Supplementary Information

A platform for the formation of uniform DNA condensate droplets using vibration-induced local vortices

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Supplementary Movies:

Supplementary Movie S1. Fluorescent particle motion at a frequency of 600 Hz in a D = 50 μm micropillar with an amplitude of 3 μm.

Supplementary Movie S2. Formation process of initial DNA aggregates during ten minutes VIF with a frequency of 600 Hz and an amplitude of 3 μm in D = 100 μm micropillars.

Supplementary Movie S3. Time-lapse images of relaxation process of DNA condensates after ten minutes of vibration at 600 Hz and 3μm amplitude. The change was recorded for 4h in D = 100 μm micropillars.

Supplementary Movie S4. Time-lapse images of the phase separation process of DNA condensates with multiple components (two Y-motifs and a linker motif) after 20 min VIF at a frequency of 600 Hz and an amplitude of 3 μm in D = 100 μm micropillar array.

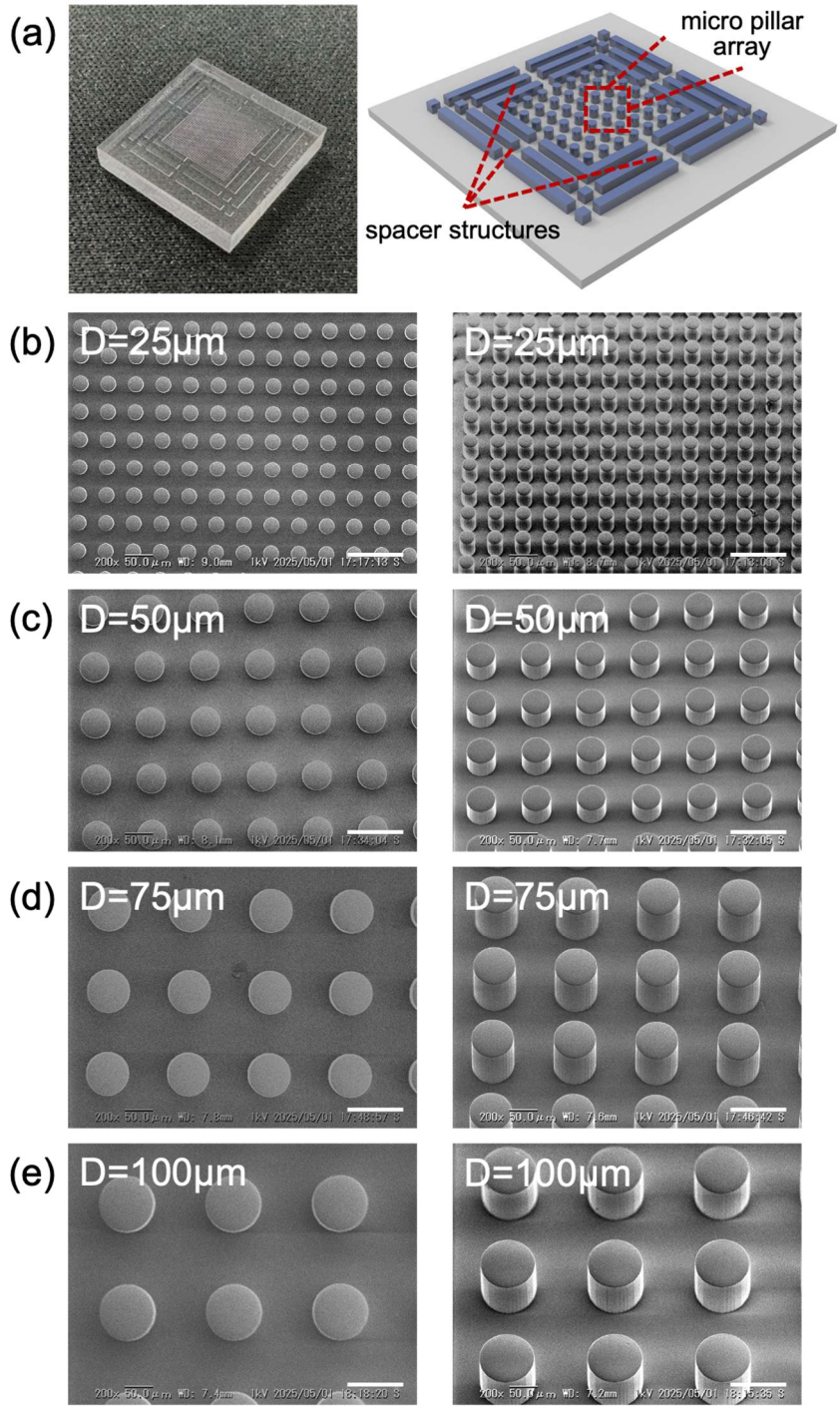

Figure S1. Design and SEM characterization of the PDMS microfluidic device.

A photographic image of the fabricated PDMS device (left) and a corresponding schematic diagram (right). The schematic highlights the central micropillar array region and the surrounding spacer structures that define the channel height. (b-e) Scanning Electron Microscope (SEM) images of the fabricated micropillar arrays with different diameters (taken with VE-9800, KEYENCE, Japan). (b) D = 25 μm, (c) 50 μm, (d) 75 μm, and (e) 100 μm; top-down (left) and 45-degree tilted (right) views. Scale bars = 100 μm.

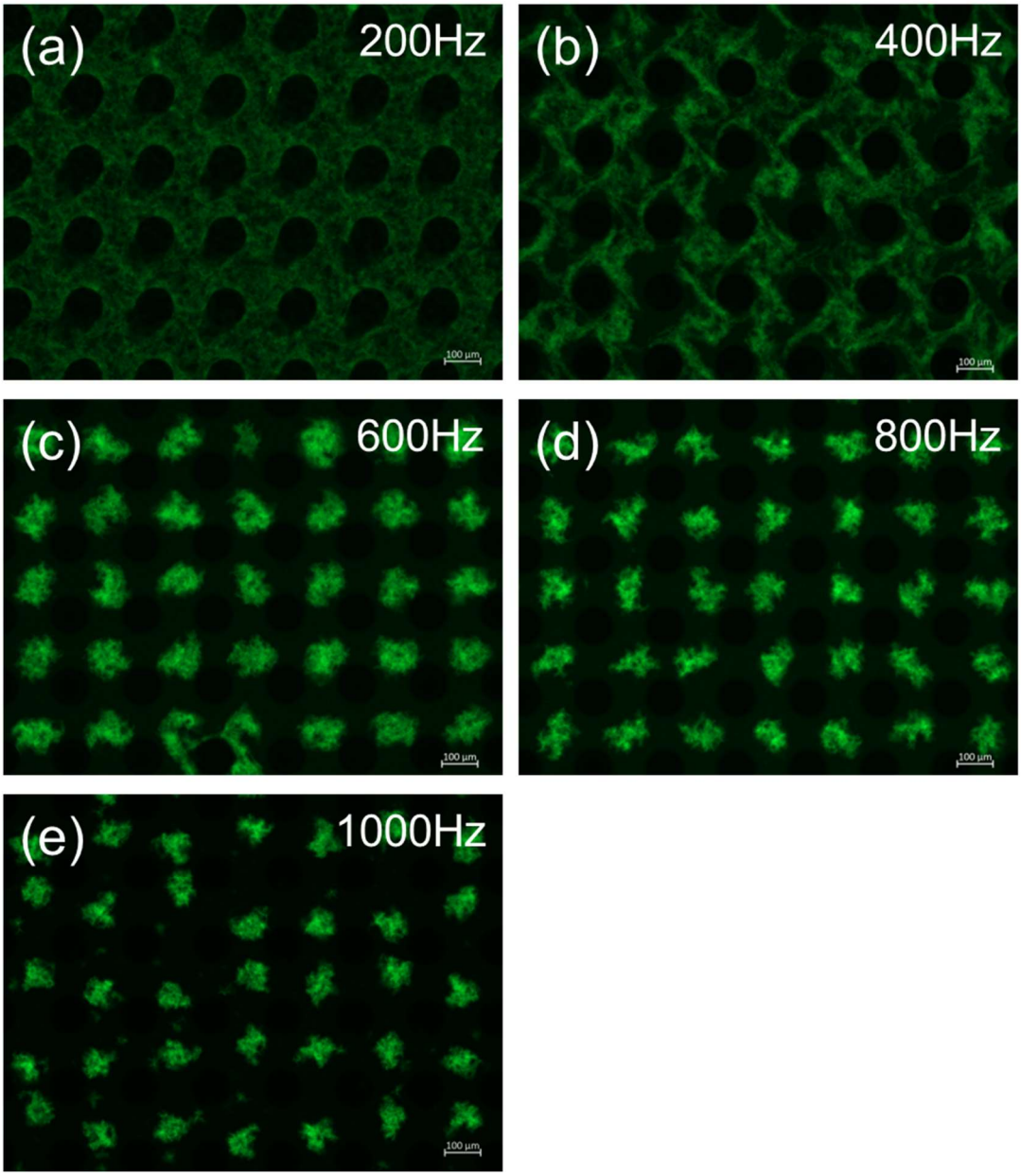

Figure S2. Effect of vibration frequency on DNA condensate formation. Fluorescence images were taken after 10 min of vibration at 3 µm amplitude. (a) At 200 Hz, separated condensates did not form, which was also the case for (b) 400 Hz. (c) At 600 Hz, uniform condensates were produced. Frequencies of (d) 800 Hz and (e) 1000 Hz yielded similar results to (b). These results establish 600 Hz as the minimum effective frequency for our system under these conditions. Scale bars = 100 µm.

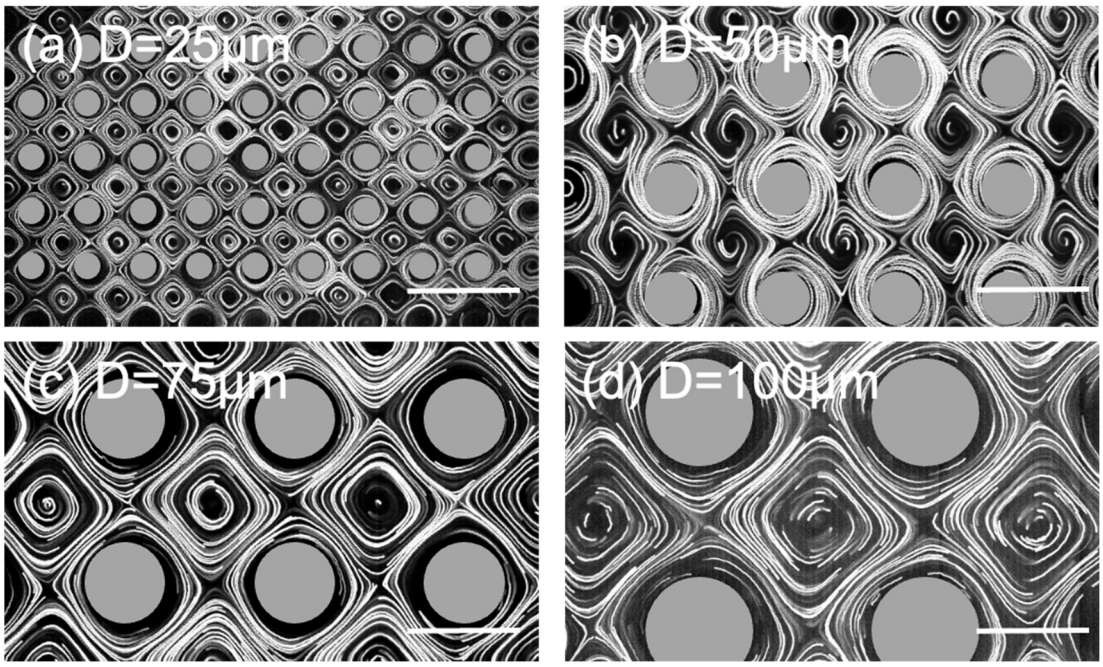

Figure S3. Characterization of the VILV flow field with different micropillar geometries driven at identical vibration conditions (600 Hz frequency, 3 μm amplitude).

The figure displays streamline plots for devices with different micropillar diameters: (a) 25 μm, generated from 400 frames at 120 fps; (b) 50 μm, from 200 frames at 150 fps; (c) 75 μm, from 400 frames at 200 fps; and (d) 100 μm, also from 400 frames at 200 fps. Scale bars = 100 μm.

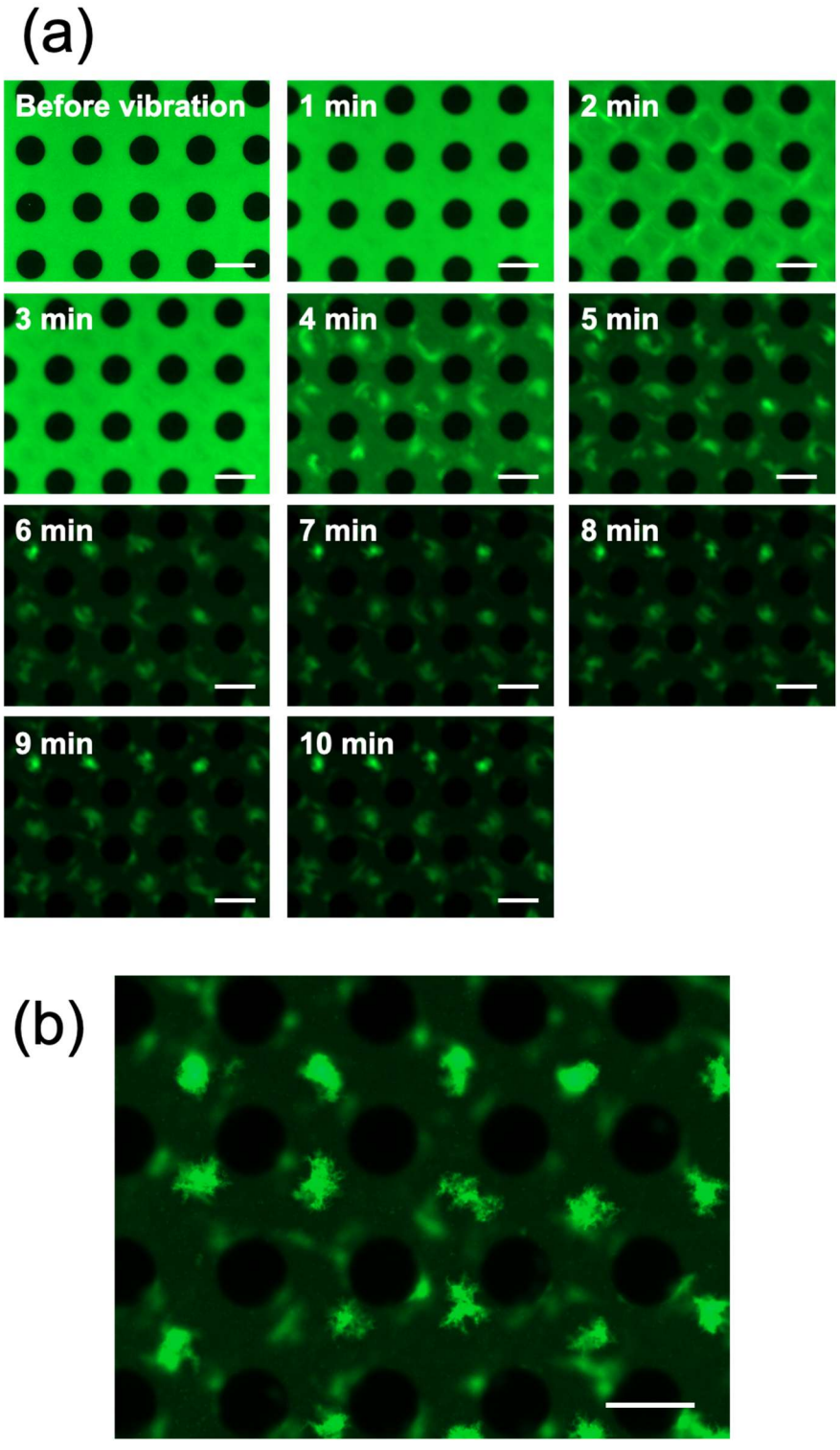

Figure S4. Fluorescent images of DNA condensation process during VIF application. (a) Time-lapse images of initial DNA aggregate formation during 10-min VIF application. (b) Still image of DNA aggregate when VIF was stopped. Scale bars = 100 μm

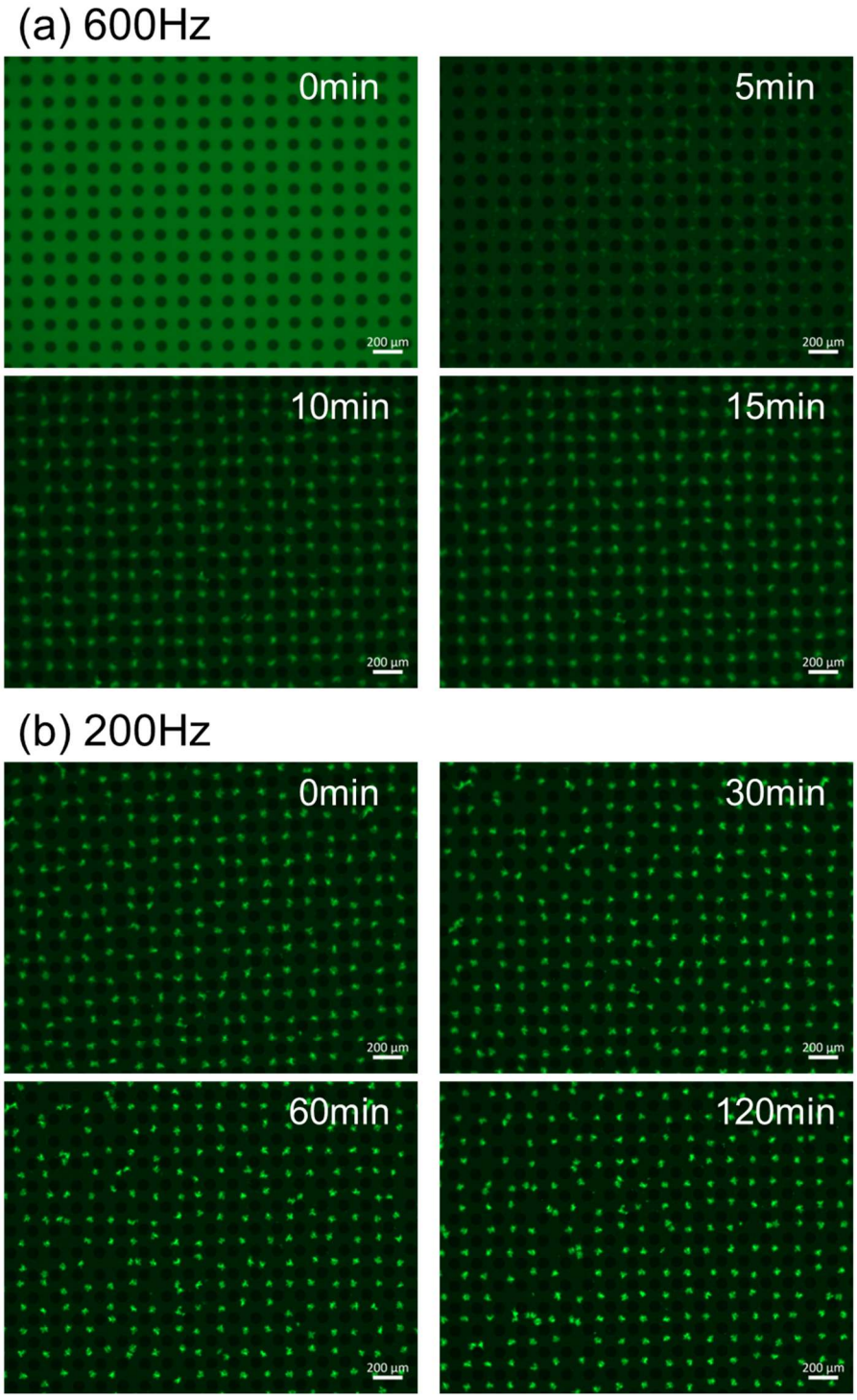

Figure S5. Preservation of condensate monodispersity using a low-frequency maintenance mode. Representative fluorescence images of 6nt-SE condensates. (a) Uniform aggregates formation with a 15-min vibration at 600 Hz. (b) The same field of view after an additional 2 h under a continuous low-frequency (200 Hz) vibration. The spatial separations of condensates were maintained, and collision was effectively prevented. Scale bars = 200 μm.

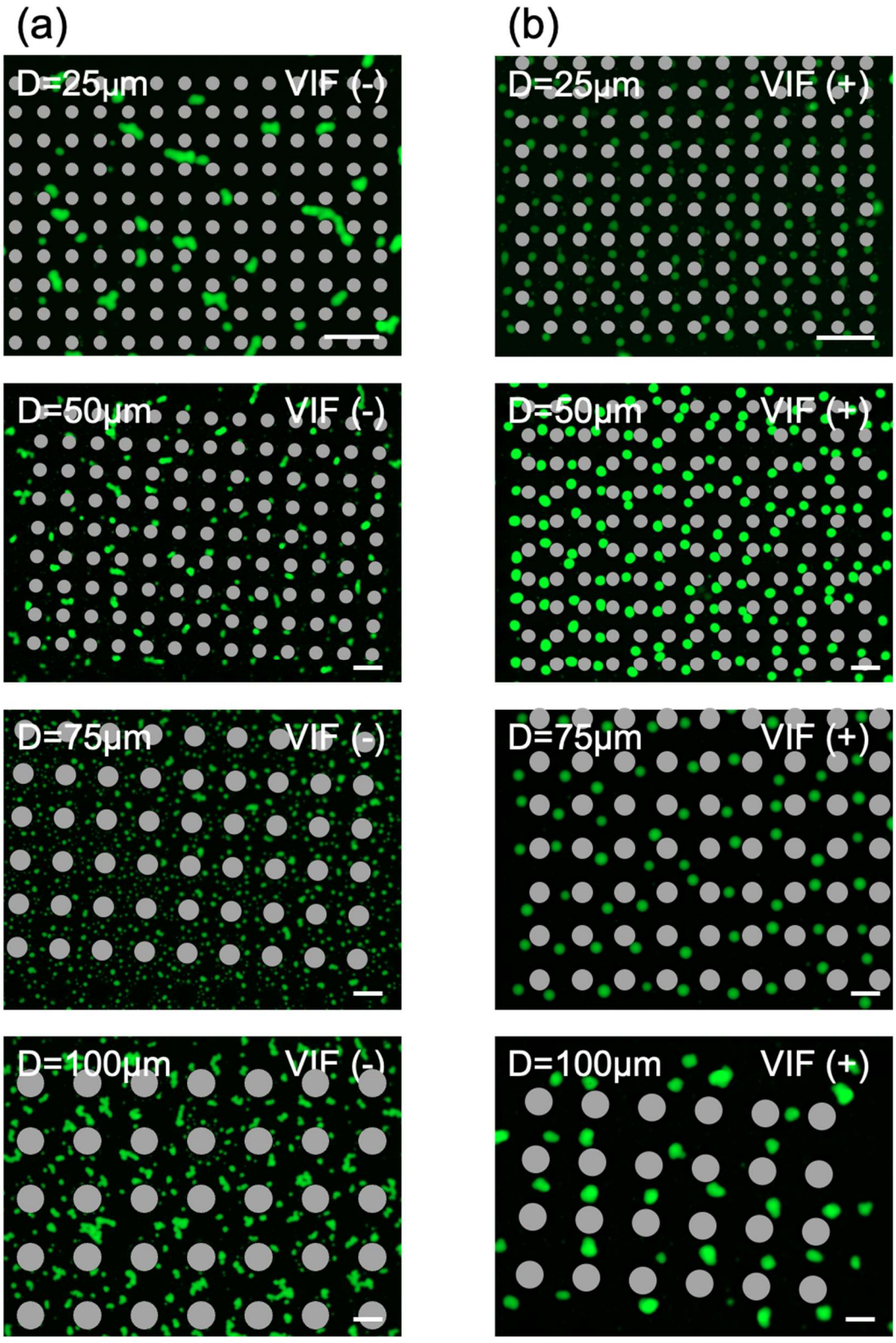

Figure S6. Comparison of the spatial distribution of DNA condensates across all four micropillar geometries (a) without and (b) with VIF application. In both panels, the images correspond to D = 25 μm, 50 μm, 75 μm, and 100 μm micropillar array (from top to bottom). Scale bars = 100 μm.

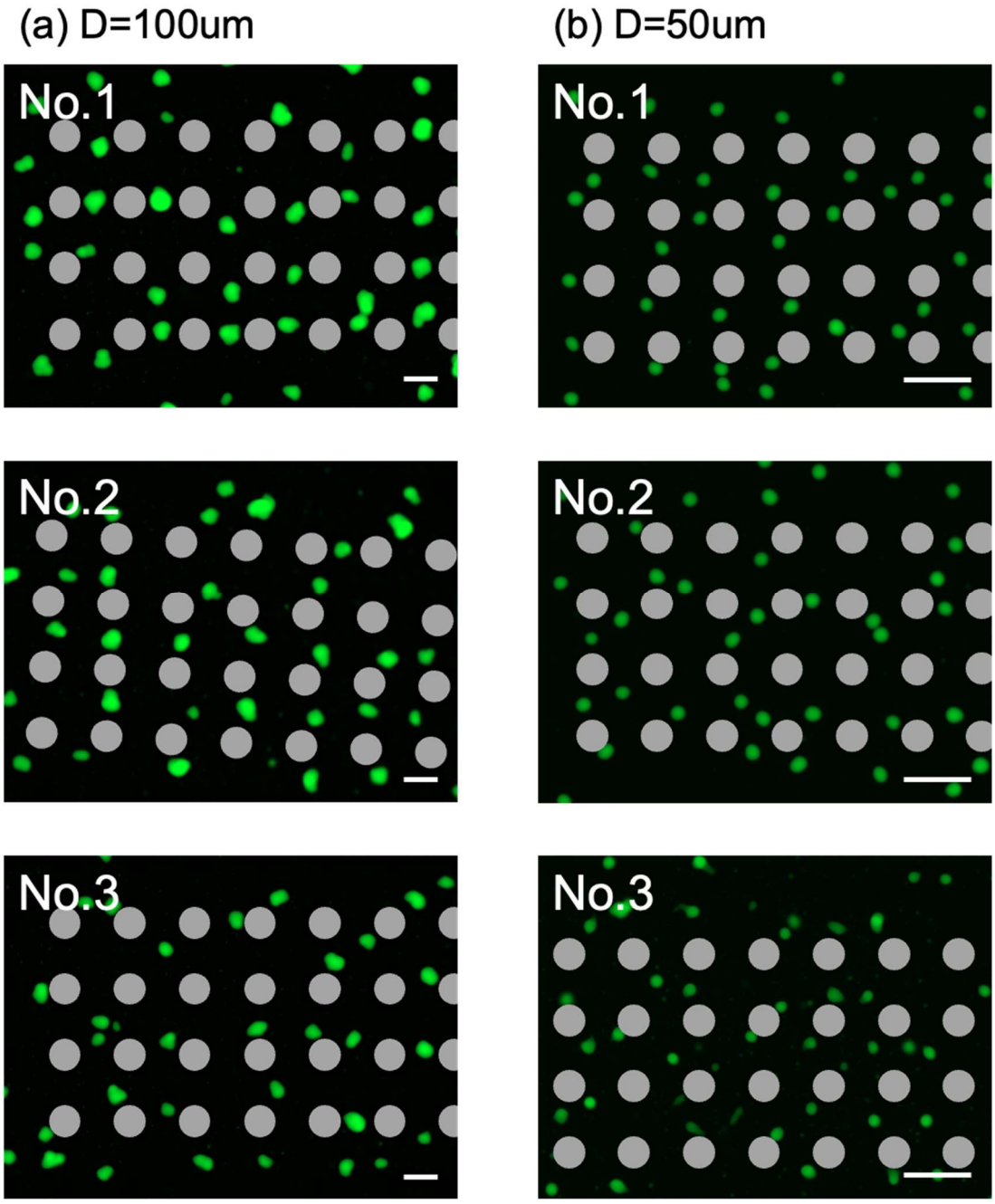

Figure S7. Demonstration of the high reproducibility of DNA condensate formation under 10 μM Y-motif at 600 Hz, 4 μm. Representative images from three independent experiments are shown for devices with *D* = (a) 100 μm and (b) 50 μm micropillar arrays. Statistics in Figure 6 (main text) were extracted from these images Scale bars = 100 μm.

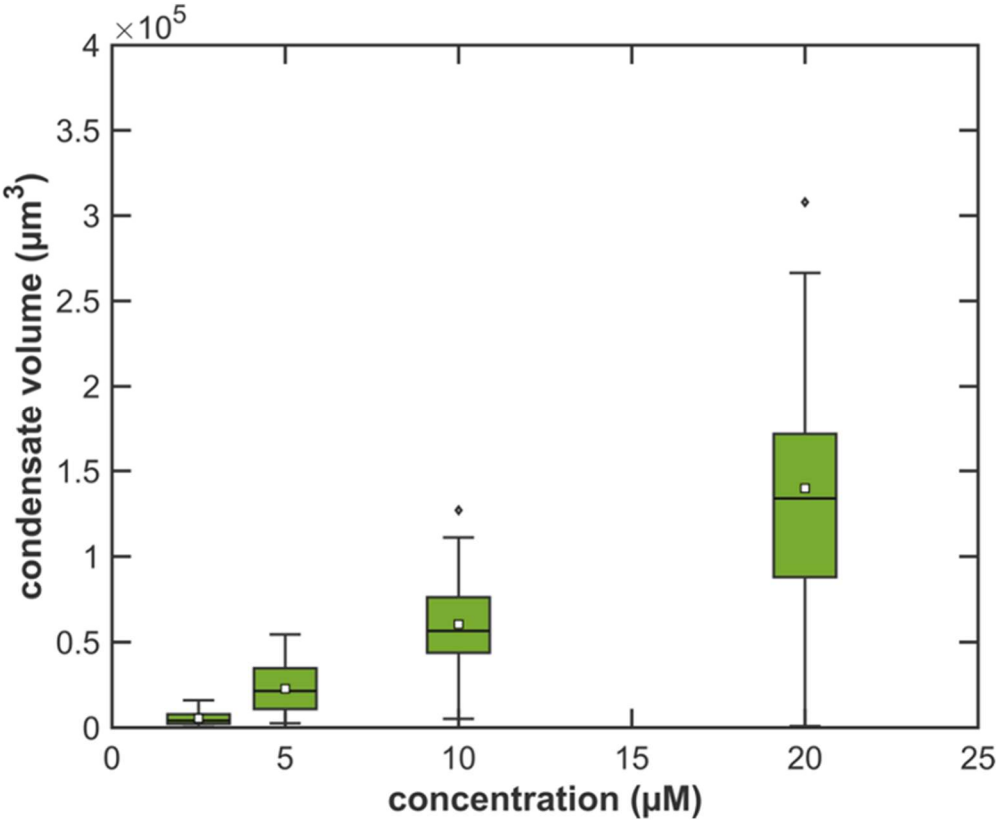

Figure S8. Statistical analysis of DNA condensate volumes.

Box plot analysis of the final condensate volume as a function of initial DNA concentration. The median volume increases with higher initial DNA concentrations. The median volumes were 4120, 21430, 56550, and 133900 μm³, with corresponding IQRs of 5623, 23710, 32440, and 83540 μm³, respectively.

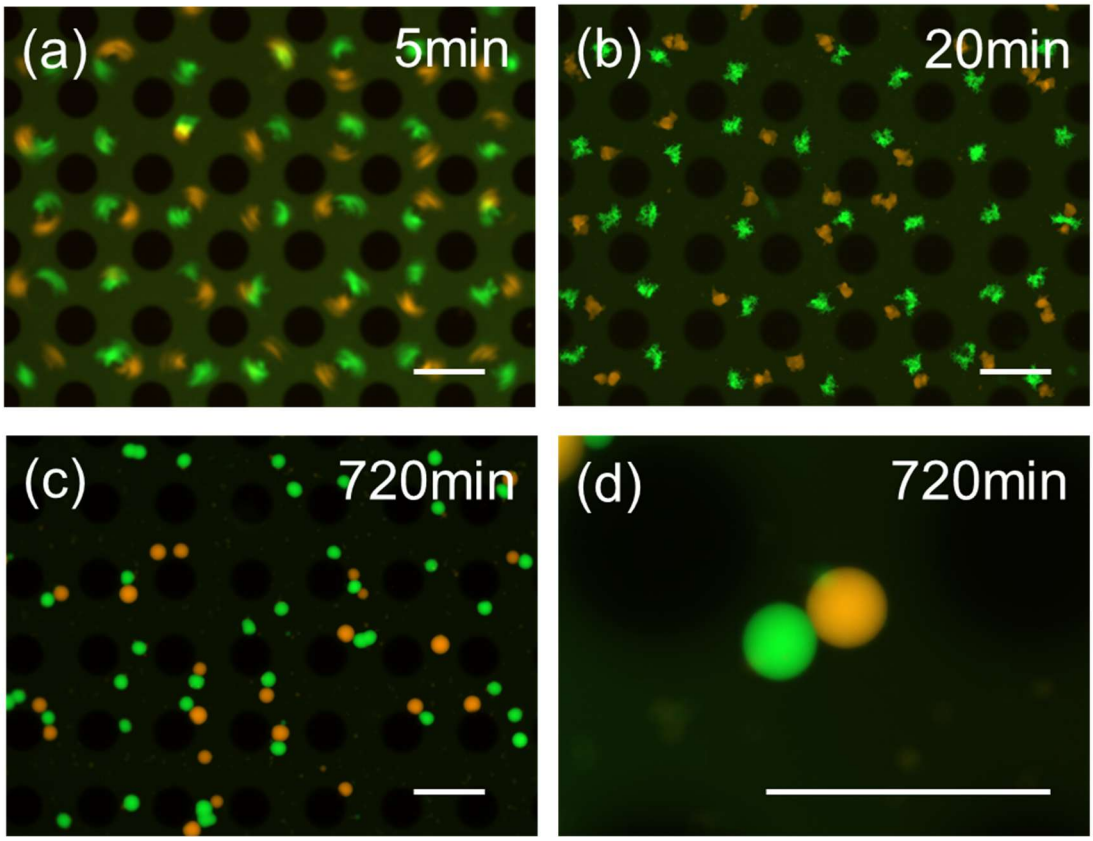

Figure S9. Control experiments confirm the crucial role of the linker motif. Under conditions where the concentration ratio of each Y-motif to linker motif DNA was reduced to 50:1(a) At 600 Hz, two distinct DNA condensates formed independently after 5 min. (b) After 20 min of VIF, both Y motifs exhibited similar sizes and states, with linker DNA not yet contributing. (c, d) Overview and close-up of Janus-type DNA condensates 720 min after VIF cessation. Over time, as linker DNA became active, some morphologically distinct, orthogonally aligned DNA condensates transformed into Janus-type condensates, showing clear differences compared to the patch-like state in Figure 6. Scale bar = 100 μm.

Table S1. Sequences of DNA oligomers

Y-motif (4nt):

Y1: GCGCCAGTGAGGACGGAAGTTTGTCGTAGCATCGCACC

Y2: GCGCCAACCACGCCTGTCCATTACTTCCGTCCTCACTG

Y3: GCGCGGTGCGATGCTACGACTTTGGACAGGCGTGGTTG

Y-motif (6nt):

Y1-6nt: GCTAGCCAGTGAGGACGGAAGTTTGTCGTAGCATCGCACC

Y2-6nt: GCTAGCCAACCACGCCTGTCCATTACTTCCGTCCTCACTG

Y3-6nt: GCTAGCGGTGCGATGCTACGACTTTGGACAGGCGTGGTTG

DNA sequences for patchy condensate:

Y-motif (4nt):

Y1: GCGCCAGTGAGGACGGAAGTTTGTCGTAGCATCGCACC

Y2: GCGCCAACCACGCCTGTCCATTACTTCCGTCCTCACTG

Y3: GCGCGGTGCGATGCTACGACTTTGGACAGGCGTGGTTG

Y2-FAM: [6-FAM]-CAACCACGCCTGTCCATTACTTCCGTCCTCACTG

^{orth}Y-motif (4nt):

^{orth}Y1: GGCCCTGGTTACACTGAGCTTTATGAACCTAGTGTGGC

^{orth}Y2: GGCCGCCACACTAGGTTCATTTCGCTTGATACGATGTC

^{orth}Y3: GGCCGACATCGTATCAAGCGTTAGCTCAGTGTAACCAG

^{orth}Y2-HEX: [HEX]-GCCACACTAGGTTCATTTCGCTTGATACGATGTC

S-motif (4nt):

S-1: GGCCGCTGGACTAACGGAACGGTTAGTCAGGTATGCCAGCAC

S-2: GGCCCTCAGAGAGGTGACAGCATTCCGTTCCGTTAGTCCAGC

S-3: GGCCCCATGGTCCCAAGTGATGTTTGCTGTCACCTCTCTGAG

S-4: GCGCCAGACGTCACTCTCCAACTTCGCAAATTTACAGCGCCG

S-5: GCGCGTGCTGGCATACCTGACTTTGTTGGAGAGTGACGTCTG

S-6: GCGCGTGCTGGCATACCTGACTTTGTTGGAGAGTGACGTCTG

*Under bars represent SE sequence.

Table S2. DNA condensate composition in each experiment

Condensation experiments (Figure 3, 4, 5)

component	concentration
Y-1	10 μM
Y-2	10 μM
Y-3	10 μM
YOYO-1	10 μM
NaCl	50 μM
Tris-HCl (pH 8)	20 μM

Condensation experiment with varying Y-motif concentrations (Figure 6)

component	concentration			
Y-1	2.5	5	10	20 μM
Y-2	2.5	5	10	20 μM
Y-3	2.5	5	10	20 μM
YOYO-1	10	10	10	10 μM
NaCl	50	50	50	50 μM
Tris-HCl (pH 8)	20	20	20	20 μM

Table S3. Condensation experiment for patchy DNA condensate (Figure 7)

component	concentration	
Y-1	5	μM
Y-2	4.5	μM
Y-2-FAM	0.5	μM
Y-3	5	μM
^{orth}Y1	5	μM
^{orth}Y2	4.5	μM
^{orth}Y2-HEX	0.5	μM
^{orth}Y3	5	μM
S-1	0.5	μM
S-2	0.5	μM
S-3	0.5	μM
S-4	0.5	μM
S-5	0.5	μM
S-6	0.5	μM
NaCl	50	μM
Tris-HCl (pH 8)	20	μM