Supporting information of

Flow-Programmable and Reversible Surface-Induced LLPS in Nanofluidic Channels

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Figure S1. Characterization of nanochannel in fabricated device

(a) Photograph of the entire assembled nanofluidic device. **(b)** Enlarged microscopic image showing the intersection between the microchannel and the nanochannels. **(c)** High-resolution deep-ultraviolet (DUV) microscope image (INM300 DUV) of the nanochannels. The measured width of the nanochannel is 750 ± 50 nm. **(d)** Depth profile of an adjacent 250-μm-wide microchannel, measured using a surface profiler (ET200A, Kosaka Laboratory Ltd.). The measured depth is 890 nm. Because the aspect ratio (~ 1) is sufficiently low for the reactive-ion etching (RIE) process on glass substrates, the aspect-ratio-dependent etching (ARDE) effect is negligible. Therefore, the depth of the adjacent nanochannels is reasonably estimated to be identical (890 nm).

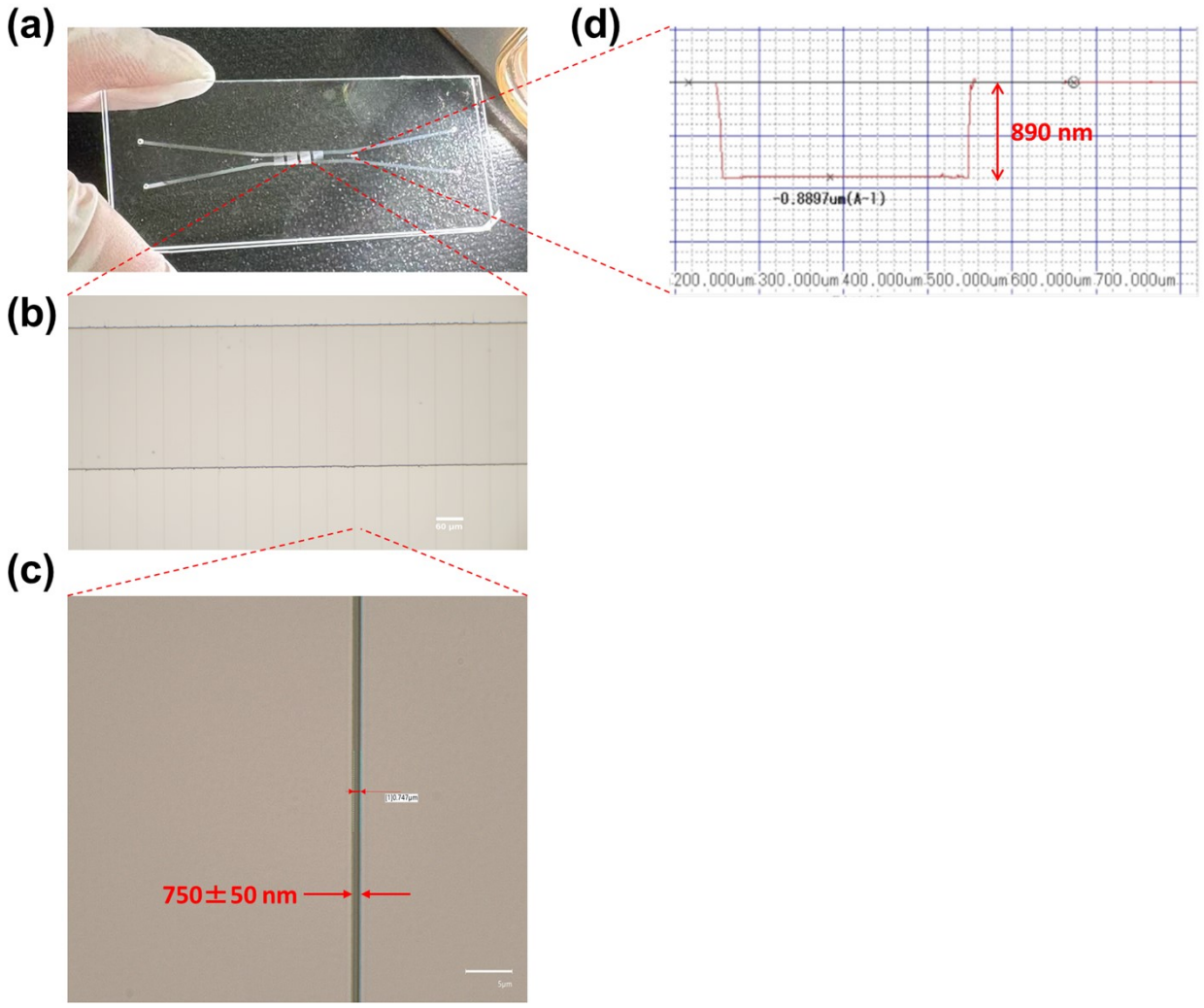


Figure ~~S1~~S2. Initial formation of an ssDNA-rich plug at the nanochannel entrance.

(Left) Schematic of the pressure configuration during ssDNA introduction. A 5 μM fluorescent ssDNA solution was introduced from the left microchannel under a 12.5 kPa pressure difference across the nanochannels.

(Right) Fluorescence image showing the emergence of a highly bright domain at the channel entrance after ~30 min of low-pressure ssDNA introduction. The accumulated ssDNA formed a cap-like obstruction that prevented further flow. The blockage could not be removed by a 100 kPa reverse pressure but was partially released by applying 400 kPa, after which several nanochannels resumed normal flow in subsequent experiments.

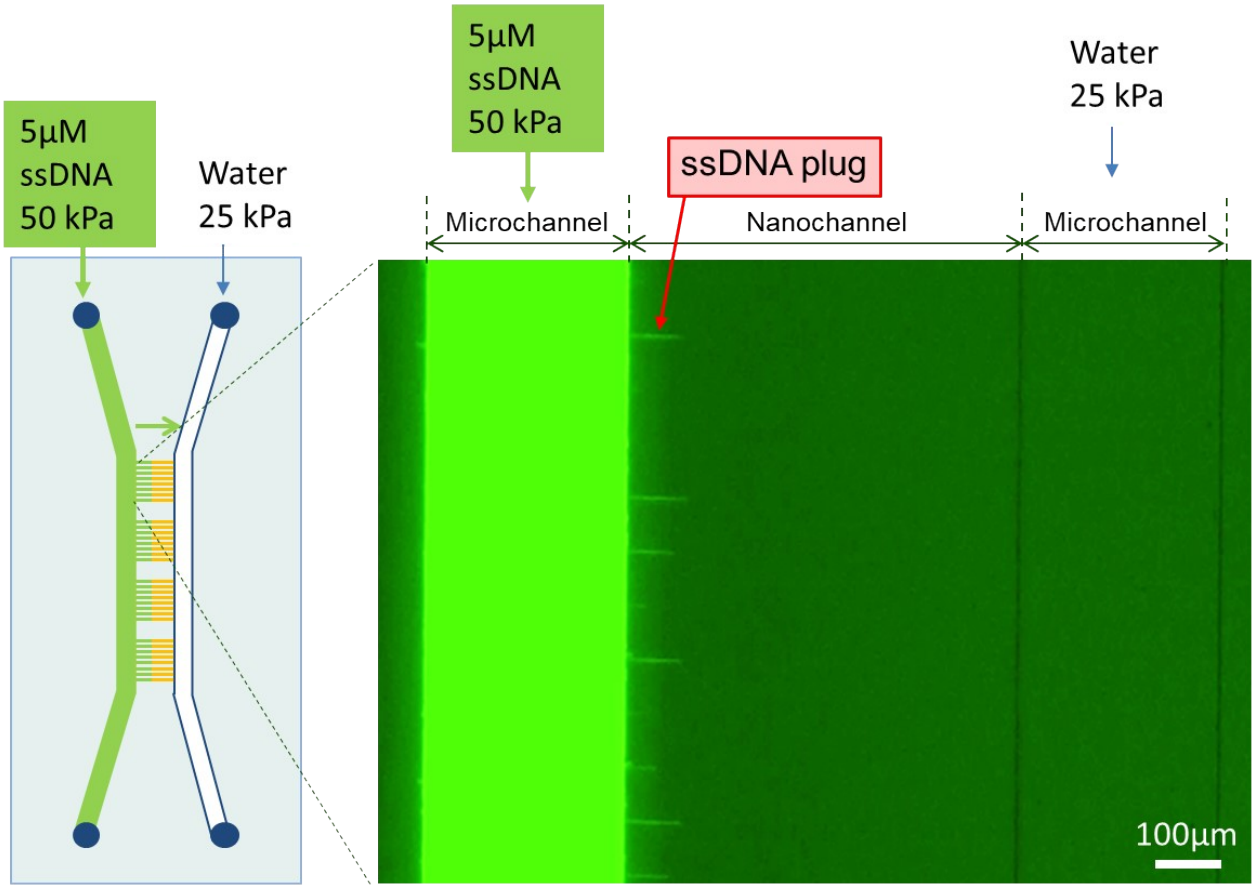
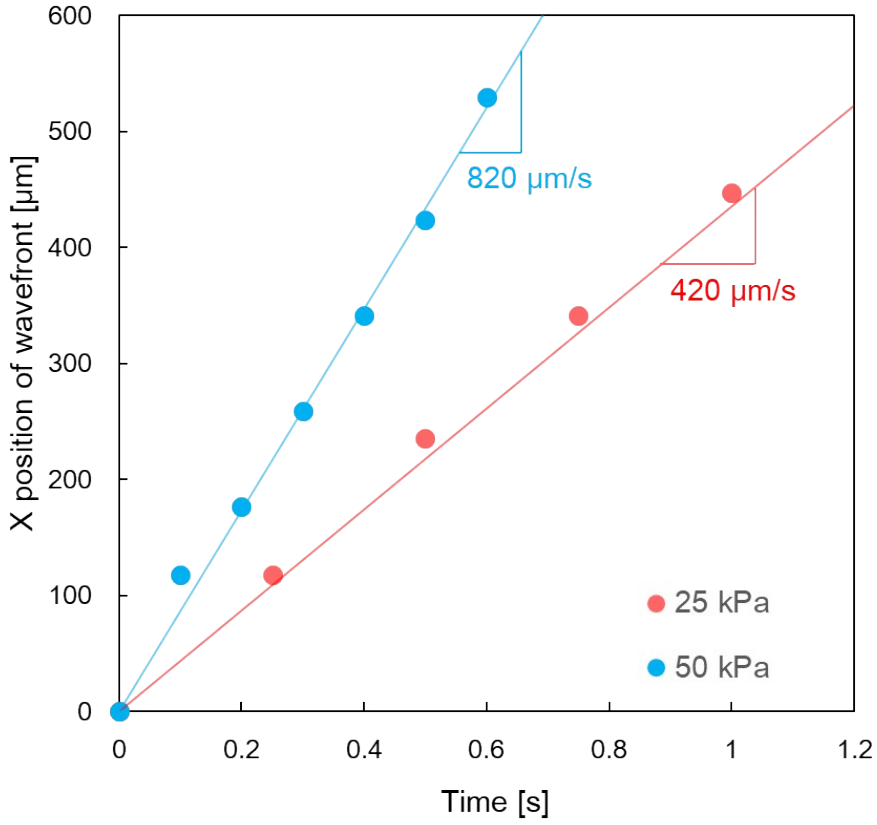


Figure S3. Experimental validation of the flow velocity within the nanochannel..

The X-position of the FITC fluorescent wavefront is plotted as a function of time during its introduction into a water-filled nanochannel under applied pressure drops of 25 kPa (red circles) and 50 kPa (blue circles). The linear fits indicate actual measured flow velocities of 420 µm/s and 820 µm/s, respectively. These experimental values are in reasonable agreement with the theoretical flow velocities (560 µm/s and 1130 µm/s for 25 kPa and 50 kPa, respectively) calculated using the Poiseuille flow model for a rectangular channel (W = 700 nm, D = 890 nm, L = 1 mm).



Videos S1-S6 : Pressure-dependent LLPS Film Formation and peel-off (Quantitative View)

These videos correspond to the experiments presented in **Figure 8**. Scale bars, timestamps, and flow directions are annotated within each video. To enable a direct quantitative comparison of ssDNA accumulation, the brightness gain for this series (Videos S1–S6) was **held constant** (Gain = 2.1). This standardization visualizes how the equilibrium fluorescence intensity varies strictly as a function of the inlet pressure (flow rate).

Video name	Pressure during introduction	Pressure during removal	Brightness gain of movie
VideoS1.mp4	450 kPa	45 kPa	2.1
VideoS2.mp4	200 kPa	45 kPa	2.1
VideoS3.mp4	75 kPa	45 kPa	2.1
VideoS4.mp4	40 kPa	45 kPa	2.1
VideoS5.mp4	12 kPa	45 kPa	2.1
VideoS6.mp4	75 kPa	95 kPa	2.1

Videos S2b-S6b : Optimized Visibility (Qualitative View)

Because the constant gain setting renders the fluorescence signal at lower pressures (e.g., Videos S5 and S6) difficult to distinguish from the background, we have provided an additional set of videos (**Videos S2b–S6b**) with the gain **optimized for visibility**. In these versions, the pixel intensity has been linearly scaled to clearly display the film behavior and dewetting dynamics. Note that these videos are for morphological observation only; quantitative intensity comparisons should be made using the original S1–S6 series.

Video name	Pressure during introduction	Pressure during removal	Brightness gain of movie
VideoS1.mp4	450 kPa	45 kPa	2.1
VideoS2b.mp4	200 kPa	45 kPa	3.0
VideoS3b.mp4	75 kPa	45 kPa	4.0
VideoS4b.mp4	40 kPa	45 kPa	4.3
VideoS5b.mp4	12 kPa	45 kPa	6.0
VideoS6b.mp4	75 kPa	95 kPa	3.0

Videos S7 : FITC result

VideoS7 shows the experimental results for FITC and corresponds to Figure 7 (left).

Video name	Pressure during introduction	Pressure during removal	Brightness gain of movie
VideoS7.mp4	75 kPa	45 kPa	3.0