Supplemental Information

Microfluidic digital focus assays for the quantification of infectious influenza virus

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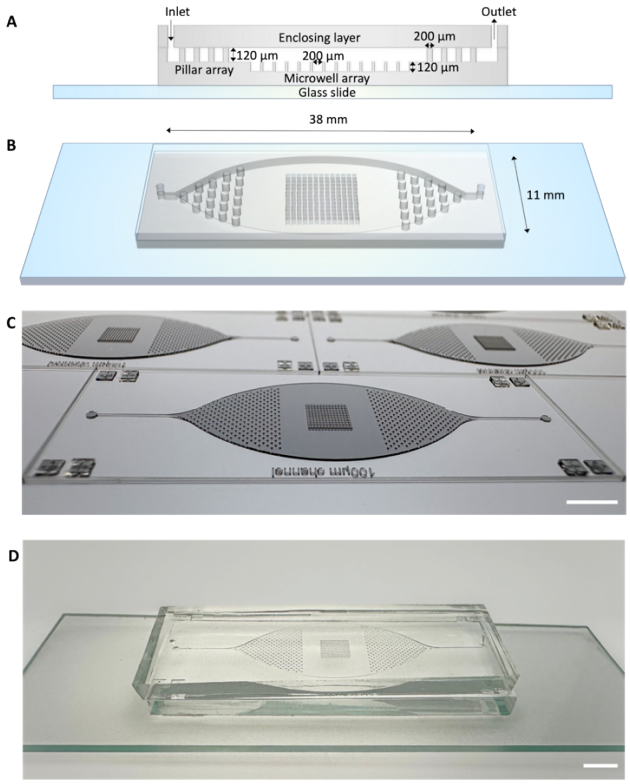

Figure S1: Overview of the dFA chip geometry. (A) Cross-sectional schematic of a PDMS chip bonded to the glass slide, with feature dimensions indicated. (B) Perspective view of a chip revealing the 13x13 microwell array and enclosing channel with inlet and outlet pillar arrays designed for improved cell seeding uniformity. (C) Image of a fabricated multi-layer SU-8 wafer with 8 devices per mold. D) An image of the enclosed PDMS microwell array chip. Scale bar: 4 mm.

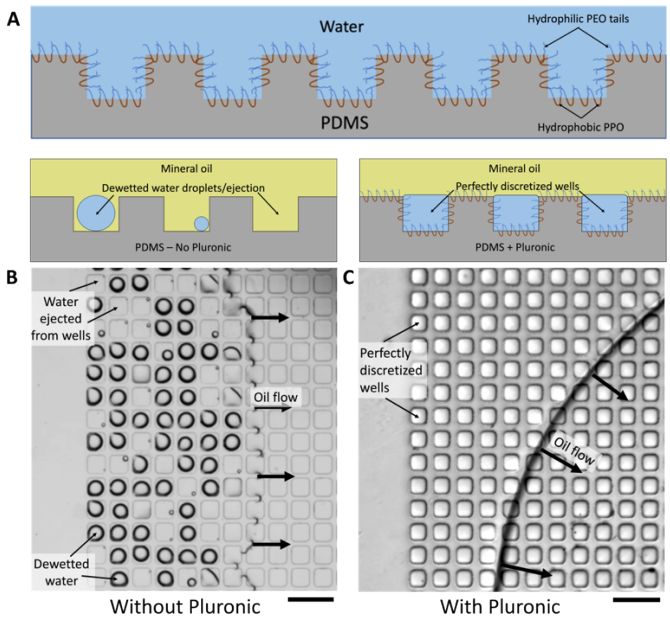

Figure S2: Hydrophilic surface modification through embedded Pluronic surfactant.
(A) Pluronic F-108 embedded into the PDMS matrix yields a hydrophilic surface after overnight exposure to water. (B) Cross-sectional schematic and image of the 200 µm PDMS microwells without embedded Pluronic F-108. Flowing mineral oil over the microwells prefilled with water caused dewetting and partial ejection of water from the microwells. (C) Cross-sectional schematic and image of the 200 µm PDMS microwells with embedded Pluronic F-108. Flowing mineral oil over the microwells yields perfect discretization of the microwells without observable loss of water. Scale bar: 500 µm.

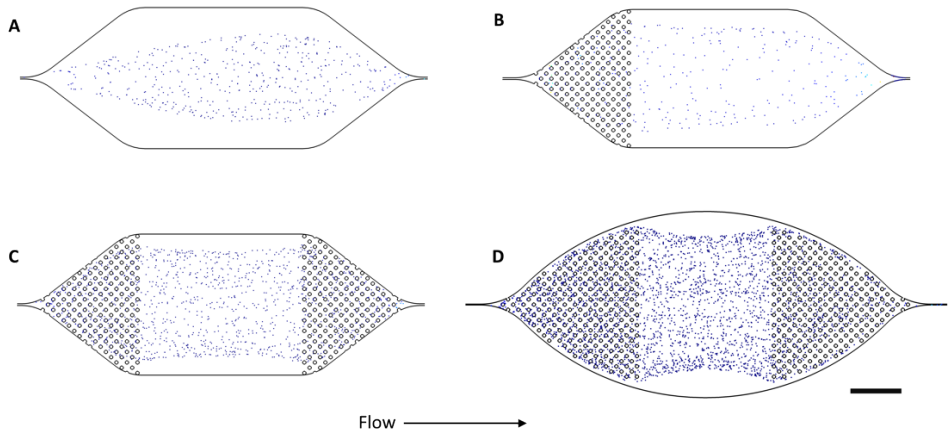

Figure S3: Cell suspension flow simulation results. COMSOL Multiphysics simulation depicting the flow behavior of cell suspension liquids through (A) a rectangular enclosing channel without pillar arrays, (B) a rectangular enclosing channel with pillar arrays on the inlet, (C) a rectangular enclosing channel with pillar arrays on both the inlet and the outlet, and (D) a continuously curving channel with pillar arrays on both the inlet and the outlet, designed to promote mixing and uniform lateral distribution of the cells to the microwell arrays. Scale bar: 3 mm.

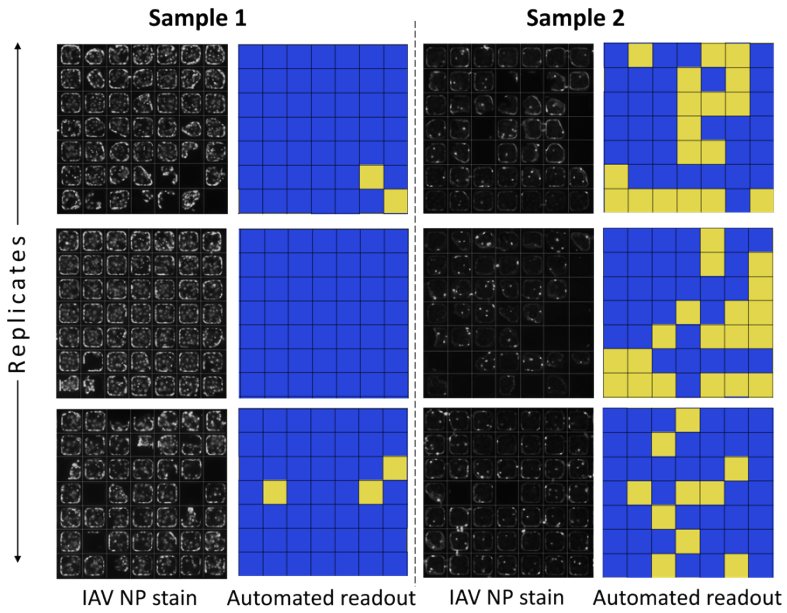

Figure S4: Fluorescence images and automated dFA readout for nasal swab specimens. Images and binary readout for a subset of wells in each of 3 replicate chips run on nasal swab specimens with high (sample 1) and low (sample 2) viral titer.