Supporting Information for

The Selective Capture and Collection of Live Target Cells Using a Photoreactive Silicon Wafer Device Modified with Antibodies via a Photocleavable Linker

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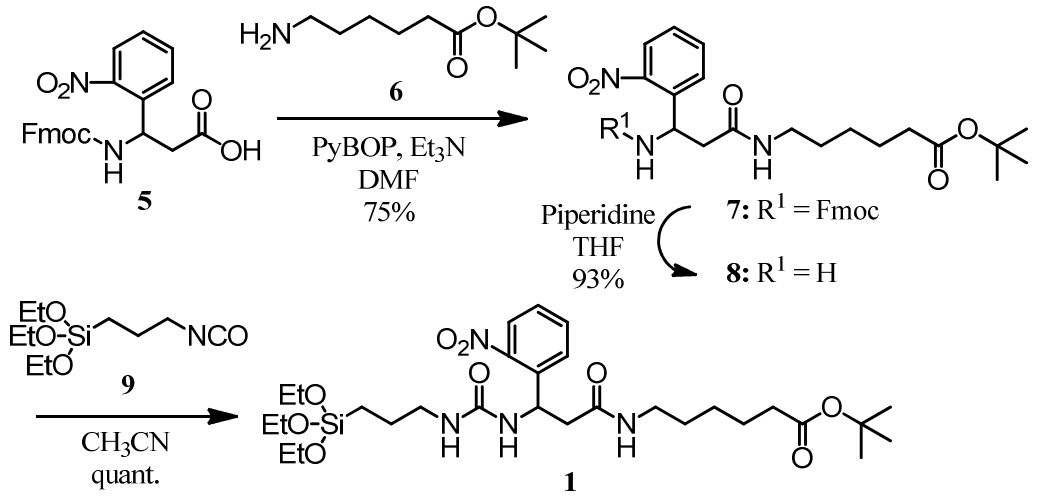
General Information. Reagents and solvents were purchased at the highest commercial quality and used without further purification. Goat polyclonal secondary antibody to mouse IgG-HL conjugated with AP (2nd IgG–AP) was purchased from Abcam. All aqueous solutions were prepared using deionized and distilled water. IR spectra were recorded on Perkin-Elmer FT-IR spectrophotometer (Spectrum100) at room temperature. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a JEOL Always 300 spectrometer. Tetramethylsilane was used as an internal reference for ^{1}H and ^{13}C-NMR measurements in $CDCl_3$. MS measurements were performed on a JEOL JMS-SX-102A and Agilent (Varian) 910-MS. Thin-layer (TLC) and silica gel column chromatography were performed using a Merck Silica gel 60 F_{254} TLC plate and Fuji Silysia Chemical FL-100D, respectively.

Scheme S1. Synthesis of the photocleavable ANP linker 1.



Synthesis of *t*Bu-Protected ANP 8. *t*-Butyl ester of *ε*-capronic acid **6** (40.5 mg, 0.216 mmol) was added to a solution of Fmoc-protected ANP **5** (81.3 mg, 0.188 mmol) in DMF (5 mL), to which Et_3N (31.5 μL, 0.216 mmol) and benzotriazol-1-yl-oxy-tris-pyrolidino-phosphinium hexafluorophosphate (PyBOP) (195.7 mg, 0.376 mmol) was added at room temperature. The reaction mixture was stirred at room temperature for 12 hr. The solvent was removed under reduced pressure and the resulting residue

was purified by silica gel column chromatography ($CHCl_3/CH_3OH$) to give the crude Fmoc-*t*Bu-protected ANP **7** as a pale yellow amorphous solid (82.3 mg, 73% crude yield), which was used without further purification for the synthesis of **8**.

Piperidine (600 μL) was added to a solution of crude **7** (82.3 mg, 0.137 mmol) in tetrahydrofuran (THF) (3 mL) at room temperature and the reaction mixture was stirred for 12 hr at the same temperature. The solvent was evaporated under reduced pressure and piperidine was removed by azeotropic distillation with CH_3OH to give a pale yellow amorphous solid. The crude material was purified by silica column chromatography ($CHCl_3/CH_3OH$) to give **8** as pale yellow amorphous solid (48.4 mg, 93% yield). IR (neat): ν = 3289, 3071, 2971, 2935, 2866, 1726, 1646, 1525, 1447, 1366, 1352, 1248, 1211, 1152, 1088, 1015, 956, 916, 855, 787, 751, 711, 668, 584 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.23–1.65 (15H, m), 2.22 (2H, t, *J* = 7.3 Hz), 2.50–2.68 (2H, m), 3.26 (2H, q, *J* = 6.6 Hz), 4.77 (1H, dd, *J* = 3.3, *J* = 9.5 Hz), 7.41 (1H, t, *J* = 8.1 Hz), 7.62 (1H, t, *J* = 7.7 Hz), 7.86–7.77 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.47, 26.19, 27.95, 29.00, 35.20, 39.03, 43.57, 46.08, 48.43, 124.21, 127.92, 133.24, 139.42, 148.39, 168.53, 170.61, 172.92. MS (*m*/*z*). Calcd for $C_{19}H_{30}N_3O_5$ ($[M+H^+]$): 380.2185 Found: 380.2180.

Synthesis of ANP Linker 1. A **s**olution of **8** (48.4 mg, 0.128 mmol) in CH_3CN (1 mL) was added dropwise to a solution of 3-(triethoxysilyl)propyl isocyanate **9** (30.4 mg, 0.128 mmol) in CH_3CN (2 mL) at 0 °C under an argon (Ar) atmosphere. The reaction mixture was stirred at room temperature for 12 hr. The solvent was removed under reduced pressure to give **1** as an orange amorphous solid (82.9 mg, quant.). IR (neat): ν = 3300, 3097, 2976, 2931, 2885, 1731, 1644, 1560, 1530, 1480, 1442, 1392, 1367, 1349, 1259, 1154, 1104, 1080, 958, 859, 790, 746 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.60 (2H, t, *J* = 8.2 Hz), 1.22 (9H, t *J* = 7.0 Hz), 1.35–1.55 (8H, m), 2.18 (2H, t, *J* = 7.3 Hz), 2.60–2.85 (2H, m), 3.10–3.22 (4H, m), 3.81 (6H, q, *J* = 7.0 Hz), 4.70 (1H, t, *J* = 4.8 Hz), 7.38 (1H, t, *J* = 7.0 Hz), 7.55–7.66 (2H, m), 7.94 (1H, d, *J* = 7.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 7.56, 18.24, 23.42, 24.38, 26.07, 28.05,

28.84, 35.19, 39.19, 41.49, 42.92, 48.45, 58.35, 124.73, 127.93, 128.52, 133.50, 138.46, 147.97, 157.17, 170.67, 173.05. MS (*m*/*z*). Calcd for $C_{29}H_{51}N_4O_9Si$ ([M+H^+]): 627.3425 Found: 627.3421.

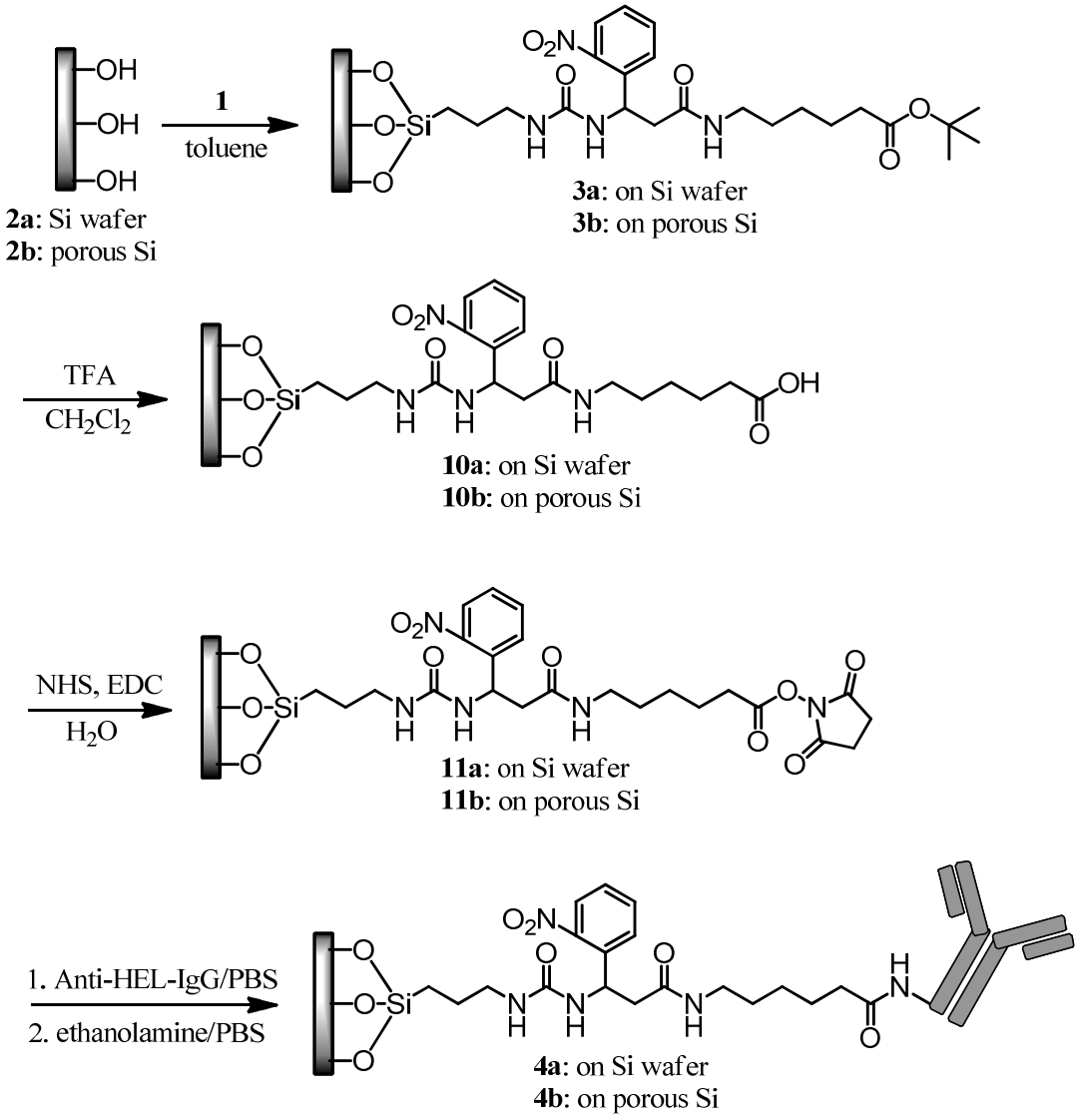
Preparation of anti–HEL–IgG. Hybridoma producing anti–HEL C2 (HyC2) was provided by Drs. Akemi Ota and Hajime Fujio of Osaka University. Anti–HEL–IgG was purified from culture supernatants using an HEL–Sepharose colum as described previously. (Oda, M.; Uchiyama, S.; Robinson, C. V.; Fukui, K., Kobayashi, Y.; Azuma, T. Reginonal and Segmental Flexibility of Antibodies in Interaction with Antigens of Different Size. *FEBS J.* **2006**, *273,* 1476–1487.)

Preparation of Si Wafer 2a. The Si wafers (100) (thickness: 525 μm, resistivity: 10~20 Ω·cm) were treated with 46 % HF solution for 1.5 min, and the wafers were then heated at 950 °C under a dried O_2 atmosphere for 30 min in an infrared heating oven (ULVAC MILA-3000) in order to thermally-oxidize the surface.

Preparation of Porous Si Wafer 2b. Double-face Si wafers (100) (thickness: 200 μm, resistivity: 0.008~0.02 Ω·cm) was treated with 46% HF solution for 1.5 min. Anode oxidation of Si wafer was carried out in electrolysis solution (ultra-pure water : 46% HF : ethanol = 5:3:2) at 10 °C for 200 seconds under 70 mA/cm^2. After Si wafer was washed with acetone, reactive ion etching (FWD: 30 W) of Si wafer was carried out under SF_6 (18 sccm) and O_2 (4.0 sccm) for 30 seconds to remove the micro-porous layer.

Modification of Si Wafer with 1. The modification of the Si wafer with the ANP linker **1** was carried out as shown in Scheme S2 in the Supporting Information. The Si wafer (7.5 mm × 7.5 mm) **2a** was reacted with **1** in anhydrous toluene at 80 °C for 3 hr. After modification, the wafer was sonicated with $CHCl_3$ and dried in a stream of Ar gas to give **3a**. *t*Bu group of **3a** were deprotected by TFA/CH_2Cl_2 (1:2) at room temperature for 3 hr and these materials were then washed by sonication in $CHCl_3$ and H_2O and dried by a stream of Ar gas to give **10a**. Next, **10a** was reacted with 5 mg/mL NHS and 50 mg/mL EDC in H_2O solution at room temperature for 3 hr and the wafer was then sonicated with H_2O and dried in a stream of Ar gas to give **11a**. Finally, **11a** was treated with PBS solution containing 2 μg/mL anti–HEL–IgG at room temperature for 12 hr and a blocking reaction with 0.1 M ethanolamine PBS solution at room temperature for 3 hr. After modification, the Si wafer was washed by gentle shaking in PBS containing 0.05% (w/v) Tween 20 (PBST) and in PBS to give **4a** in PBS. Similarly, the porous Si (15 mm × 15 mm) **2b** was reacted with **1** and then converted to **4b**. Porous Si at each modification steps was not washed by sonication. Rather, these samples were washed by gentle shaking so as not to destroy their microstructure.

Scheme S2. Modification of Si wafers with the ANP linker 1.



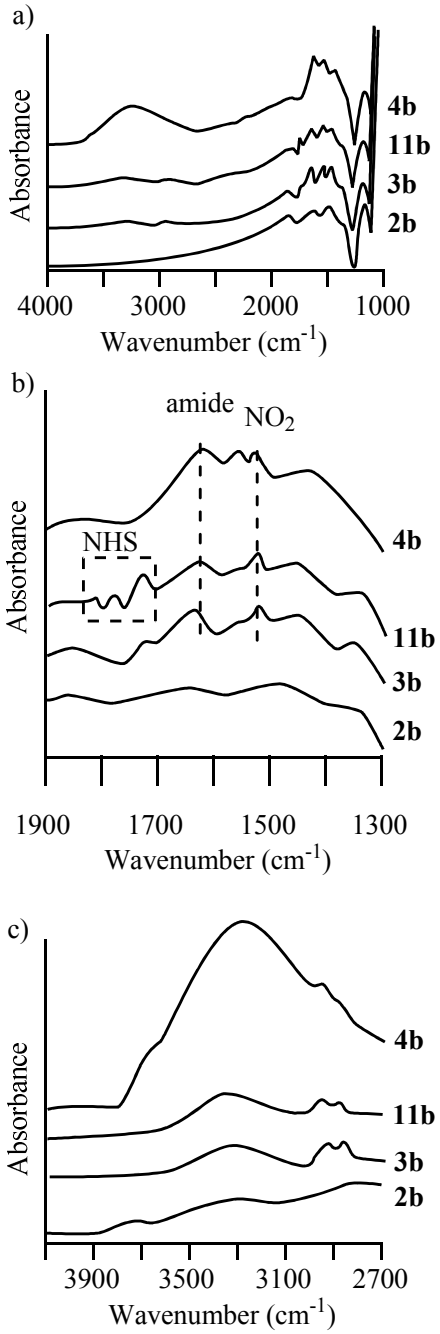


Figure S1. FTIR-ATR spectra of IgG-modified porous Si **4b** in the 4000 to 1000 cm^{-1} (a), 1900 to 1300 cm^{-1} (b) and 4000 to 2700 cm^{-1} (c)

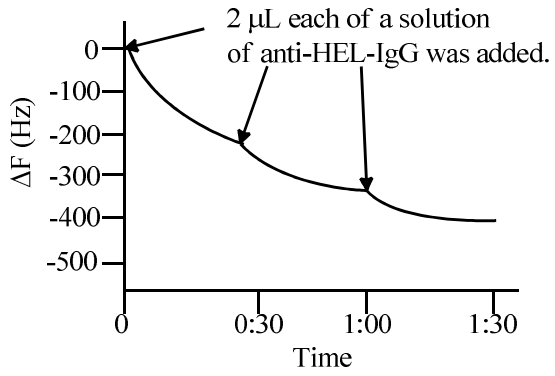
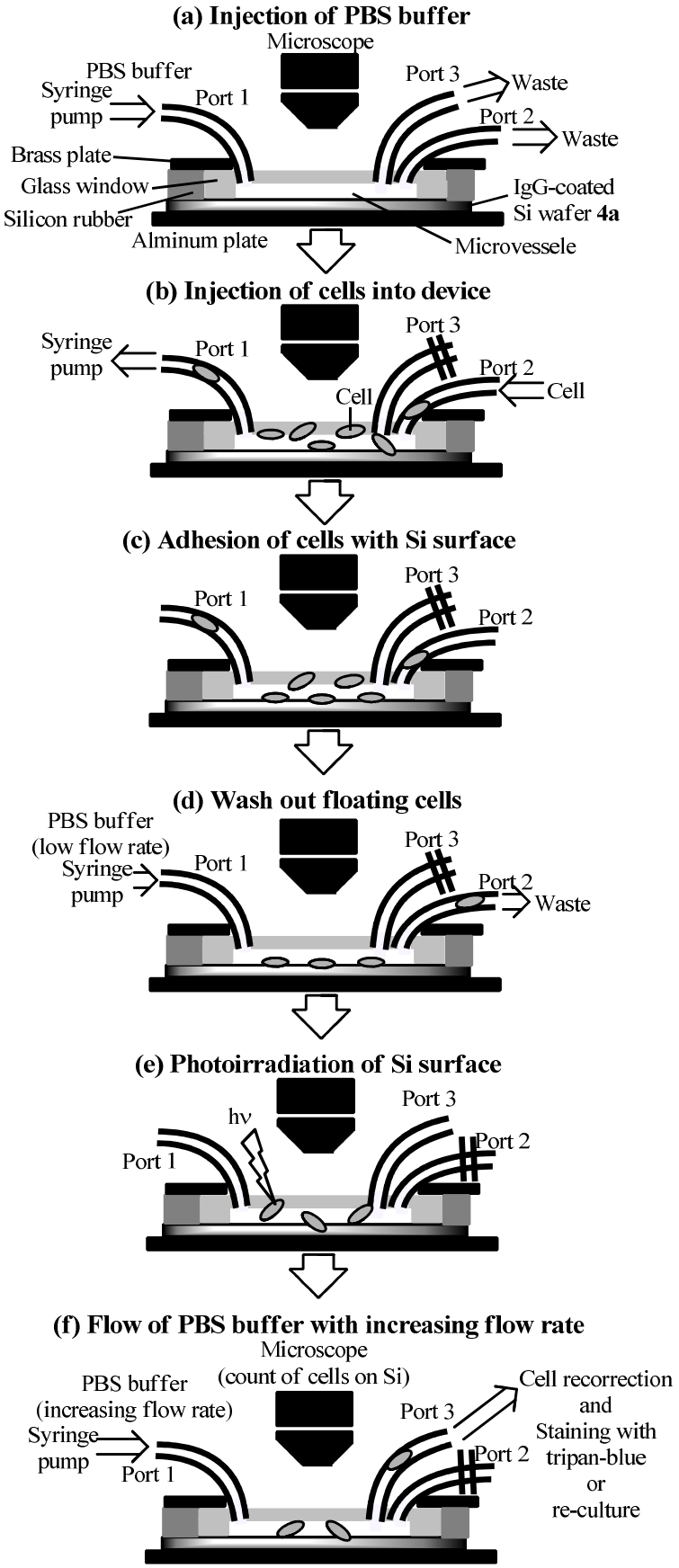


Figure S2. 27 MHz QCM results for the interaction between anti–HEL–IgG and HEL. Time course for frequency change of QCM. The association constant K_a was estimated to be 1.35×10^8 [M^{-1}] (dissociation constant K_d = 7.4 nM) from these curves (Ref. 54 in the text).

Scheme S3. Selective cell capture and recollection of SP2/O–HEL cells and SP2/O cells on the Si wafer device shown in Figure 3.



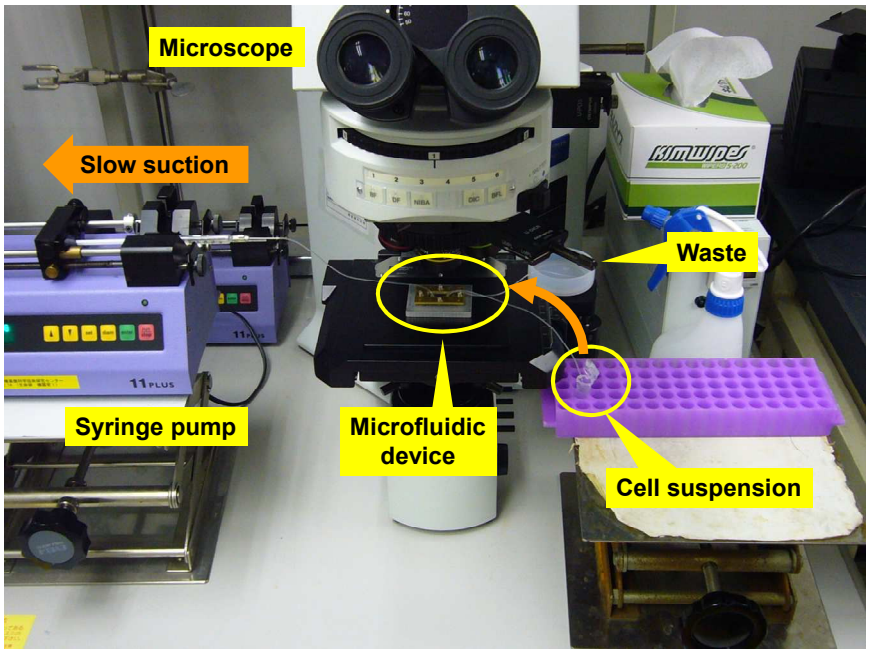


Figure S3. Whole system including microfluidic device, microscope, and syringe pump.

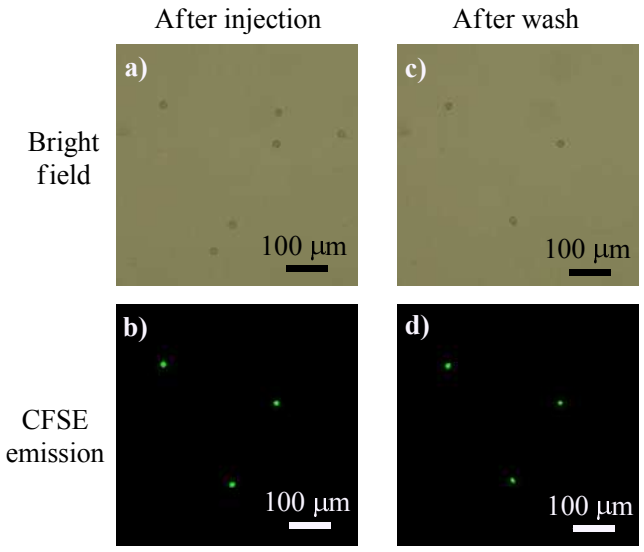


Figure S4. Brightfield (a) and fluorescent (b) images of SP2/O–HEL–CFSE and SP2/O on **4a** before wash and brightfield (c) and fluorescent (d) images of **4a** after wash process.

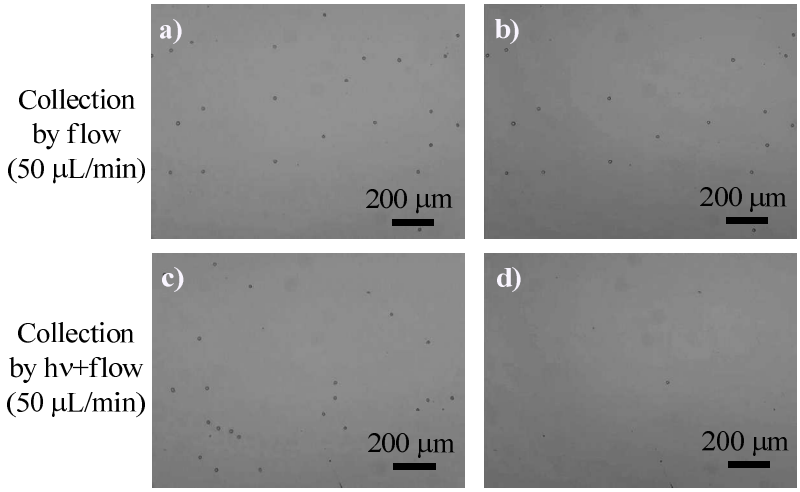


Figure S5. Microscopic images showing the collection of SP2/O–HEL by flow only and flow + photoirradiation. Microscopic images of SP2/O–HEL on **4a** after incubation for 30 min under dark conditions (a) and after successive flow of PBS at a rate of 50 μL/min (b). Microscopic images of SP2/O–HEL on **4a** after photoirradiation at 365 nm for 30 min (c) and after successive flow of PBS at a rate of 50 μL/min (d).

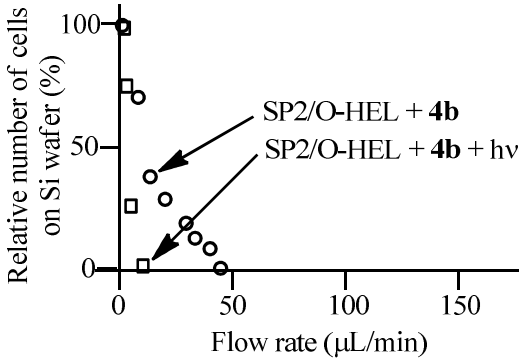


Figure S6. Relative numbers of cells observed on the porous Si wafer with increasing PBS buffer flow rate. Relative numbers of SP2/O–HEL on porous silicon wafer **4b** before or after photoirradiation are shown as open circles and open squares, respectively.