted carefully into the back end of the

nanopipette and the nanopipette was filled with solution by slowly pushing solution into the tip while continuously drawing the microloader back from the beginning of the tip to the back of the pipette. Second, in order to ensure the solution have reached to the tip totally, the centrifugal method was adopted (5 min, 8000 rpm) with Centrifuge 5430 (Eppendorf). Blue tack was used to immobilize the nanopipette so as to prevent the tip from destroying.

5. Preparation of Electrodes

Silver wire was used to prepare for the electrodes, cut up about 4cm in length and incubated in $FeCl_3$ solution overnight. They were then carefully connected with the nanopipette body liquid and another end for nanopipette experiments was placed in the bath solution.

6. Methods

To form the electrical connection, a silver electrode was connected with the solution in the pipette body, and another end dived into the solution fulfilled with cells (Fig. S1). Further up, the Ag/AgCl electrode was placed into the nanopipette as working electrode while another Ag/AgCl electrode immersed in the bath solution as reference/counter electrode. The nanopipette electrodes consist of pulled quartz nanopipettes backfilled with fluorescence molecules to a density enough to generate the nanopipette's narrow opening.

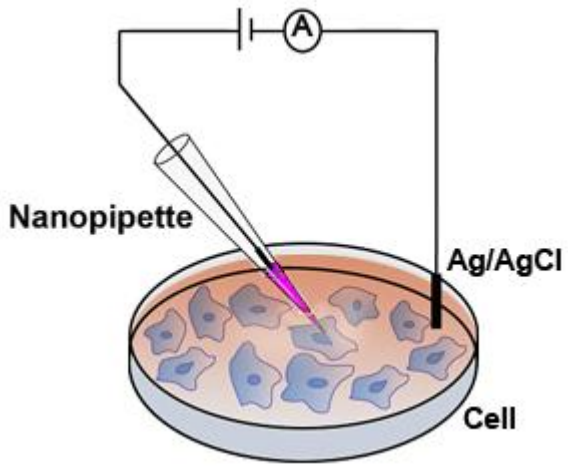

Fig. S1 Schematic illustration of the RhB-filled nanopipette connected with a DC voltage source for the electroosmotic flow-triggered injection of RhB into a single MCF-7 cell. An Ag/AgCl electrode was inserted into the RhB solution at the tip of the nanopipette as the working electrode. Another Ag/AgCl electrode was immersed into the bulk solution as the reference electrode.

In detail, a cell of interest was selected using white light transmission microscopy on the inverted microscope platform by adjusting and controlling step by step, and then the nanopipette tip can be positioned with the same method, when approaching a single cell for injectioning begins by positioning the tip manually about 50 μm above the solution surface via coarse tuning. This provides sufficient abundant height for approaching the nanopipette to the single cell. At this time, fine tuning instead till the tip of nanopipette insert the cell. And the process can be observed by imaging system. After the nanopipette is positioned inside the cell, transmission of fluorescent probes begins by regulating the voltage, basing on the charge of the fluorescent probes.

Results and Discussion

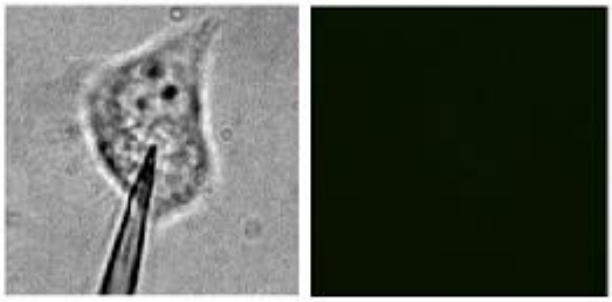

Fig. S2 Nanopipette injected into the cell (left: bright-field) and the fluorescent image (right) of the cell after the nanopipette was pulled out.

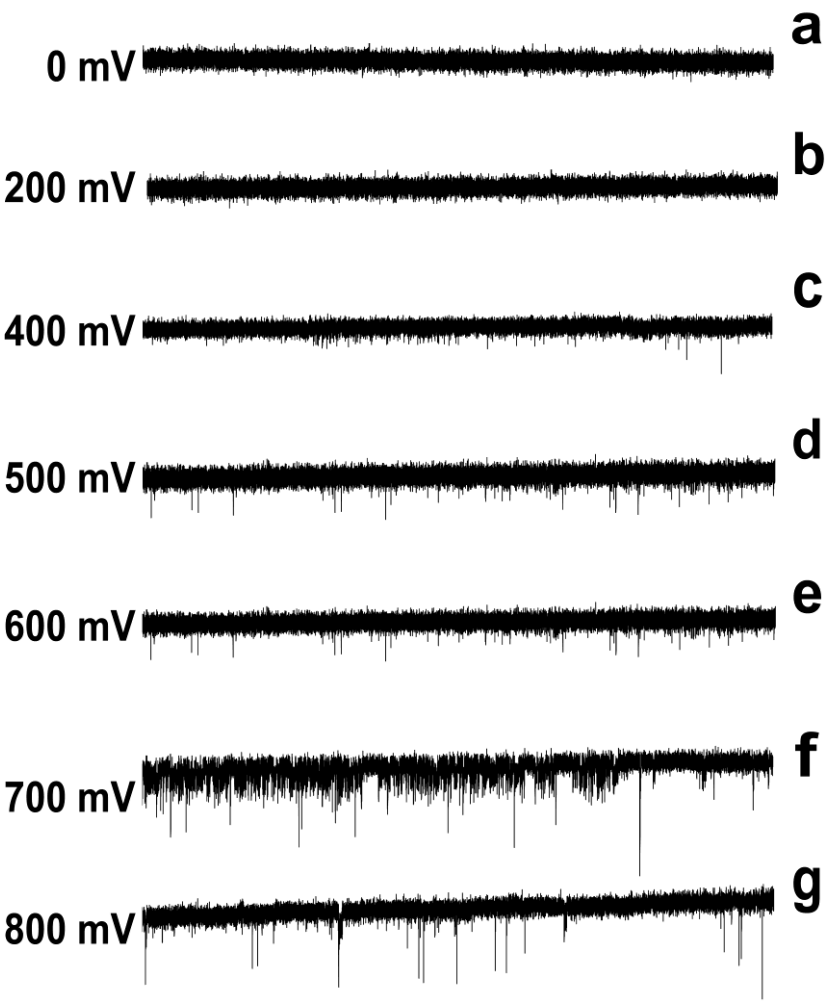

Fig. S3 Current changes during the injecting process. From a to g: 0, 200, 400, 500, 600, 700 and 800 mV.

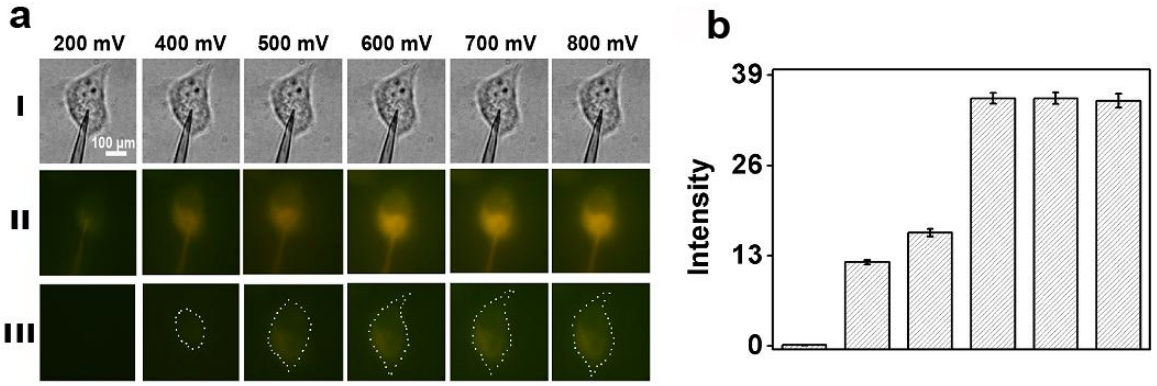

Fig. S4 (a) Microscopic images of the nanopipette inserted MCF-7 cell treated under different voltages for 20 min. (b) Histogram showing the average fluorescence intensities within the cell area. (I) Bright-field images. (II) Fluorescent images (ex: 552 nm; em: 610 nm). (III) Fluorescent images (ex: 552 nm; em: 610 nm).

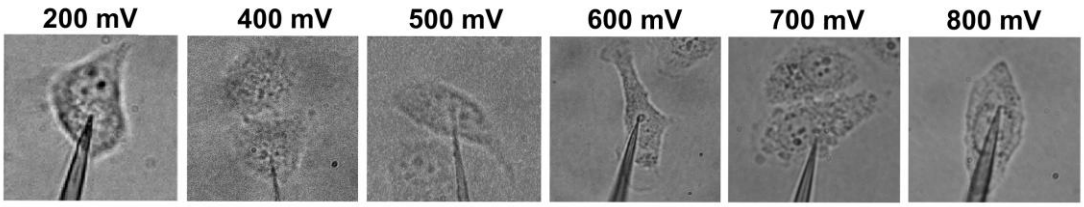

Fig. S5 Microscopic images of the nanopipette inserted MCF-7 cell treated under different voltages for 20 min.

Reference

(S1) M. Karhanek, J. T. Kemp, N. Pourmand., R. W. Davis, C. D. Webb, *Nano. Lett.*, 2005, **5**, 403-407.