Voltage Controlled Nano-injection System for Single-cell Surgery

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Supporting Information

Experimental Section

SICM: Our SICM consists of a low-noise amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, CA) for nanopipette bias and current measurement, a micromanipulator (MP-285, Sutter Instrument Company, Novato, CA) for coarse control in the X, Y, and Z directions, a piezo actuator (Nano-cube, Physik Instrumente) for fine control in the X, Y, and Z directions, and a Field Programmable Gate Array (FPGA) (PCIe-7851R, National Instruments) for hardware control of the system. Our system is further modified with a low-noise high voltage source (Model 2100 Isolated Pulse Stimulator, A-M Systems), and a custom relay for switching between the low voltages and low noise required for feedback control, and the high voltages needed for material ejection (Fig. S1). The system is controlled using custom coded software written in LabVIEW.

Single cell injection protocol: We accomplished the nanopositioning by using a computer controlled feedback system originally developed as a Scanning Ion Conductance Microscope (SICM). The basic SICM consists of a nanopipette back-filled with an electrolyte solution and immersed in an electrolytic bath[1; 2; 3]. An electrode is placed in the nanopipette and a ground

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electrode is placed at some distance away in the bath. The current flowing through the nanopipette depends on the tip-substrate separation, and it acts as a feedback signal to precisely control the height of the nanopipette at a predefined distance above a surface. If the nanopipette is then scanned across a surface, a topographical image can be rendered[1; 4]. Cell injection was performed using a double-barrel nanopipette and automated using custom software, controlling the height of the pipette, applied voltage, and voltage duration. The double-barrel configuration negates the need for an external ground electrode, as is common with single-barrel nanopipettes, and has the advantage of eliminating the introduction of a transmembrane potential during injection, which can cause destructive changes in cell membrane properties including ion permeability or electroporative effects. A double-barrel nanopipette was fabricated by laser pulling a theta quartz capillary and filled with a 0.1M PBS solution containing 1mM carboxyfluorescein and 0.01% surfactant (Pluronic F-68) to aid in filling. An Ag/AgCl wire was inserted in each barrel, with one barrel biased relative to the other, which acts as the ground electrode.

Double-barrel nanopipette fabrication: Double-barrel nanopipettes were fabricated from theta quartz capillaries with an outer diameter of 1.2 mm and an inner diameter of 0.90mm (QT120-90-7.5; Sutter Instrument Co.). The capillary was pulled using a P-2000 laser puller (Sutter Instrument Co.) programmed with a two-line program to fabricate nanopipettes. Parameters used were: (Line 1) Heat 650, Fil 4, Vel 20, Del 170, and Pul 0; (Line 2) Heat 750, Fil 4, Vel 40, Del 170, and Pul 200. The resulting nanopipette tips have inner diameters ~50nm (Fig. 1B) and the septum running all the way through the nanopores (Fig S3).

Nanopipettes were backfilled with 0.1M PBS, .01% Pluronic F-68, and the working molecule, typically a fluorescent dye. Nanopipettes were centrifuged for 10 seconds to ensure that solution

reached the very tip of the nanopipette. The blunt end of the nanopipette was subsequently silanized with trichloromethylsilane in vapor phase for 30 seconds in order to prevent any electrolyte bridging across the pipette septum at the blunt end, which can cause an electrical short.

Cell Culture: All cells were cultured in 5% CO2 at 37°C.. Human BJ fibroblasts were a gift from Dr. S. Salama (UCSC) and cultured in FibroGRO™-LS Complete Media Kit (Millipore), supplemented with Pen/Strep L-Glutamine mix (BioWhittaker) on untreated ibidiGRID u-Dish 35mm plates coated with 1% gelatin.

Reagents: Poly-l-lysine (PLL; 19320-A) was purchased from Electron Microscopy Sciences (Hatfield, PA). Dulbecco Modified Eagle's media (MT10017CV), fetal bovine serum (BW14502F), sodium pyruvate (BW13115E) and Pen/Strep/Glu (SV3008201) and gelatin were purchased from Fisher. Polydimethylsiloxane was purchased from Dow Corning, (Sylgard® 184 silicon elastomer kit). Carboxyfluorescein succinimidyl ester (CFSE) was purchased from AnaSpec Inc. Sulforhodamine 101 (#284912) was purchased from Sigma. PBS solutions at pH 7.4 were prepared using standard methods. Aqueous reagents were prepared using MilliQ water with >18MΩcm^{-1} resistance.

Fluorescence Microscopy: Cells injected with fluorescent molecules were visualized using an Olympus IX71 inverted microscope with epifluorescence. Images were captured with a Hamamatsu CCD camera and image analysis was performed with ImageJ and Matlab.

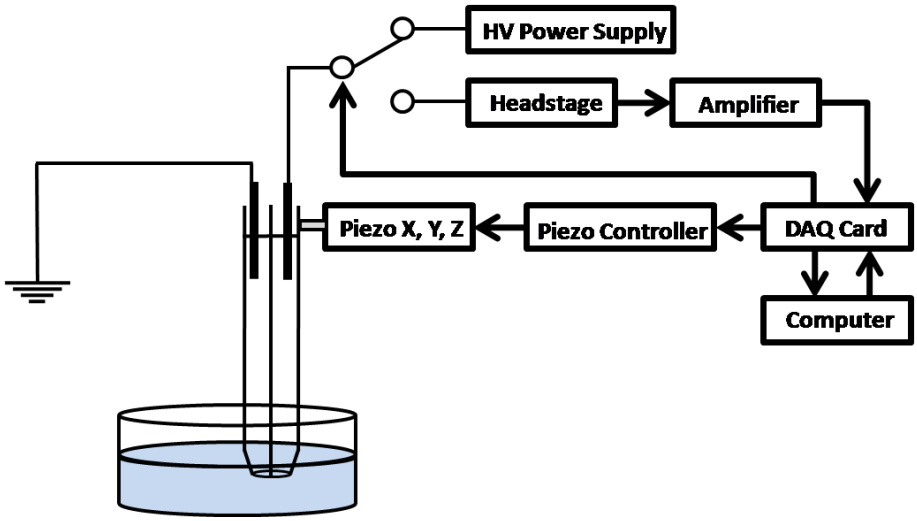

Figure S1. A schematic of the cell injection system. A standard Scanning Ion Conductance Microscope (SICM) is modified with a HV power supply and a custom relay for switching between the low voltages required for feedback control and the high voltages needed for material ejection. The system is controlled using custom-coded software written in LabVIEW.

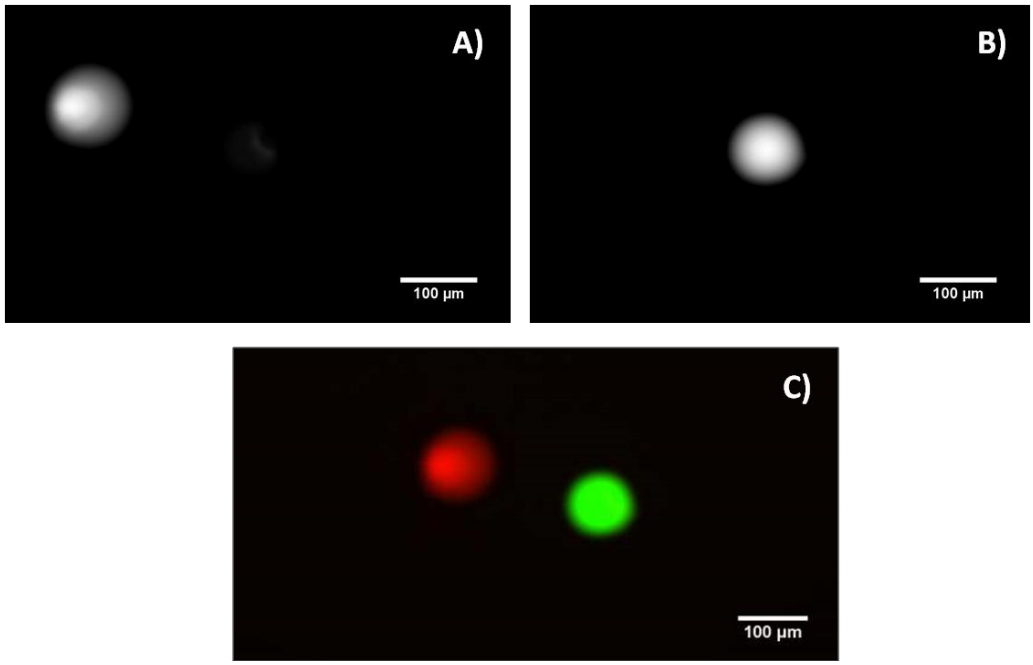

Figure S2. Fluorescent image of texas red (left/red spot) and carboxyfluorescein (right/green spot) spots deposited on a glass slide in mineral oil. A) red channel, B) green channel and C) False color image of both channels overlaid showing negligible cross-talk.

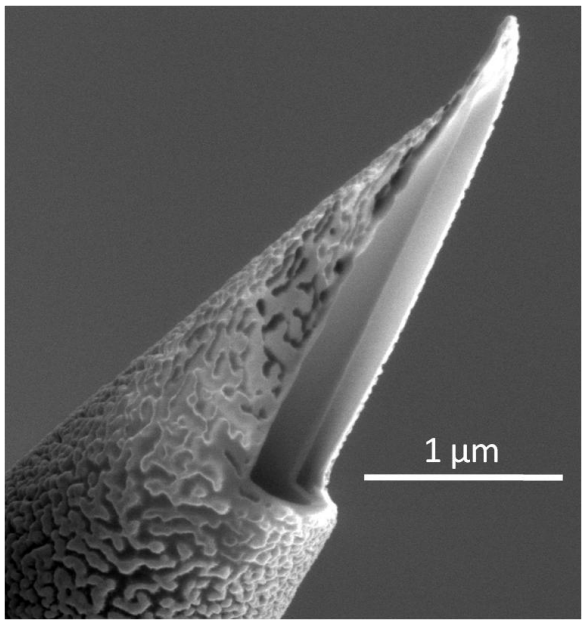

Figure S3. SEM micrograph of a Au-coated double barrel nanopipette after milling with a focused ion beam. One can clearly see the two barrels and the septum running all the way through the nanopores.

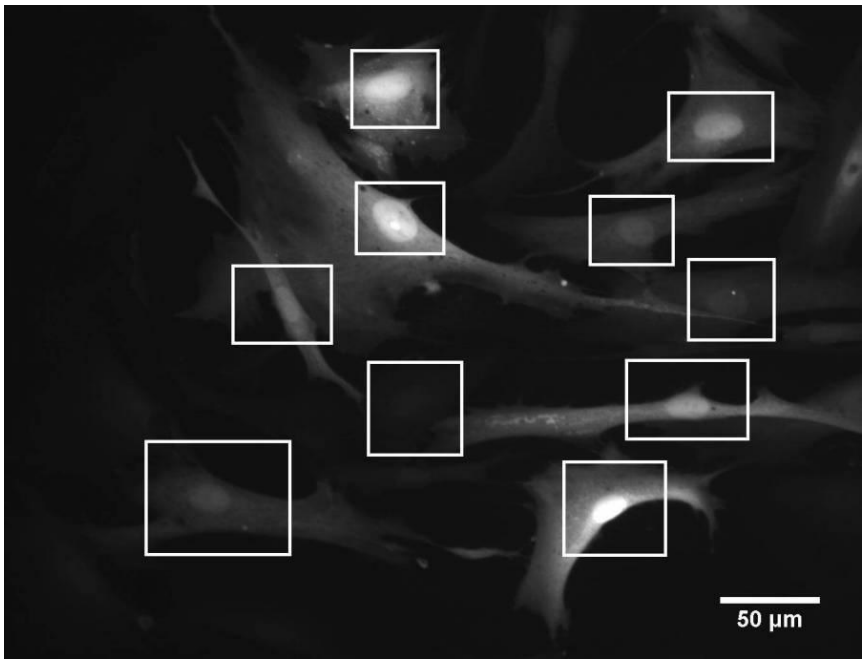

Figure S4. Fluorescent micrographs of adherent fibroblasts injected with a feedback-controlled system. All ten injected cells show detectable fluorescence (box highlights the nucleus). Entire procedure was completed in less than 5 minutes

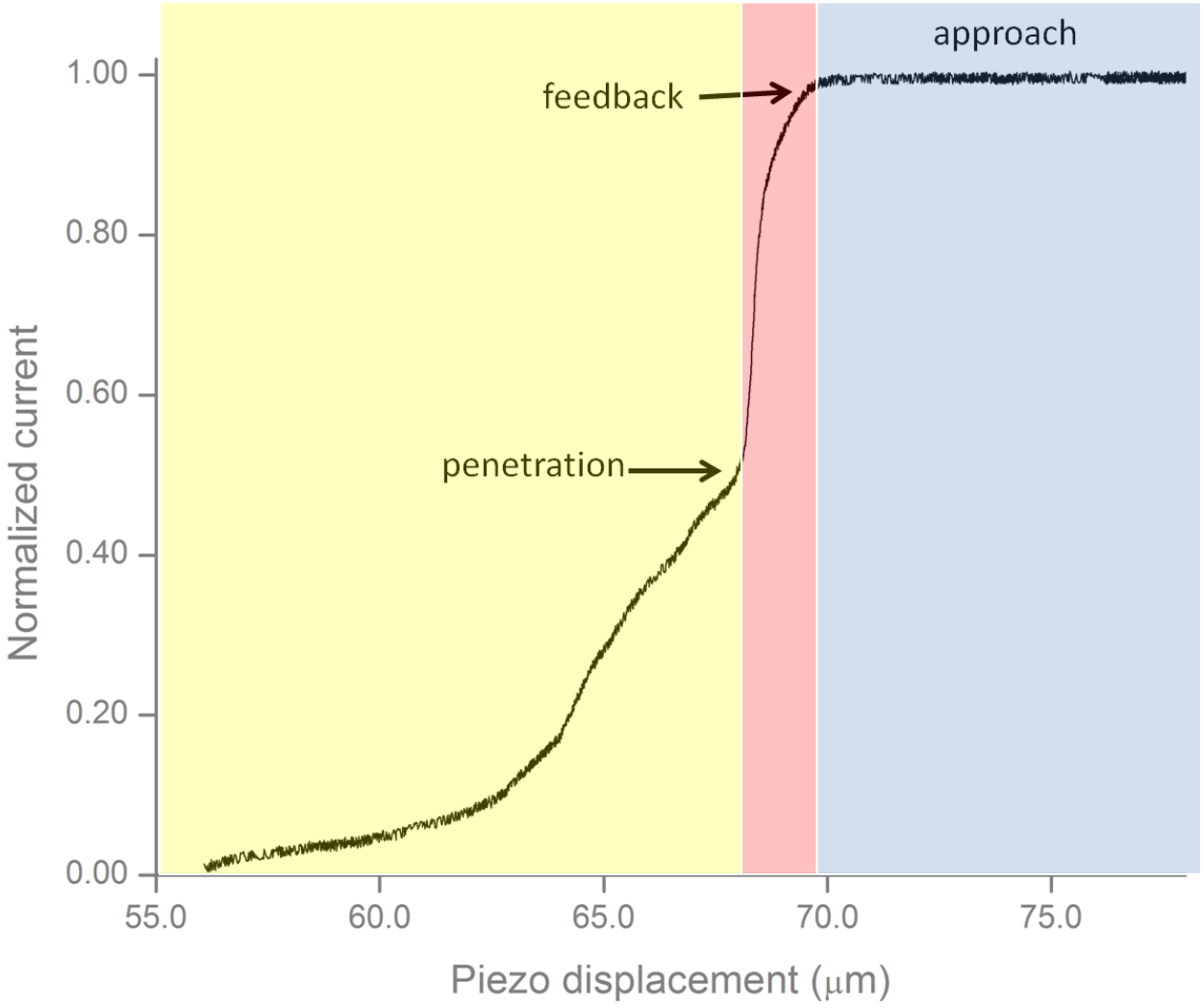

Figure S5. Approach curve of a double-barrel nanopipette where normalized ion current is plotted as a function of piezo displacement. One can indentify three separate regions. A steady-state region (approach) in which the ion current is unaffected by nanopipette-cell separations. A feedback region in which ion current is impeded by the proximity of the cell surface and a penetration region , in which the nanopipette crosses the cell membrane. Eventually the current goes to 0 when the nanopipette touches the bottom of the Petri dish where cells are cultured.

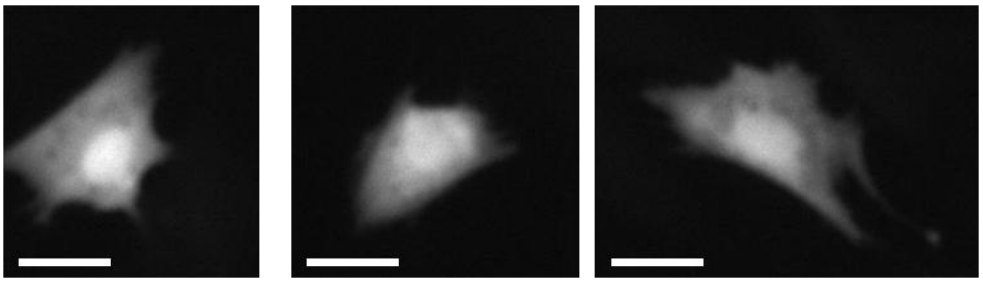

Figure S6. Fluorescent micrographs of human fibroblasts after injection (10V, 500ms) with CFSE. Scale bar 25 µm.

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