Supporting Information

Double-Sided Microwells with a Stepped Through-Hole Membrane for High-Throughput Microbial Assays

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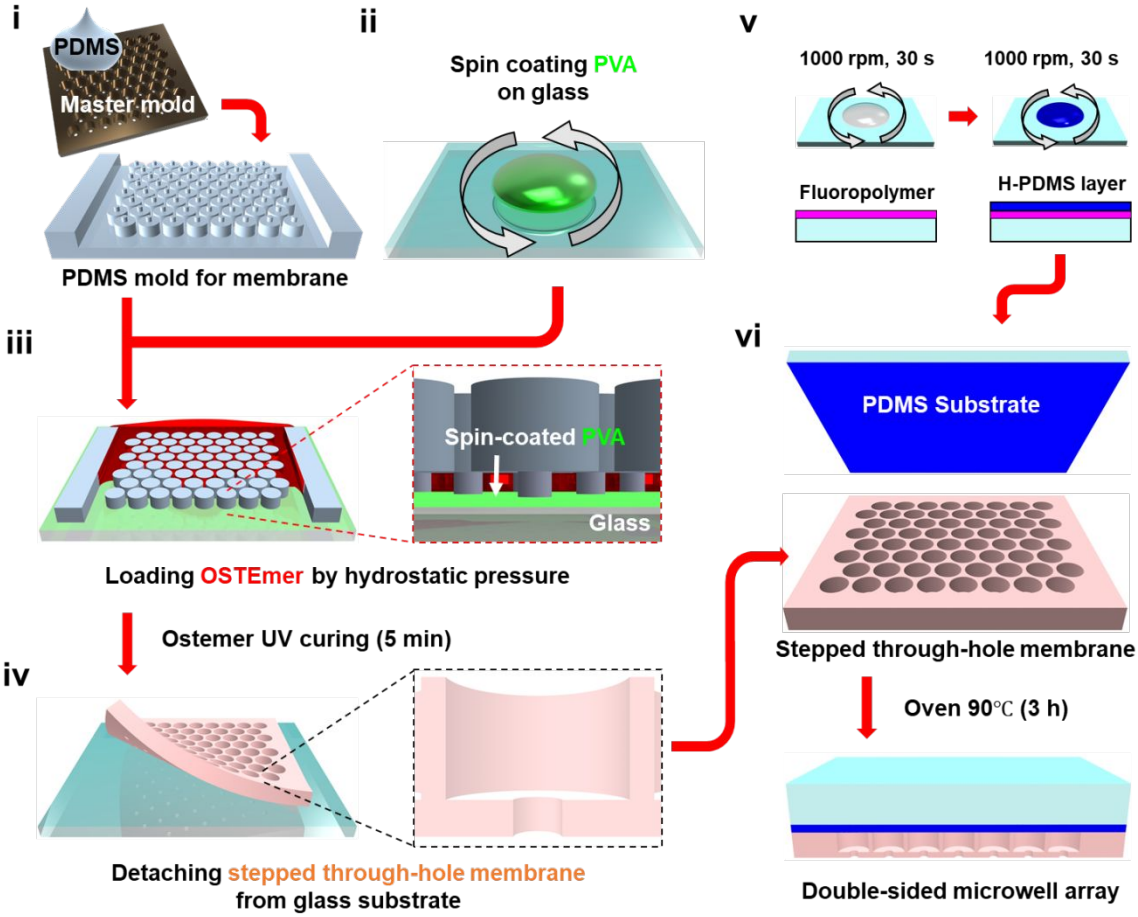

Figure S1. Fabrication processes of the double-sided microwell array (i) The PDMS mold of the culture membrane is prepared by pouring the PDMS mixture on an SU-8 master mold. (ii) PVA is spin-coated on clean glass at 1000 rpm for 30 s. PVA functions as a water-soluble sacrificial layer. (iii) The PDMS mold is placed on PVA-coated glass; then, the Ostemer resin is loaded by hydrostatic pressure. (iv) UV curing allows the OSTEmer resin to remain solid but soft. The PVA sacrificial layer is removed by dipping the glass in water. When the PVA is completely dissolved in the water, the Ostemer membrane can be easily disassembled from the glass. (v) PDMS is spin-coated with a fluoropolymer followed by H-PDMS at 1000 rpm for 30 s. (vi) H-PDMS is treated with oxygen plasma and, then, vapor-deposited with GPTMS. The membrane was covered with H-PDMS and baked in an oven at 90 °C for 3 h for tight bonding. The baking process also fully cures and hardens the membrane.

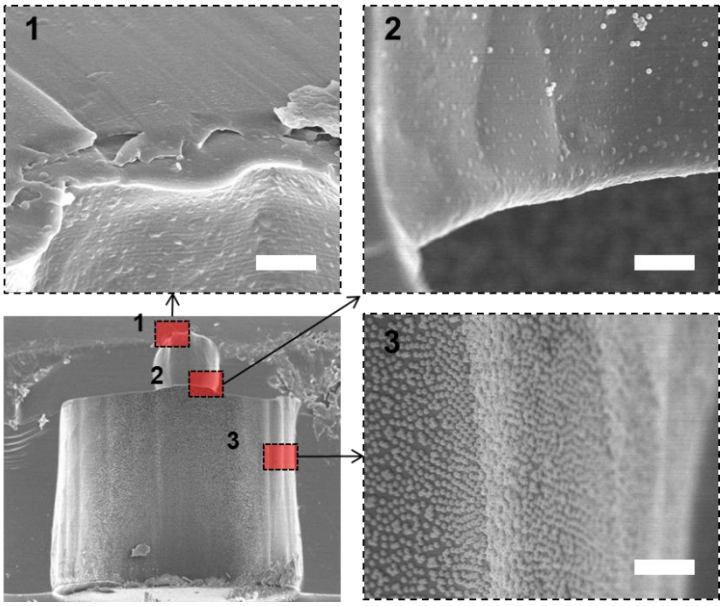

Figure S2. Surface functionalization of the fabricated hydrophilic-in-hydrophilic microwell. SEM image shows the cross-sectional side view of a microwell. The microwell was treated with oxygen plasma, and the amine group was attached to the treated surface with aqueous 10% APTES solution. Then, the device was baked in an oven at 85 °C for 15 min. To confirm the surface functionalization with APTES, we loaded a suspension of negatively charged 200-nm carboxylated polystyrene particles into the microwell and, then, washed the suspension thoroughly with water. As a result, only the interior of the microwell had many electrostatically attached particles (image 3) due to the positively charged amine group, whereas the exterior surfaces were clean and not functionalized with APTES (image 1). Partially attached particles in image 2 suggest that the liquid-air interface of 3M Novec 1720 is formed in the vicinity of the inlet holes during the solution-processed fluoropolymer coating. Scale bars indicate 10 μm.

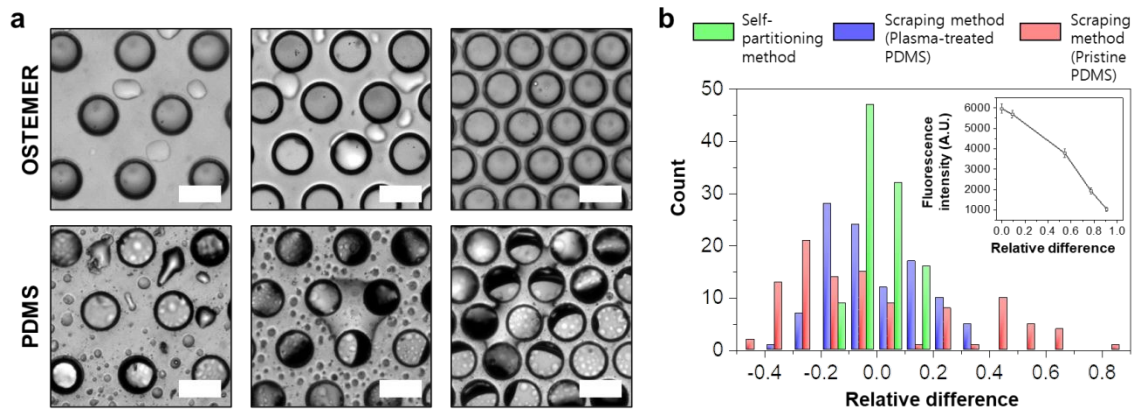

Figure S3. Partitioning of bacterial samples using the common scraping method. (a) One member of our group, who had no experience with microwell arrays, conducted the common scraping-based partition of bacterial samples. The images show that many microwells interconnected with the bridged liquid were formed as the density of the microwells increased. In addition, in case of the PDMS-based microwell array, the scraping force deformed the microwell, resulting in deteriorated loading of liquid in the microwells. Therefore, the partition requires delicate control of scraping by proficient experts. The scale bar indicates 100 μm. **(b)** Relative difference in partitioned sample volumes in microwells for three different conditions. Fluorescence intensity of FITC solution (50 μM FITC, 1× PBS, 0.002% pluronic F-127) was measured to quantify the relative volume of the partitioned samples in microwells. Inset graph shows the calibrated relation between known volumes of FITC solution and intensity values. The calibration was done with different heights of microchannels filled with the FITC solution. Structure of the microchannels was mimicked to the microwell structure. We set volume of a microwell as the reference volume. Plasma-treated PDMS is hydrophilic while pristine PDMS is hydrophobic. The self-partitioning method developed in this work has less deviation than the two scraping methods, demonstrating higher uniformity.

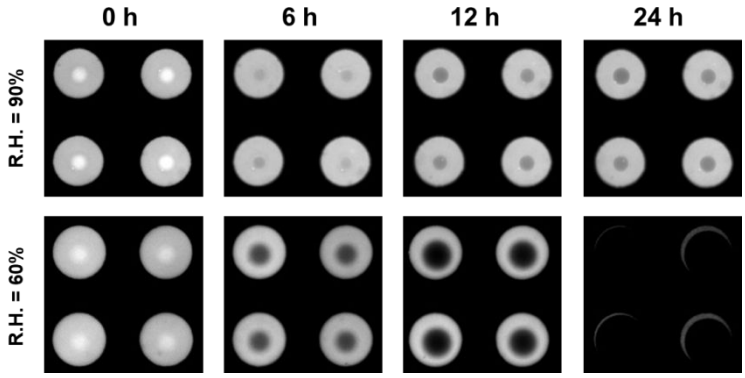

Figure S4. Partitioned FITC solution in different humidity conditions. The fluorescence images show partitioned FITC solution in different humidity conditions. There was no noticeable evaporation of liquid at high humidity (RH = 95%) over 24 h, in contrast to that at low humidity (RH = 60%).

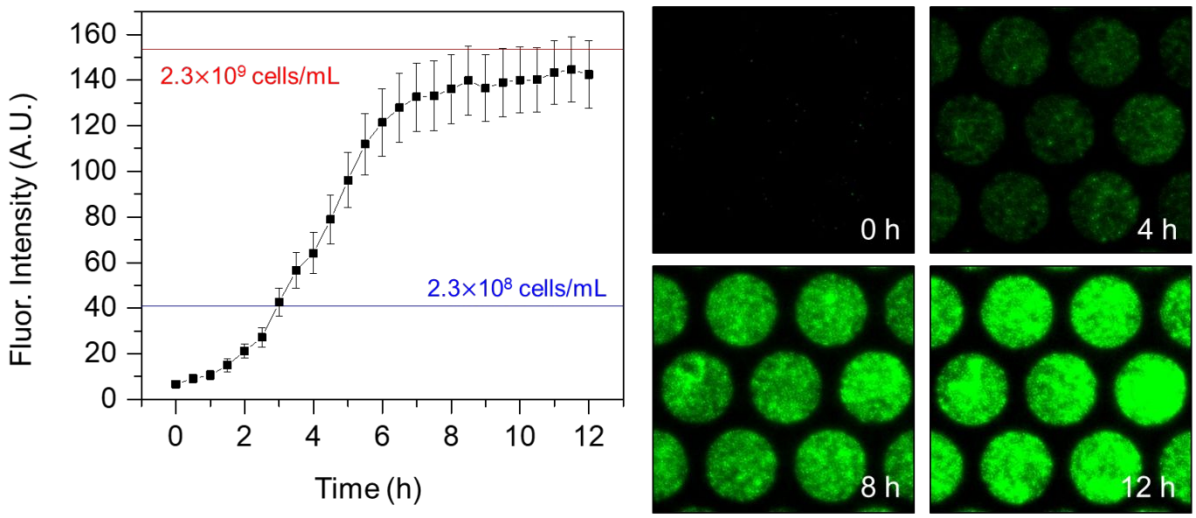

Figure S5. Growth curve of bacterial cells in the microwell array. The graph shows the fluorescence intensity of GFP cells in each microwell over 12 h of culture. Error bar is standard deviation of microwells in a chip. Red and blue lines indicate the fluorescence intensities of cells corresponding to 2.3×10^9 and 1.9×10^8 cells mL^{-1} respectively. For the substitution of bacterial density with the fluorescence intensities, we loaded the cells in a microwell array with known bacterial density and measured their fluorescence intensity.

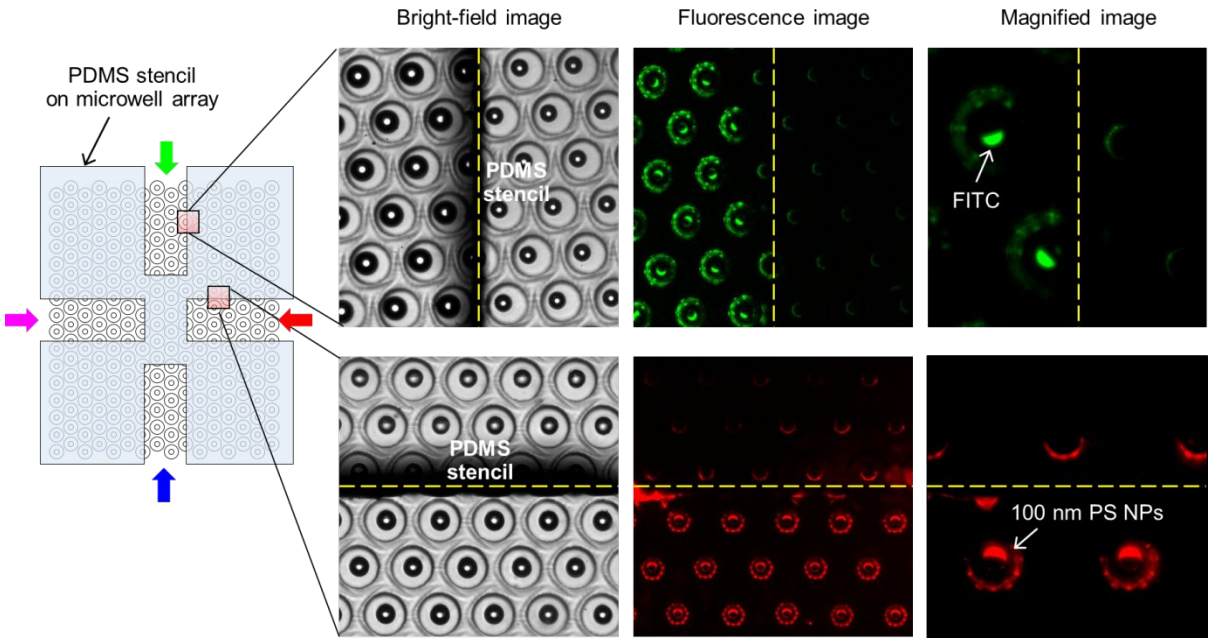

Figure S6. Stencil-assisted injection of multiple chemicals on a chip. Schematic illustration depicts that the side of small holes is covered with a PDMS frame with four rectangular window areas. The PDMS frame works as a stencil to aid loading of different solutions into the microwells separately. FITC solution (250 μM FITC, 1× PBS, 0.002% Pluronic F-127) and 100 nm NP suspensions (0.1% carboxylated PS NPs, 0.005% Pluronic F-127) were individually loaded and aspirated by micropipette. The bright-field and fluorescence images show that FITC molecules and nanoparticles are partially injected onto the rim of the small holes without dissolving in the partitioned aqueous samples. The molecules and nanoparticle are systematically introduced into the microwell via condensation. The results mean that further development of microfabricated stencils having microchannel arrays such as gradient generators would enable to inject various chemical conditions in a high-throughput, simple, and one-step manner with a single stencil.

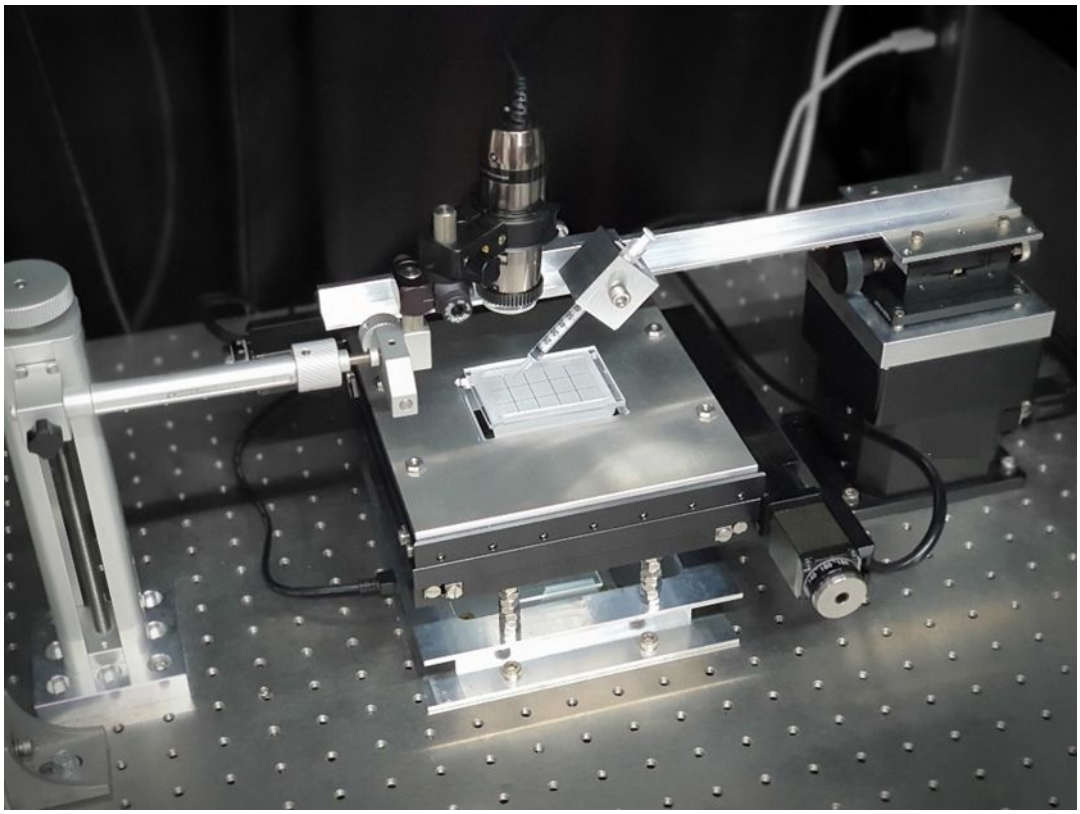

Figure S7. Experimental setup for the hatching-like extraction.

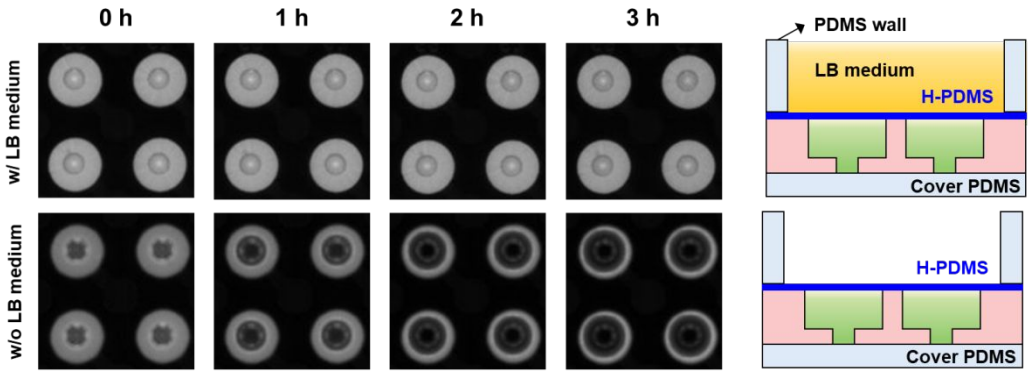

Figure S8. Preventing the permeation of water via H-PDMS. After preparing the microwell array for the hatching-like extraction as illustrated in the schematics, we compared two conditions (with/without LB medium). The presence of the LB medium on the H-PDMS layer was critical in preventing the evaporation of water in microwells. As most automated extraction processes are difficult to perform in humid conditions, the technique involving covering of the LB medium is of great importance.

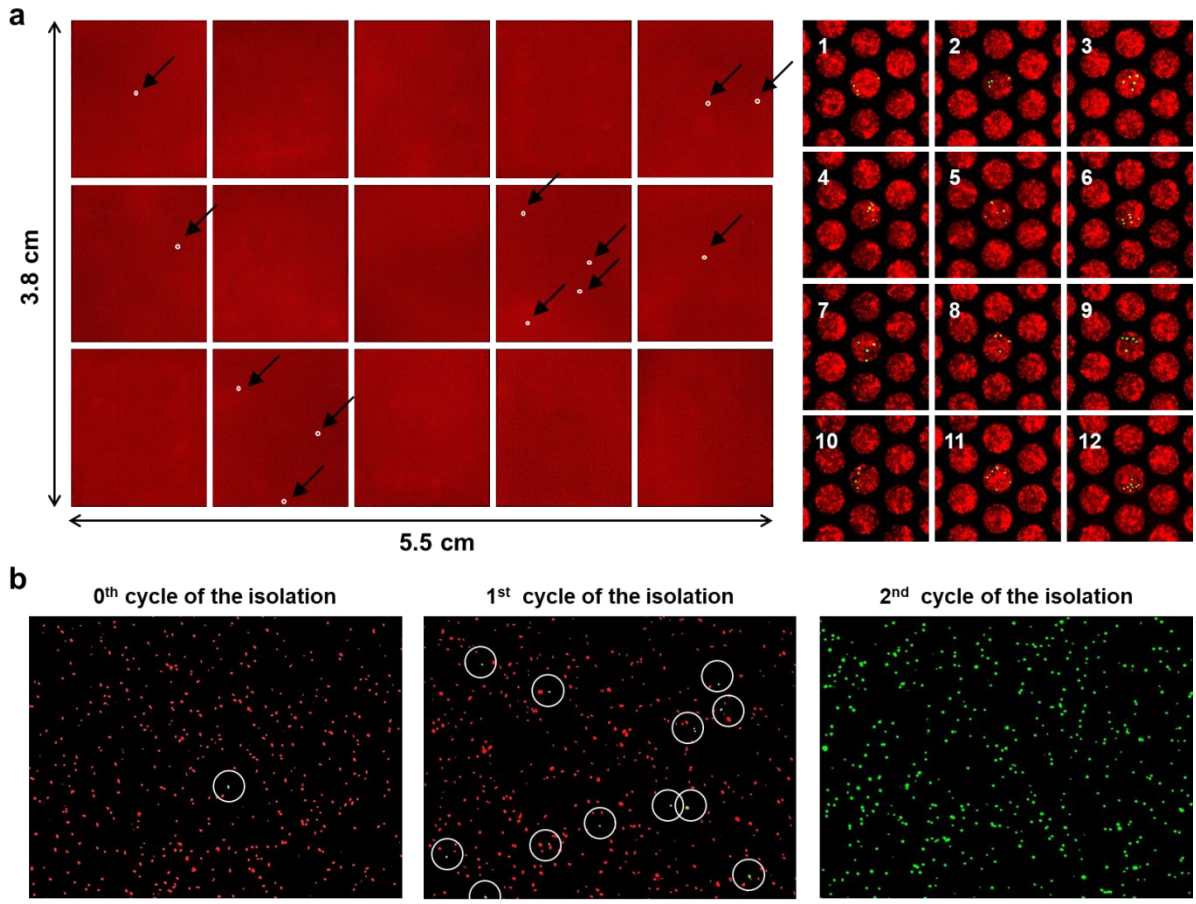

Figure S9. Rare cell isolation in a high-throughput manner. (a) In total, 150,000 microwells were made in a 3.8 cm × 5.5 cm area of the device. Fluorescent images of the entire area from the first cycle of isolation were captured and stitched into a single image. Black arrows indicate the identified microwells containing GFP cells. The GFP cells were detected in twelve microwells, which are shown in magnified images of the microwells corresponding to the black arrows. **(b)** Fluorescence images showing an overview of bacterial composition after the 0^{th}, 1^{st}, and 2^{nd} cycle of isolation. We captured the images of three bacterial samples in 10-μm-high microchannels. White circles highlight the identified GFP cells in images.

Supporting Movies

Supporting Movie 1. Rate of aqueous sample loading depending on the wettability of devices. The device with hydrophilic-in-hydrophobic (HIH) surfaces loaded the samples in 5 min, whereas the device with only hydrophobic surfaces loaded the samples in more than 10 min. For fabrication of a device with only hydrophobic surfaces, we coated the entire surface of the device with a fluoropolymer.

Supporting Movie 2. Operation of automated cell extractor. This video shows that the setup can punch any recorded coordinates of microwells. To visualize whether the hole was punched, microwells were filled with a green-dye solution. After cracking the H-PDMS layer, we observed the device for 4 min to visualize the diffusion of the green dye via the cracked H-PDMS layer. In the end, the punched chambers became whitish.

Supporting Movie 3. Extraction of bacterial cells via cracked H-PDMS. The cracked H-PDMS layer serves as a vertical microchannel. Many cells in the microwell burst through the crack, despite of the fact that the cells were not motile in the culture environment.

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