Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Computational Fluid Dynamics of Sample Digitization

Two physical models were involved in the models: First, the laminar two-phase flow, level set model was used to track the interface between two immiscible fluids. It solved Navier-Stokes equations for the conservation of momentum and a continuity equation for the conservation of mass. The interface position was tracked by solving a transport equation for the level-set function in COMSOL:

$$\rho\frac{\partial \boldsymbol{u}}{\partial t}+\rho(\boldsymbol{u}\cdot\nabla)\boldsymbol{u}=\nabla\cdot[-\rho\boldsymbol{I}+\mu(\nabla\mathbf{u}+\nabla\mathbf{u})^{T}]+\rho\boldsymbol{g}+\boldsymbol{F}_{st}+\boldsymbol{F}$$

$$\nabla\cdot\boldsymbol{u}=0$$

$$\frac{\partial\phi}{\partial t}+\boldsymbol{u}\cdot\nabla\phi=\gamma\nabla\cdot\left[\epsilon_{ls}\nabla\phi-\phi(1-\phi)\frac{\nabla\phi}{|\nabla\phi|}\right],\phi=\mathrm{phils}$$

Second was the two-phase Darcy's law model which simulated the air permeation to the interstices in a porous PDMS substrate medium surrounding the chamber. It solved Darcy's law for the total pressure and the transport of the fluid content for one fluid phase in COMSOL:

$$\frac{\partial\rho\epsilon_{p}}{\partial t}+\nabla\cdot(\rho\boldsymbol{u})=0$$

$$\frac{\partial c_{1}\epsilon_{p}}{\partial t}+\nabla\cdot(c_{1}\boldsymbol{u})=\nabla\cdot(D_{c}\nabla\mathrm{c}_{1})$$

Table S1. Parameters involved in the two-dimensional multiphase fluid model of the sample digitization in COMSOL

	Channel-chamber with straight conjunction	Channel-chamber with 45° conjunction	Straight main channel	180° curved main channel
Geometry	Channel: w=100μm, h=50μm. Chamber: w=h=80μm	Channel: w=100μm, h=50μm. Chamber: w=h=80μm	Channel: w=20mm, h = 0.1mm	Channel: l=20mm, h = 0.1mm, d_1 =6.42mm, d_2=6.32mm
Physical model 1	Laminar Two-Phase Flow, Level Set	Laminar Two-Phase Flow, Level Set	Laminar Two-Phase Flow, Level Set	Laminar Two-Phase Flow, Level Set
Fluid properties	Fluid 1: air. Fluid 2: water. T=293.15K, built-in density and dynamic viscosity	Fluid 1: air. Fluid 2: water. T=293.15K, built-in density and dynamic viscosity	Fluid 1: air. Fluid 2: water. T=293.15K, built-in density and dynamic viscosity	Fluid 1: air. Fluid 2: water. T=293.15K, built-in density and dynamic viscosity
Inlet condition	Laminar inflow V=0.05m/s	Laminar inflow V=0.05m/s	Laminar inflow V=0.001m/s	Laminar inflow V=0.001m/s
Outlet condition	Pressure P_0= 0, Suppress backflow	Pressure P_0= 0, Suppress backflow	Pressure P_0= 0, Suppress backflow	Pressure P_0= 0, Suppress backflow
Wall condition	Wetted wall: θ_w=90°, β=5μm	Wetted wall: θ_w=90°, β=5μm	No slip	No slip
Physical model 2	Two-phase Darcy's law	Two-phase Darcy's law	N/A	N/A
Porous media properties	D_c=500μm^2/s, K_{r1}=0.2, K_{r2}= 0.01	D_c=500μm^2/s, K_{r1}=0.2, K_{r2}= 0.01	N/A	N/A
Inlet condition	Normal inflow V=0.5mm/s	Normal inflow V=0.5mm/s	N/A	N/A
Outlet condition	Pressure P_2= -0.01atm	Pressure P_2= -0.01atm	N/A	N/A
Mesh	1849 domain & 207 boundary elements	2144 domain & 219 boundary elements	7256 domain & 1458 boundary elements	3046 domain & 1010 boundary elements
Time stepping method	generalized-alpha	generalized-alpha	generalized-alpha	generalized-alpha
Time step size	0.01s	0.01s	0.1s	0.1s
Time	10s	10s	10s	10s

Table S2. Target and primer sequences for synthetic DNA oligos.

CDKN2A Unmethylated (0/13)	TGTTTTTGGTGTTGTTTATTTTTTTGTGAGTTGTGGGATGTGAATTATGA AAATTTTTATTTGTGGTGGGTTGTATGTGTGTTGAATTTGGAGGGTTATT AAGAATTTGTGTATTATGT
CDKN2A 4/13 Methylated	CGTTTTTGGCGTTGTTTATTTTTTTGTGAGTCGTGGGATGTGAATTACGA AAATTTTTATTTGTGGTGGGTCGTATGTGTGTCGAATTTGGAGGGTTATT AAGAATTTGCGTATTATGT
CDKN2A 9/13 Methylated	CGTTTTTGGCGTTGTTTATTTTTTCGTGAGTCGTGGGATGTGAATTACG AAAATTTTTATTCGTGGCGGGTCGTATGCGTGTCGAATTCGGAGGGTTA TTAAGAATTTGCGTATTATGT
CDKN2A 13/13 Methylated	CGTTTTTGGCGTTGTTTATTTTTTCGTGAGTCGCGGGATGTGAATTACG AAAATTTTTATTCGCGGCGGGTCGTACGCGCGTCGAATTCGGAGGGTT ATTAAGAATTTGCGTATTATGT
CDKN2A Forward Primer	CGTTTTTGGCGTTGTTTATTTT
CDKN2A Reverse Primer	ACATAATACGCAAATTCTTAATAACCCTC
BRCA1 2/5 Methylated	CGCGGGAATTATAGATAAATTAAAATTGTGATTGCGCGGTGTGAGTTTG TTGAGATTTTTTGGACGGGGGA
BRCA1 3/5 Methylated	CGCGGGAATTATAGATAAATTAAAATTGTGATTGCGCGGCGTGAGTTTG TTGAGATTTTTTGGACGGGGGA
BRCA1 5/5 Methylated	CGCGGGAATTATAGATAAATTAAAATTGCGATTGCGCGGCGTGAGTTCG TTGAGATTTTTTGGACGGGGGA
BRCA1 Forward primer	CGCGGGAATTATAGATAAATTAAAATTG
BRCA1 Reverse primer	TCCCCCGTCCAAAAAATCTCAA

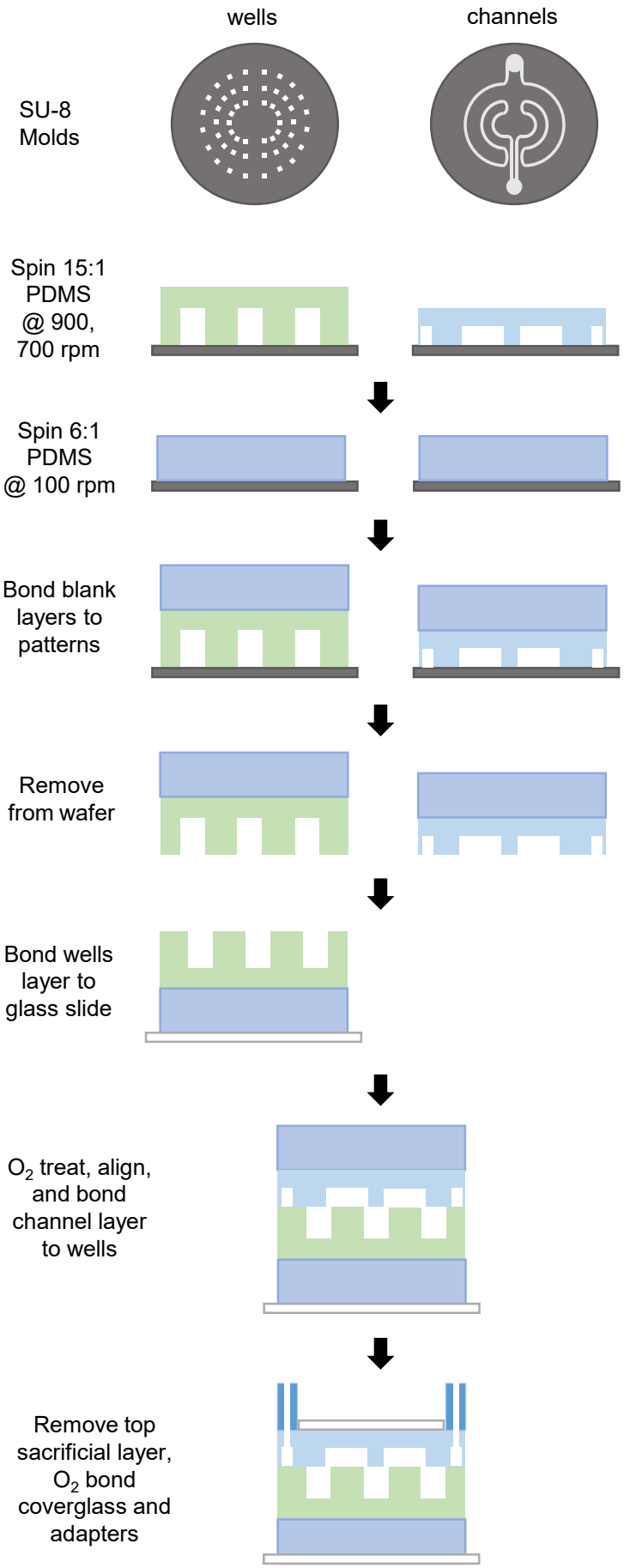

Figure S1. Ultra-thin layers of PDMS were spun on each pattern mold, and removed by temporary bonding of a sacrificial layer. The two patterns were then oxygen-plasma treated, aligned, and bonded. Finally, a coverglass and adapters where oxygen-plasma bonded.

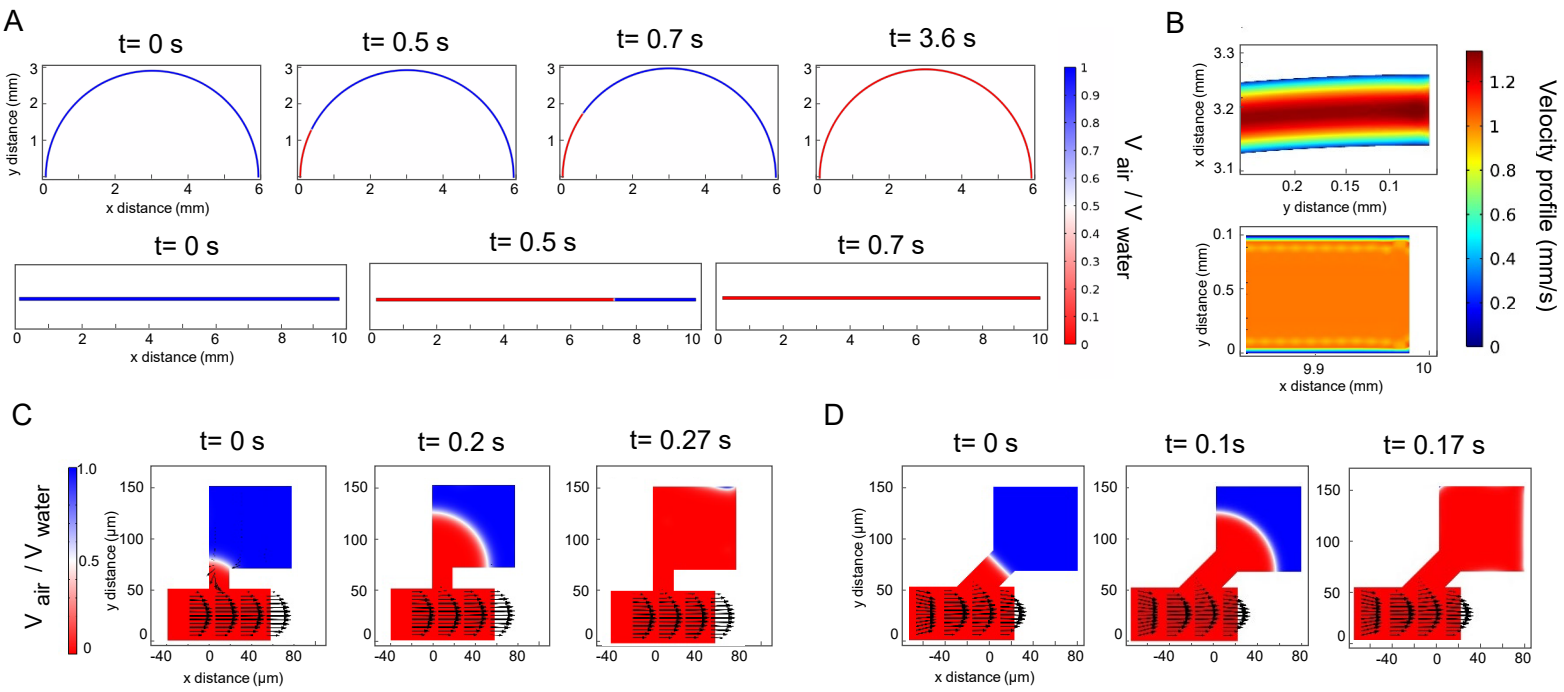

Figure S2. (A) COMSOL simulation results of sample loading in 180° curved vs. linear main channels with same length by two-dimensional water-air multiphase flow CFD analysis. (B) Aqueous sample velocity profiles of each channel geometry after the channel was filled with the aqueous sample. COMSOL simulation results of sample digitization by two-dimensional water-air multiphase flow CFD analysis before (t_0), (C) Straight (D) 45° inclined chamber filling; shown are the color contours of the water volume fraction ranging from blue = air to red = water (refer to the color legend) and the water/air interface. The vectors show the flow velocity in the whole geometry.

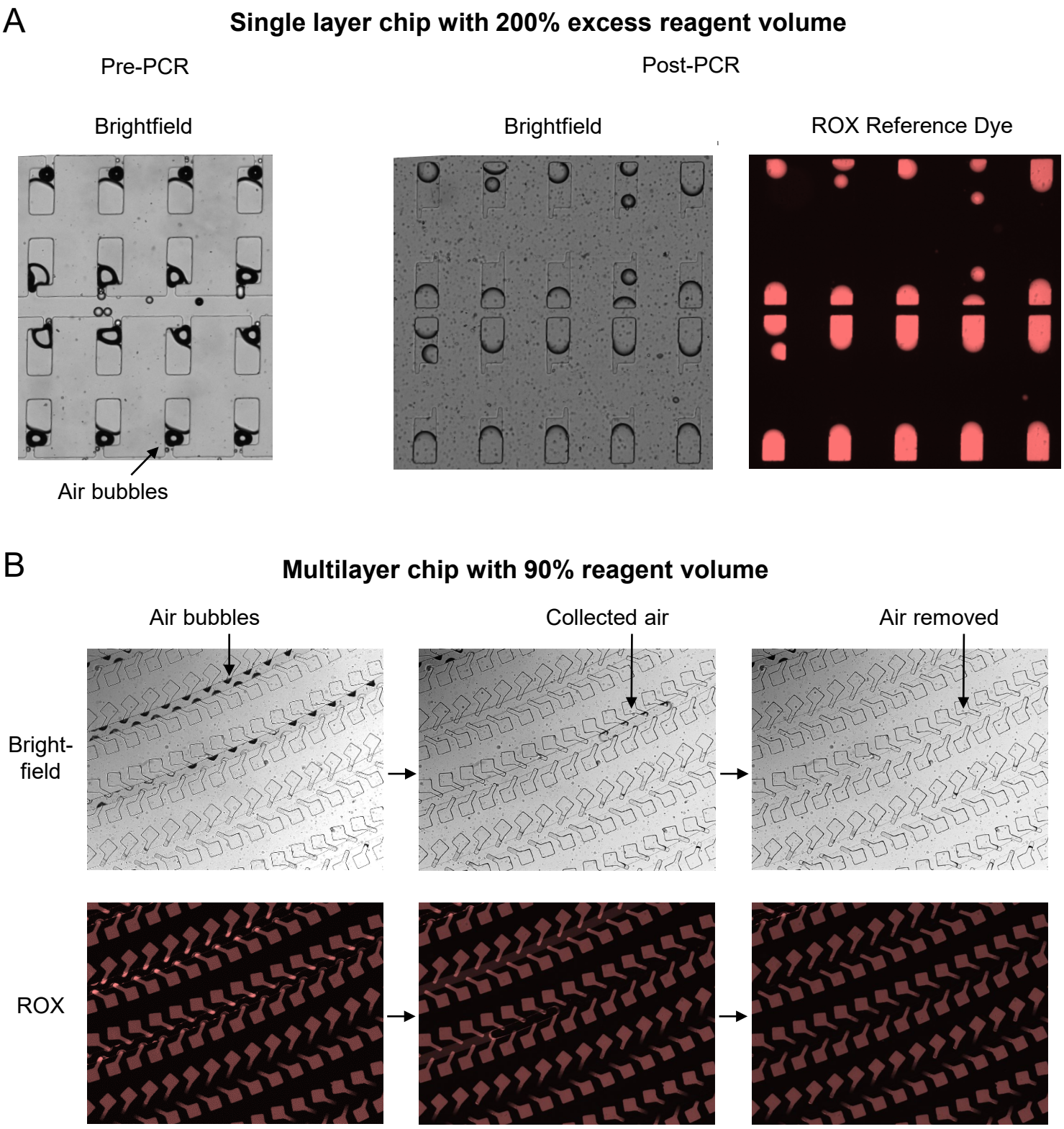

Figure S3. (A) Loading and partitioning of single-layer microfluidic device. The device has a capacity of 4 µL and was loaded with 8 µL. Air entered the chip after loading and resides in the wells after partitioning. This air caused significant evaporation and sample loss during heating steps. (B) Loading and partitioning of multilayer device. The device was loaded at less than full capacity to minimize loss. Air collects in the channels and is removed by oil partitioning.

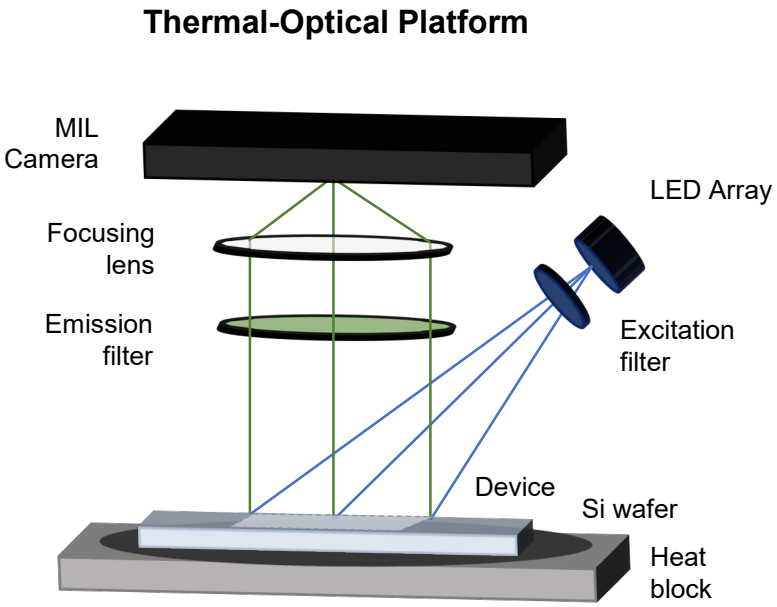

Figure S4. A commercial heater was modified by securing a 96-well to flat block adaptor and silicon wafer to the surface. The device, which is secured to the wafer, is illuminated by an LED array and an excitation filter. The fluorescence emission passes through an emission filter and focus lenses to the MILC.

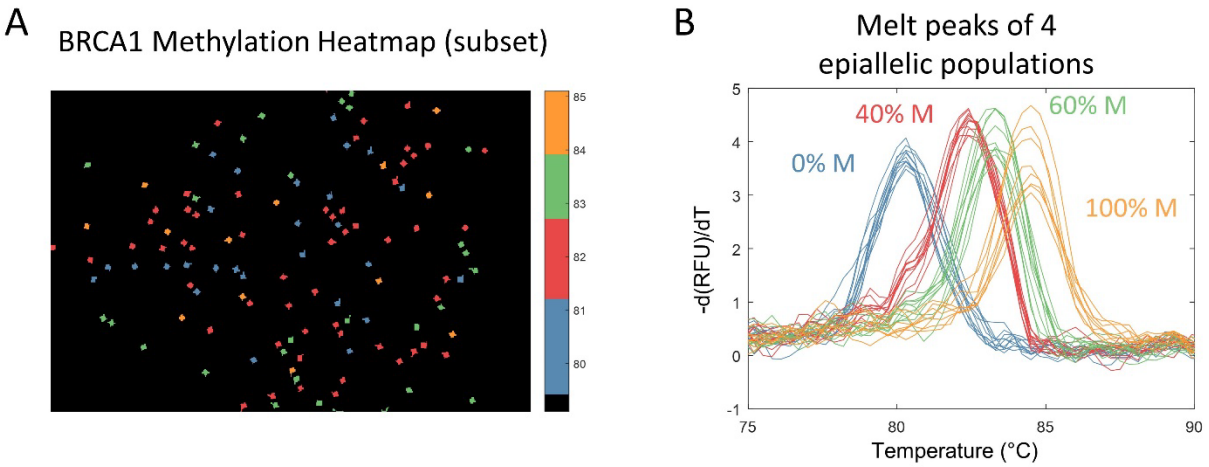

Figure S5. Synthetic sequences representative of 40%, 60% and 100% methylated bisulfite-converted BRCA1 were spiked into human male genomic DNA (bisulfite-converted) from random donors. After digitization, amplification, and melt analysis, a heatmap shows the distribution of the 4 epiallelic populations on the device (A). (B) Representative melt curve derivatives for the genomic unmethylated and synthetically methylated populations.