Supplemental Materials

An Ultra High-Efficiency Droplet Microfluidics Platform using Automatically Synchronized Droplet Pairing and Merging

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Microfabrication of the vertically curved droplet transition junction

Two-photon lithography, also commonly termed two-photon polymerization (2PP), is a relatively new form of lithography that overcomes the limitations of conventional 2-dimensional microstructure fabrication method. Allowing the fabrication of sub-micrometer resolution microstructures in three-dimensional (3D) space similar to that of 3D printing and stereolithography, albeit at a much higher resolution, 2PP has emerged as a powerful high-resolution 3D fabrication method. Two-photon polymerization utilizes two near-IR photons focused on the same voxel to polymerize a photosensitive resin by scanning a femtosecond laser beam, creating 3D structures having a resolution in the tens to hundreds of nanometers. Given the relatively easy fabrication process and high-resolution 3D spatial control, 2PP fabrication has begun to be utilized in the generation of novel microfluidic structures that require complex geometries to enhance not only continuous flow microfluidic capabilities, but also droplet microfluidic system capabilities.

For the vertically curved microstructure shown here, a 3D computer aided drawing (CAD) was made using Solidworks 2018 (Dassault Systemes SolidWorks Corp., MA) to replicate the exact footprint of the
step-shaped droplet transition junction. The final Solidworks file was then exported as an .STL file and loaded to DeScribe (Nanoscribe GmbH software, Germany) in order to prepare the .STL file for path and job recognition. The prepared file was then uploaded to a two-photon polymerization tool, the Nanoscribe Photonics Professional GT (Nanoscribe GmbH, Germany), and the microstructure fabricated using a negative photoresist (IP-S, Nanoscribe GmbH, Germany) using a power scaling of 1.0, tetrahedron inner scaffold, base scan speeds of 50,000, base laser power of 60%, shell/scaffold scan speeds of 100,000, and shell/scaffold laser power intensities set at 70%. All designs were fabricated on 4-inch silicon wafers. Following the microfabrication run, the wafers were removed from the tool and set to develop in propylene glycol monomethyl ether acetate (PGMEA, Millipore Sigma, MA) for 6 min. Following the PGMEA development, the wafers containing the microstructures was set for a fine development in 99% proof isopropyl alcohol (IPA, VWR, PA) for 10 mins. Following the IPA development, the microstructures were dried with nitrogen gas and inspected under a microscope for quality assurance. This microstructure becomes the master mold for the subsequent soft lithography step.

Following inspection, the patterned wafers were coated with (tridecafluoro-1,1,2,2 tetrahydrooctyl) trichlorosilane (United Chemical Technologies, Inc., Bristol, PA) for 20 min to preventing pattern removal during the polymer replication process. A thin poly(dimethylsiloxane) (PDMS, Sylgard® 184, Dow Corning Corp., MI) layer (thickness: 30 µm) was spin-coated on the patterned glass slide (Micro Slides 2947-75x50, Corning Inc., NY) at 3,000 rpm for 30 sec to obtain a hydrophobic bottom surface that is necessary for droplet generation. For all other layers, the PDMS microchannels were replicated from the master molds by pouring 20 g of PDMS mixture (1:10 curing agent to polymer ratio) onto the master molds. After PDMS crosslinking at 85 °C for 4 hr, the released PDMS layers were bonded to the PDMS-coated glass slide using oxygen plasma treatment (Plasma cleaner, Harrick Plasma, Ithaca, NY) for 90 s.

![Fig. S1](image_url) The microfluidic channel design for evaluation of the droplet transition junctions. The device with (a) conventional step-shaped transition junction and (b) 2PP-printed vertically curved droplet transition junction.
Fig. S2 The cone-shaped PDMS chamber for droplet storage, cultivation, and reflow. (a) Empty chamber, (b) after blue color dye-encapsulated droplet collection, and (c) during droplet reflow into the outlet tubing. Generated droplets are collected from either or both inlet 1 and/or 2. At the droplet collection phase, the outlet is pinched, and the oil tube is open to discharge extra oil. At the droplet reflow phase, the inlet 1 and 2 are pinched, and oil flows into the chamber from the bottom oil tube to push the droplets out from top side.

COMSOL Multiphysics simulation parameters

COMSOL Multiphysics 5.2a (COMSOL Inc., Palo Alto, CA, USA) was used for the velocity profile simulation. Three assumptions were made to mimic the actual flow condition, as following: (1) the fluid is Newtonian, (2) no-slip boundary condition, and (3) the flow within the channel is incompressible. The 3D models were created initially in AutoCAD 2016 (Autodesk Inc., San Rafael, CA, USA), and then imported into the COMSOL library. The simulation was performed using physical interfaces laminar flow (spf) under the stationary study model. The inlet flow rate was set to 20 μL/h. The material was set to Novac® oil having density of 1614 kg/m³ and dynamic viscosity of 0.00125 Pa.s at 25°C.
Fig. S3 COMSOL simulation of the velocity profile in the droplet transition junctions. Side view of the velocity profile in (a) 2PP-printed vertically curved droplet transition structure and (b) conventional step-shaped transition structure. Top view of the velocity profile in the (c) 2PP-printed vertically curved droplet transition structure and the (d) conventional step-shaped droplet transition structure. For (c) and (d), the data was collected at the bonding interface of two PDMS layers.
**Fig. S4** Micrographs showing the effects of (a) aqueous solution flow speed and (b) aqueous channel width on the size of the cleaved droplets using the auto-synchronizing droplet cleaving system.

**Fig. S5** Summary of the droplet cleaving and merging efficiency at different throughputs (continuous aqueous channel width was 75µm for cleaving and 100µm for merging).
Fig. S6 Frame-by-frame micrograph images of an example of missed cleaving (dotted red circle) when using a conventional step-shaped droplet transition structure.

Fig. S7 Micrograph images showing the droplet cleaving and droplet merging events using GFP-*Salmonella* as the aqueous solution flow and droplets containing DAPI fluorescent beads with 1 mg/mL of gentamycin. (a) GFP channel showing the continuous flow of GFP-*Salmonella*. (b) DAPI channel showing the droplet pairs after cleaving. (c) & (e) Paired droplets generated and collected but without applying electric field, where the two different droplet populations (DAPI bead-containing gentamicin droplets and GFP-*Salmonella*-containing droplets) can be seen without merging. (d) & (f) The merged droplets with and without antibiotic after turning on electrical field. (e) The blank small droplets and the cleaved large droplets containing *Salmonella* before merging. (f) All of the merged droplets containing *Salmonella*. 
**Table S1** Statistic analysis of antibiotic discovery functional assay (N=50)

<table>
<thead>
<tr>
<th>Groups</th>
<th>GFP intensity range*</th>
<th>Mean intensity</th>
<th>SD</th>
<th>Z-score</th>
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<tr>
<td>DAPI+ (1:1)</td>
<td>21.47 to 34.56</td>
<td>27.58</td>
<td>2.46</td>
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<td>DAPI- (1:1)</td>
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<td>DAPI- (1:100)</td>
<td>68.15 to 114.43</td>
<td>97.12</td>
<td>12.93</td>
<td>0.201</td>
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</tbody>
</table>

*The GFP intensity was calculated based on the mean grey value of each droplet.

**References**