Supporting Information

Facile and Low-Waste Self-Digitization of Samples by Oil-Triggered, Template-Confined Dewetting on a Networked Microwell Array

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S1. Calculation of Sample Utilization Rate during Self-Digitization with the NMA Chip

Considering the oil-triggered, template-confined dewetting mechanism demonstrated in the main text, only the part of the sample volume filled in the main channel of a NMA chip is not partitioned into microwells, i.e., wasted. Thus, we define the sample utilization rate as:

\[
\text{Sample utilization rate} = 1 - \frac{\text{Sample volume in the main channel}}{\text{Total sample volume in the channel-well space}} \times 100\%
\]

For our NMA chip, the total numbers of microwells and connecting channels are 40,233 and 121,468, respectively. According to the dimensions illustrated in Figure 1 of the main text, the volumes of each well (100 μm in diameter and 100 μm in depth) and each connecting channel (52 μm in length, 20 μm in width, and 20 μm in depth) are about 0.785 nL and 0.021 nL, respectively. The volume of the main channel (132 mm in length, 60 μm in width, and 100 μm in depth) is about 0.79 μL. Thus, it can be inferred that the sample utilization rate is about 97.74%.

\[\text{Figure S1.} \] Schematic diagrams for the fabrication procedure of spherical colloidal photonic crystals using our self-digitization method. (a) degassing of the chip in a vacuum chamber; (b) removing the chip from vacuum conditions, adding an aliquot of PS colloidal suspension in the inlet and a few drops of oil in the oil reservoir; (c) aspiration of the sample into the microchannels under the negative pressure created by the degassed PDMS substrate; (d) sample priming of all channels and wells; (e) peeling off the cover layer from the microstructured layer; (f) discretization of the primed sample into all wells induced by oil-assisted dewetting; (g) evolution of the discretized sample to spherical droplets induced by evaporation and energy minimization; (h) evaporation-induced colloidal assembly for the generation of close-packed spherical colloidal photonic crystals.
**S2. Measurement of Oil/Water/PDMS Three-Phase Contact Angle**

The relationship between the three-phase contact angle and interfacial interactions is given by Young–Laplace equation equation [1]:

\[
\cos \theta = \frac{\gamma_{PO} - \gamma_{PW}}{\gamma_{WO}}
\]  
(1)

where \( \theta \) is the angle of the three-phase interface, and the \( \gamma_{ij} \)s are the interfacial free energies between phases \( i \) and \( j \). The subscripts \( P \), \( W \), and \( O \) correspond to the PDMS, water, and oil phases, respectively. Surface energies were calculated using the two-liquid Owens-Wendt equation [2]:

\[
\gamma_{PW} = \gamma_P + \gamma_W - 2(\sqrt{\gamma_P^{d}\gamma_W^{d}} + \sqrt{\gamma_P^{p}\gamma_W^{p}})
\]  
(2)

\[
\gamma_{PO} = \gamma_P + \gamma_O - 2(\sqrt{\gamma_P^{d}\gamma_O^{d}} + \sqrt{\gamma_P^{p}\gamma_O^{p}})
\]  
(3)

where \( \gamma_i^{d} \) and \( \gamma_i^{p} \) are the dispersive and polar components from surface energy of phases \( i \), respectively. The surface energies of DI water, silicone oil, and hexadecane can be found in Table S1. The dispersive and polar surface energy components of DI water, silicone oil, and hexadecane are listed as following:

- **DI water:** \( 21.8 \text{ mN/m}, 51 \text{ mN/m} \)
- **Silicone oil:** \( 20.8 \text{ mN/m}, 0 \)
- **Hexadecane:** \( 27.6 \text{ mN/m}, 0 \)

**S3. Measurement of E. coli JM109 Stock Concentrations via Plating**

The *E. coli* JM109 stock sample was diluted with LB broth down to \( 4.38 \times 10^{5} \text{ CFU/mL} \), \( 1.75 \times 10^{2} \text{ CFU/mL} \), \( 7.00 \times 10^{2} \text{ CFU/mL} \). One-hundred \( \mu \text{L} \) from each of the 3 dilutions was plated in an LB agar plate, resulting in three plates with expected CFU counts of 4, 18 and 70, respectively. All LB agar plates were incubated at 37 °C for 12 h. After incubation, the number of colonies on each plate was manually counted and recorded directly on the plate. Each measurement was repeated for 3 separate samples, resulting in a total of 9 data points (i.e., 3 titrations in triplicate). The counted number of colonies was plotted against the expected number of colonies, and a linear fit was performed. The linearity was examined and the slope was calculated. Finally, if necessary, the slope of the linear fit line was used to recalculate the bacterial sample concentration. According to the fit line, the concentration of this *E. coli* stock is recalculated as \( 2.8 \times 10^{9} \text{ CFU/mL} \).
Figure S2. Measurement of *E. coli* JM109 stock concentration via plating. (a) An aliquot of a 2.8 × 10⁹ CFU/mL *E. coli* stock (originally estimated via a single plate) is divided into 3 titrations (4.38 × 10⁰, 1.75 × 10¹, and 7.00 × 10² CFU/mL), and 100 µL of each titration is plated in LB agar plates, resulting in expected CFU counts of 4, 18, and 70, respectively. After 12 h, 37 °C incubation, the number of colonies from each titration is manually counted and recorded directly on the plate. (b) After repeating the same experiment for 3 separate aliquots (i.e., 3 titrations in triplicate), the counted number of colonies is plotted against the expected number of colonies. The slope of the linear fit line is 1.036. Consequently, the concentration of this *E. coli* stock is recalculated as 2.8 × 10⁹ CFU/mL. Error bars represent standard deviations from triplicated experiments.
Table S1. The materials and physical parameters used for the numerical simulation.

| Fluid Composition | Oil Phase | Aqueous Phase |
|-------------------|-----------|---------------|
|                   | Silicone  | Hexadecane    | Water        |
| Density (Kg/m$^3$) | 960       | 770           | 1000         |
| Viscosity ($\times 10^{-3} \text{ Pa}\cdot\text{S}$) | 50        | 3.34          | 2.98         |
| Surface Tension ($\times 10^{-3} \text{ N/m}$) | 20.8      | 27.6          | 72.8         |
| PDMS Surface Energy Density ($\times 10^{-3} \text{ J/m}^2$) | 20.78     | 27.25         | 40.71        |

Reference

[1] Young, T., An essay on the cohesion of fluids. *Philos. Trans. R. Soc. London* **1805**, (95), 65-87.

[2] Owens, D. K.; Wendt, R. C., Estimation of the surface free energy of polymers. *J. Appl. Polym. Sci.* **1969**, **13** (8), 1741-1747.
Legends of Videos

Video S1: Demonstration of the operation of the NMA chip for sample digitization.

Video S2: Demonstration of the dynamic behavior of the oil-triggered, templated dewetting in a NMA chip (bird's eye view).

Video S3: Demonstration of the deformation/evolution of oil/water interfaces after introducing an oil phase on the surface of a NMA chip (Cross-sectional view) (AVI).

Video S4: Demonstration of the dynamic pressure mapping of the two-phase system in a NMA chip during the self-digitization process (Front view) (AVI).