Supporting Information
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3D Bioelectronic Interface: Capturing Circulating Tumor Cells onto Conducting Polymer–Based Micro/Nanorod Arrays with Chemical and Topographical Control

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Experimental Procedures

1. Materials and Reagents

PEDOT Film Fabrication and Bioconjugation. 3,4-Ethylenedioxythiophene (EDOT, 97%), iron(III) *para*-toluenesulfonate hexahydrate [Fe(III)TOS], sodium persulfate (Na_2SO_8), and hydrazine (N_2H_4) were obtained from Sigma–Aldrich. Carboxyl-functionalized EDOT (EDOT-Ac) was synthesized according to a literature procedure (S.-C. Luo, E. M. Ali, N. C. Tansil, H. H. Yu, S. Gao, E. A. B. Kantchev, J. Y. Ying, *Langmuir* **2008**, *24*, 8071). Imidazole (IM) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were obtained from TCI; *N*-hydroxysuccinimide (NHS) was obtained from Sigma. Streptavidin (SA, 1 mg mL^{-1}) and biotinylated anti-human EpCAM/TROP1 antibody (Goat IgG) were obtained from R&D Systems.

Cell Studies. The breast cancer cell line (MCF7), lung cancer cell lines (A549, HCC827), prostate cancer cell line (PC3), glioma cell line (U87), cervical cancer cell line (HeLa), and white blood cells (WBC) were purchased from American Type Culture Collection. Streptavidin (1 mg mL^{-1}), GlutaMAX-I, DAPI, live/dead assay [calcein acetoxymethyl (Calcein AM) for live cells; ethidium homodimer-1 (EthD-1) for dead cells], Vybrant® DiD and DiO cell–labeling solution, DMEM, EMEM, and RPMI-1640 growth medium were obtained from Invitrogen. Fetal bovine serum (FBS) was obtained from Lonza BioWhittaker. Sodium citrate (10%, w/w) was used to prevent coagulation during blood collection.

2. Fabrication and Characterization of Silicon Micro/Nanorod Arrays

The surfaces of the (100)-oriented silicon (Si) wafers were made hydrophilic through the following procedure. The Si wafers were ultrasonicated in acetone and EtOH at room temperature for 10 and 5 min, respectively, to remove contamination from organic grease. The degreased Si substrates were placed in boiling Piranha solution [H_2SO_4/H_2O_2, 4:1 (v/v)] and RCA solution [$NH_3/H_2O_2/H_2O$, 1:1:5 (v/v/v)] for 1 h each and then rinsed several times with deionized water. The clean Si wafers were spin-coated with photoresist (TMHR ip3650, TOK) and then patterned using I-line projection photolithography (FPA-3000i5+ Stepper, Canon). Two designed metal masks were applied: with 10- and 2-μm square array patterns and period of 2 and 0.8 μm, respectively. After

photoresist development, the Si micro/nanopillar arrays were formed through inductively coupled plasma dry etching (ICP; MESC Multiplex, STS). Relevant parameters for ICP: power source, 600 W; bias power, 11.5 W; two working gases per duty cycle (25 sccm of SF_6 for 7 s; 75 sccm of C_4F_8 for 7 s). The positive Si micro/nanorod arrays were cleaned in boiling Piranha solution [H_2SO_4/H_2O_2, 4:1 (v/v)] to remove the remaining photoresist; treated with 1*H*,1*H*,2*H*,2*H*-perfluorooctyltrichlorosilane (FOTS; Alfa Aesar) as the anti-stiction coating; and then diced into silicon masters for subsequent negative poly(dimethylsiloxane) (PDMS) replica-molding.

3. PDMS Replicates

PDMS prepolymer was first prepared by combining the PDMS base and curing agent at a 10:1 (w/w) ratio (Sylgard 184, Dow Corning) and then blending with an electrical mixer. The mixture of PDMS prepolymer was poured onto the Si micro/nanorod arrays master, followed by pumping and thermal curing processes. The pumping process helped the PDMS prepolymer fill the nanostructures of the Si master; thermal curing conditions: 70 °C for 3 h. Finally, the obtained negative PDMS hole array replicates were ready for the subsequent transfer printing technique.

4. Poly(3,4-ethylenedioxythiophene) (PEDOT)-Based Micro/Nanorod Arrays

Chemical Oxidative Polymerization of PEDOT:TOS and PEDOT-Ac:TOS Films. Solutions of IM (1.47 M; inhibitor) and Fe(III)TOS (2.56 M; oxidizing agent) were first prepared in MeOH in separate flasks at 60 °C. The PEDOT precursor was prepared by mixing a separate vial containing the EDOT derivative (44 mg of EDOT or 67 mg of EDOT-Ac) with IM solution (1.8 mL) and the Fe(III)TOS solution (1.8 mL). For chemical oxidative polymerization, the mixture was spun (2000 rpm, 10 s) onto air plasma–treated negative PDMS replicas and then placed directly on a hotplate (105 °C) with the covered glass Petri dish. Air plasma treatment (10 mtorr, 30 s) of the PDMS replicates was performed using a plasma cleaner (Harrick Plasma, PDC-32G). After 10 min of polymerization, the coated wet film turned from a yellow solution into a dark-green/blue solid

film. This film was cooled to room temperature and washed three times with MeOH to remove excess Fe(III)TOS and unreacted EDOT monomers. Consequently, a demolding step with a biocompatible UV-curable polyurethane precursor (NOA65, Norland) was used as an adhesive between the PEDOT and glass substrate, thereby obtaining a uniform PEDOT-based micro/nanorod array films through PDMS transfer printing techniques.

Bioconjugation on PEDOT Films. The film surface was modified with EDC (0.2 M) and NHS (0.05 M) in water. The PEDOT-Ac:TOS film was treated with SA (10 μg mL^{-1}) for 90 min. Biotinylated anti-human EpCAM/TROP1 antibody (10 μg mL^{-1} in 1X PBS containing 0.1% BSA and 0.09% NaN_3; 25 μL) was placed onto the substrate, which was then incubated (60 min) at room temperature. The PEDOT films were washed several times with 1X PBS and then immersed in 1X PBS for 1 h prior to performing the cell experiments.

5. **Scanning Electron Microscopy (SEM)**

A scanning electron microscope (FEI Nova NanoSEM 200; accelerating voltage: 10 keV) was used to observe the surface morphologies of the Si masters **(Figure S1)**, the PEDOT-based micro/nanorod arrays (**Figure 2**), and CTCs on chips (**Figures S2**d and **S2**e). The biological and PEDOT-based samples were dehydrated in ascending grades of EtOH (25, 50, 75, and 100%; each dehydration time: 20 min), subjected to critical point drying with liquid CO_2, and sputter-coated with gold (<3 nm) prior to SEM examination.

6. **Surface Energies**

Contact angles were determined on each PDMS replica through geometric mean approximation (GMA) with two standard liquids—water (H_2O) and diiodomethane (CH_2I_2)—for each measurement; each condition was repeated three times. The surface energies of the pristine and air plasma–treated PDMS replicas were calculated using the GMA method.

6. Raman Spectroscopy

The structural phases of the PEDOT:TOS and PEDOT:PSS films were characterized using a micro-Raman spectrometer (alpha 300, WITec Instruments, Germany; resolution: 1 cm^{-1}; laser excitation: 514.5 nm). The 2D Raman mapping image of the PEDOT:TOS microrod array structure was observed according to the intensity of the signal at 1445 cm^{-1}.

7. <u>Cell Capture Experiments</u>

Biotinylated anti-EpCAM [10 μg mL^{-1} in PBS with 1% (w/v) BSA and 0.09% (w/v) NaN_3; 50 μL] was placed onto a substrate (1.5 cm × 2 cm), incubated (30 min), and then washed with PBS. The substrates were placed into a size-matched two-well Lab-TekTM Chamber Slide (Thermal Fisher) and then in cell suspensions (5×10^4 cells mL^{-1}, 1 mL) or artificial CTC samples, where the cancer cells and white blood cells, previously stained with DIO and DID, respectively, were loaded. After incubating the PEDOT device setup (1 h, 37 °C, 5% CO_2), the substrate was gently washed with 1x PBS (at least five times). Finally, imaging (**Figures 3**b–c) and counting (**Figures 4**a–b) of cells was performed using a fluorescence microscope (Nikon TE2000). Cell counts for dilution experiments were determined by counting three aliquots of cells in succession using a hemacytometer, which provided a cell-count accuracy within 10%. For the cell capture experiments from artificial CTC-containing blood samples, the samples were prepared by spiking EMEM medium (10 μL) of pre-stained MCF7 cells into serum (1 mL) from a healthy donor, with final MCF7 cell concentrations in the collections of artificial CTC-containing samples of 10, 50, 100, 200, 500, and 1000 cells mL^{-1}, respectively. For ease of detection, MCF7 cells were stained with DiO green fluorescent dye (Invitrogen) prior to adding them to the blood samples. The experiments were performed using the cell capture procedure described above. Color, brightness, and morphometric characteristics (cell size, shape, and nuclear size) were considered in identifying potential CTCs. As indicated in the inset to **Figure 4**b, cells that stained positively (green: DiO) and met the phenotypic morphological characteristics were scored as CTCs; they also possessed similar morphologies in SEM observations (**Figure S2**e).

8. Cell Viability and Live/Dead Assay for Captured Cells on PEDOTAc

With the high cell capture efficiencies presented by the **PEDOTAc** platform, cell viabilities were conveniently detected using a cell viability analyzer (Vi-CELL™ XR, Beckman Coulter). Following the capture procedure described above, the substrate-modified cancer cells were trypsinized and collected. The collected cells on each **PEDOTAc** system were finally prepared to a 1.0-mL cell suspension, which was then carefully transferred to a special sample container for the Vi-CELL™XR apparatus. The cell viability was analyzed in triplicate (**Figure 4**c). Under the optimized cell capture conditions, the cell viability using anti-EpCAM–bound **PEDOTAc** was as high as 97%; this substrate was optically transparent and conducive to subsequent molecular biological diagnosis of cells and their culturing for long-term study. Furthermore, a Live/Dead fluorescence staining assay (**Figure S3**) was employed, identifying live cells using calcein AM (green) and dead cells using EthD-1 (red).

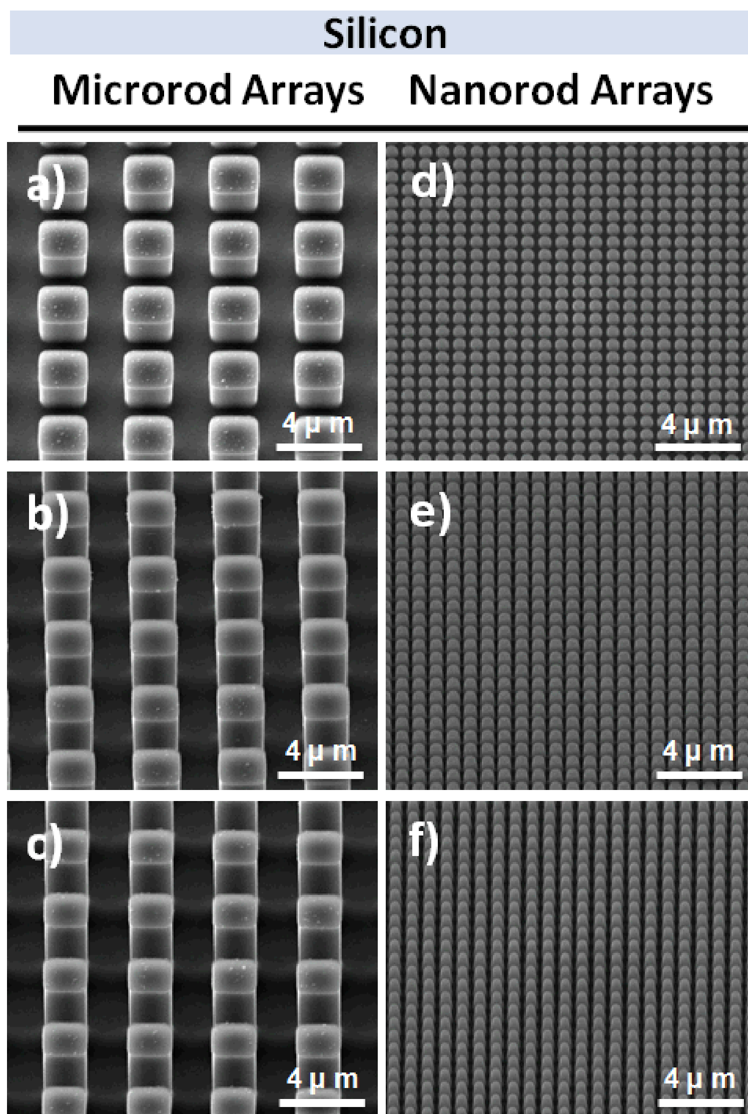

Figure S1. SEM images of Si micro/nanorod arrays: a) **Si-*2***, b) **Si-*4***, c) **Si-*6***, d) **Si-*0.4***, e) **Si-*0.8***, and f) **Si-*1.2***.

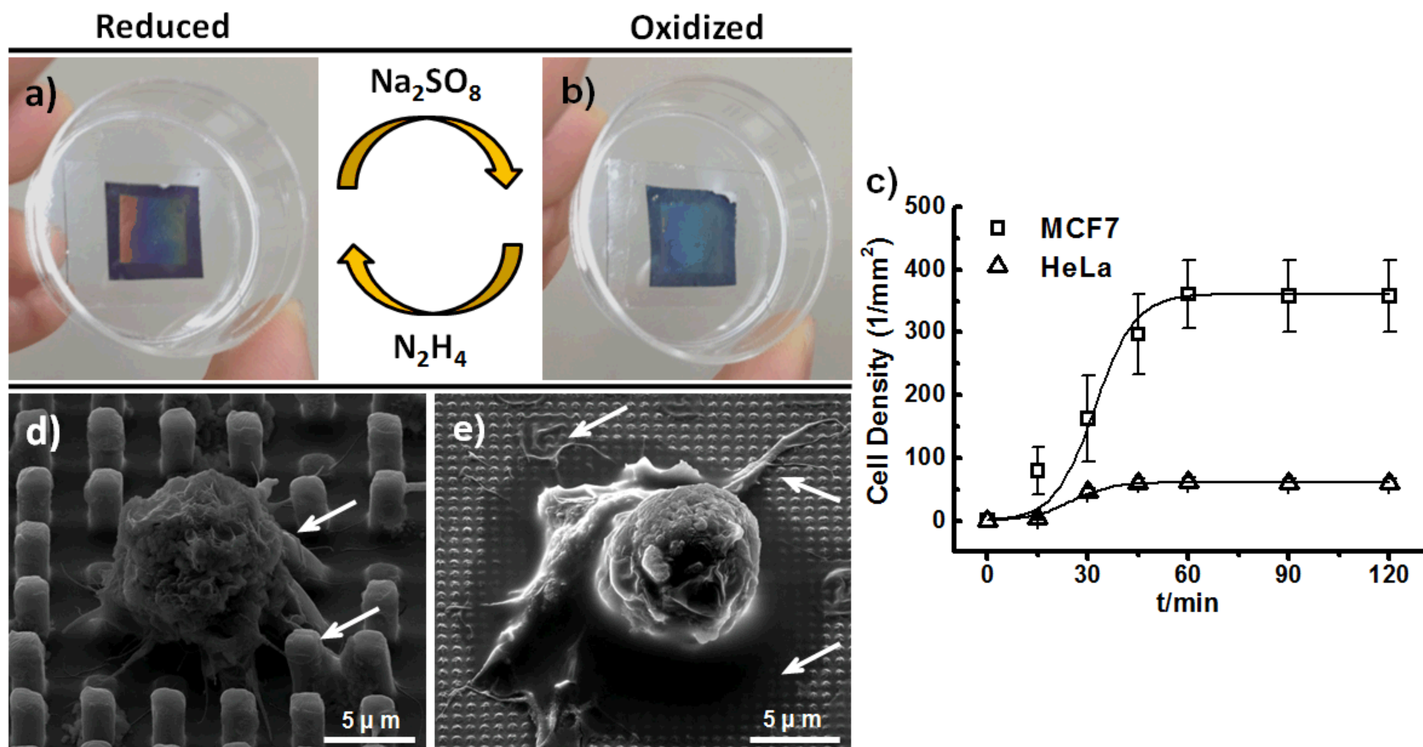

Figure S2. a, b) Oxidation/reduction states of **PEDOTAc** films: a) reduced state (treated with N_2H_4); b) oxidized state (treated with aqueous Na_2SO_8). c) Quantitative evaluation of cell-capture yields at different capture times. Each plot and error bar represents a mean standard deviation from three repeats. d, e) SEM images of captured MCF7 cells on d) **PEDOTAc-*6*** and e) **PEDOTAc-*0.4***.

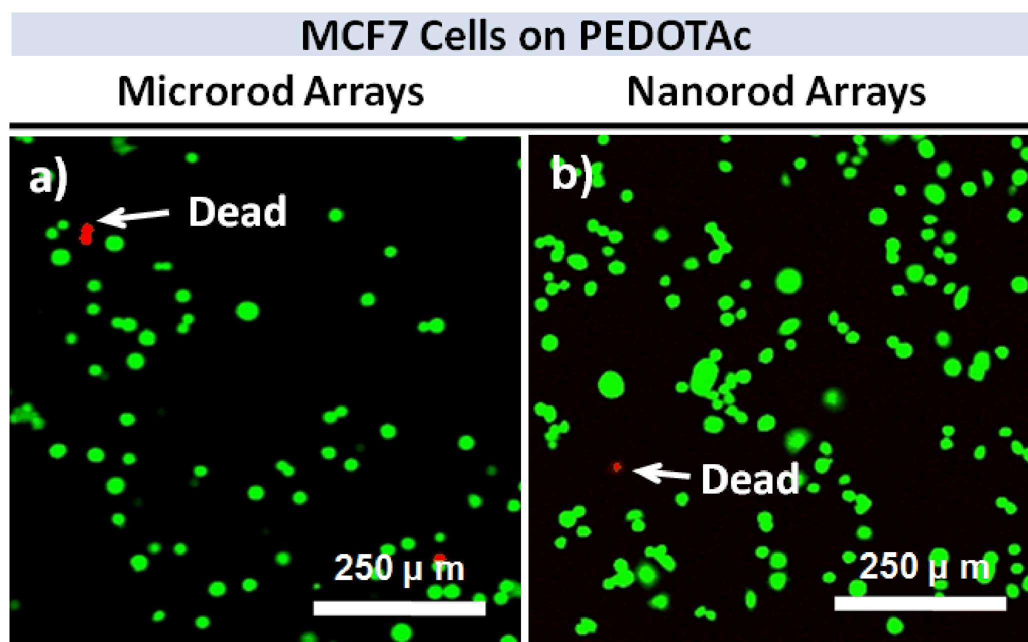

Figure S3. Viability of captured MCF7 cells on PEDOT-based micro/nanorod array films: a) **PEDOTAc-*6***; b) **PEDOT-*0.4***.

Table S1. Contact angles for H_2O and CH_2I_2 and corresponding surface energies (γ) of plasma-treated PDMS

Plasma applied on PDMS	Contact angle (deg)		γ^a (mJ mm^{-2})
	H_2O	CH_2I_2	
None	129.1	108.2	8.67
Air	24.1	58.6	27.92

a: Calculated using the GMA from contact angle measurements of H_2O and CH_2I_2.

Table S2. Geometric parameters of Si micro/nanorod arrays masters

Entry **Si-*X*** [a]	Geometry [c]		
	Width (W; μm)	Period (P; μm)	Height (H; μm)
Si-*FLT*	–	–	–
Si-*2*	2	2	2
Si-*4*	2	2	4
Si-*6*	2	2	6
Si-*0.4*	0.4	0.8	0.4
Si-*0.8*	0.4	0.8	0.8
Si-*1.2*	0.4	0.8	1.2

a: Values of W, P, and H of the Si micro/nanorod arrays were identified using SEM.