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

Supplementary Information

An automated centrifugal microfluidic assay for whole blood fractionation and isolation of multiple cell populations using an aqueous two-phase system

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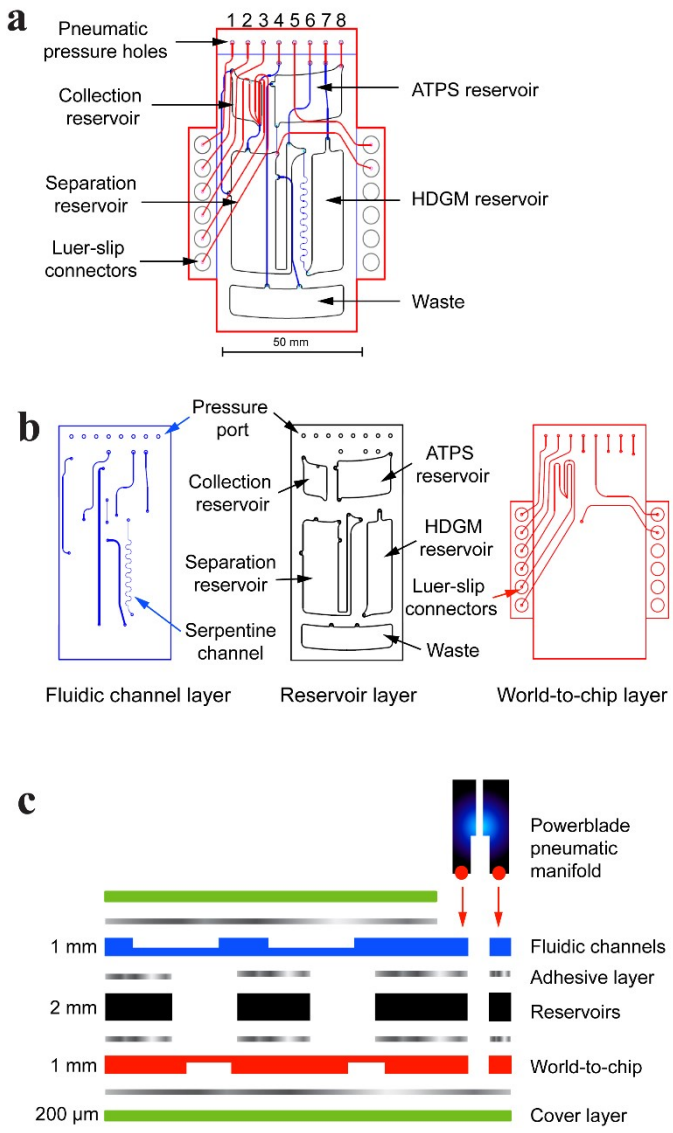

Figure S1. (a) Schematic drawing of the assembled device. The device assembly incorporates three layers of fluidic channels (top layer), reservoirs (middle layer) and world-to-chip channels (bottom layer). The pressure ports are located at the top side of the device and are numbered 1-8 from left to right. The color represents the same layer in (b) of the three different individual layers. Two layers containing microfluidic channels (blue and red) and one layer with reservoirs (black). All the fluidic features in these layers are milled in-house by CNC machining. The plastic used for these layers is Zeonor 1060R which is injection molded in the form of 6-inch diameter discs either 1 mm or 2 mm thick. Two additional layers of flat, unstructured Zeonor films with a thickness of 200 µm are used to seal the microfluidic device on the top (fluidic) and bottom (world-to-chip) layers. Alignment and sealing of all these plastic layers in the microfluidic stack along with the Luer connectors are performed manually. Two types of double-sided adhesive are employed to fully seal the devices: 100 µm thick 3M (3M, Saint-Laurent, Canada) for the bonding of the three fluidic layers (channels, world-to-chip and reservoirs) as well as the Luer connectors;

and a 25 µm thick acrylic based adhesive (ARcare® 92524, Adhesive Research, Glen Rock, PA, USA) for the two flat Zeonor sheets at the top and bottom. The footprint of the device is 50 mm x 100 mm. There are five reservoirs and eight pneumatic ports. The pneumatic ports are used to feed in the applied air pressure. The eight luer slip connectors are used to deliver fluids and air pressure onto the device. (c) Cross-sectional view of the device. The thickness of fluidic channels, reservoirs and world-to-chip channels are 1 mm, 2 mm and 1 mm, respectively. A 200 µm thick plastic film covers the top and bottom of the device to seal the fluidic channels. To apply pressure from the PB system to the device, an o-ring sealed pneumatic manifold on the PB rotor is used to distribute the pressure source into the microfluidic device.

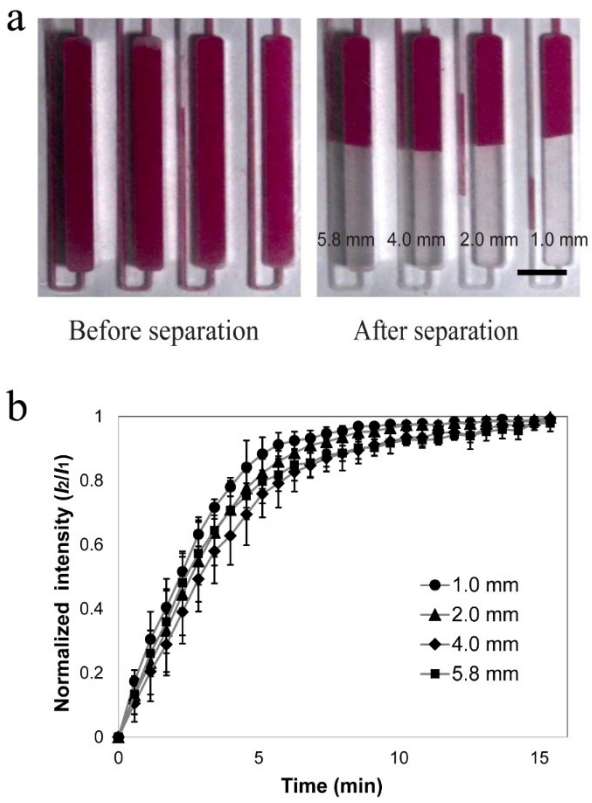

Figure S2. ATPS phase-separation on the PB system. (a) Images of the pre-mixed solutions and the separated ATPS solutions. Four different chamber depths are shown: 1.0, 2.0, 4.0 and 5.8 mm in depth. To visualize the phase-separation, 1 µm polymicrobeads (density: ~ 1.05 g/mL, Polysciences Inc.) are added in the pre-mixed solution. We observe that once the centrifugal force is applied, the microbeads move to the PEG solution immediately due to their affinity to PEG. A PEG-DEX interface becomes clearly visible within 15 min after centrifugation commences. Scale bar = 5 mm. (b) Plot of the normalized intensity (I_2/I_1) versus centrifugation time ($n=4$ devices). We plot four different experimental conditions with different chamber depths. We do not observe any significant difference of the separation time between the four different chamber depths. Here, the centrifugal force is applied using 600 rpm rotation

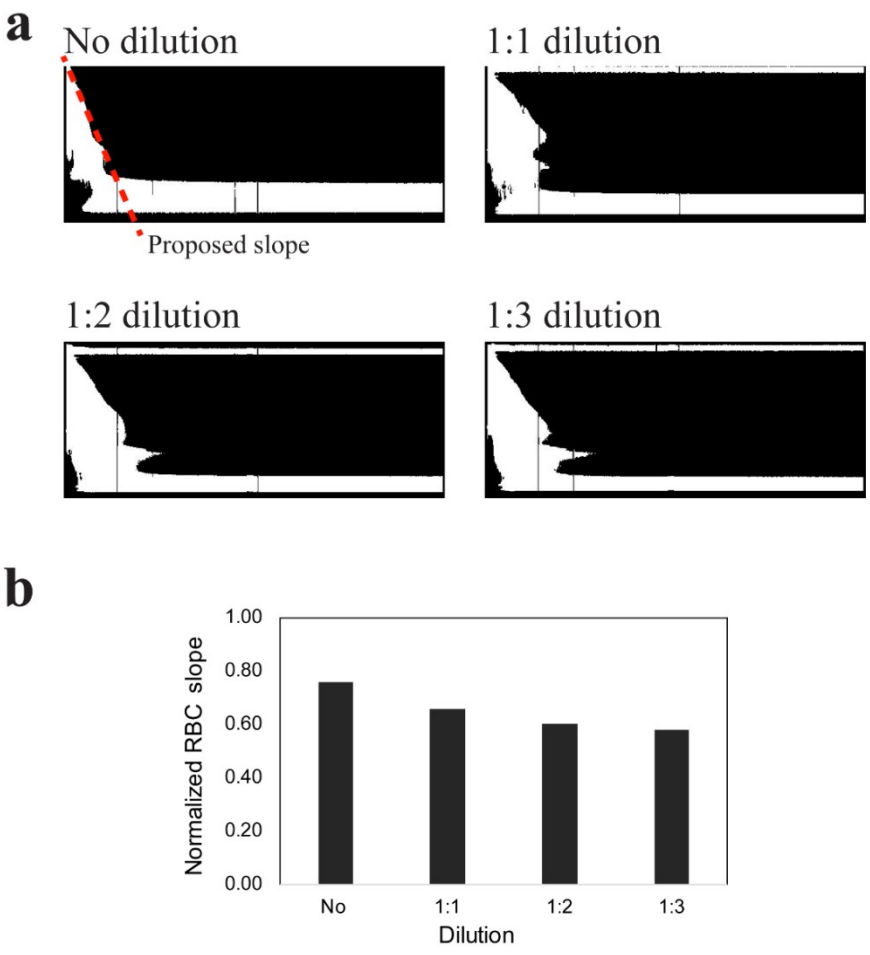

Figure S3. A proposed image analysis process using (a) Huang threshold and (b) normalized RBC slope with dilution factors. To analyze the orthogonal view images, the images are first converted to 8 bit using ImageJ with the default "Huang" setting selected to convert the images into black and white. The slope of the images is also calculated using ImageJ.

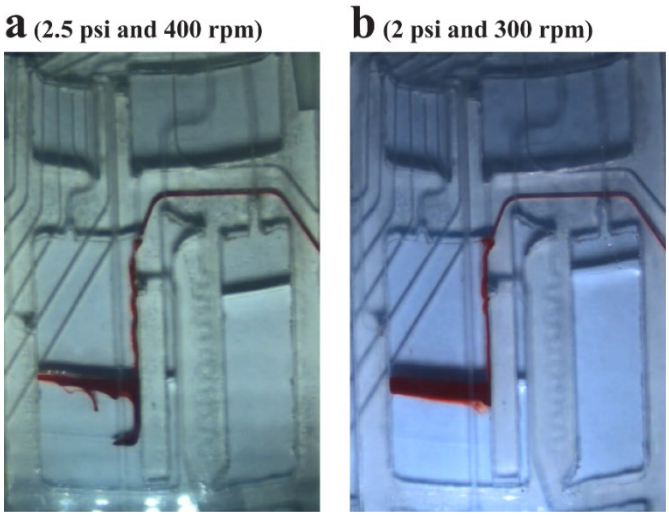

Figure S4. Experimental images of different blood layering behavior on the microfluidic platform. During transport of the whole blood into the device and onto the phase-separated ATPS solution, the applied pressure and centrifugal force both critically influence the layering characteristics onto the PEG phase. Higher pressures and higher centrifugal forces can create unwanted disruptions in the smooth layering of whole blood on the ATPS. Here, the applied pneumatic pressures and rotation speeds are (a) 2.5 psi and 400 rpm (b) 2 psi and 300 rpm, respectively.

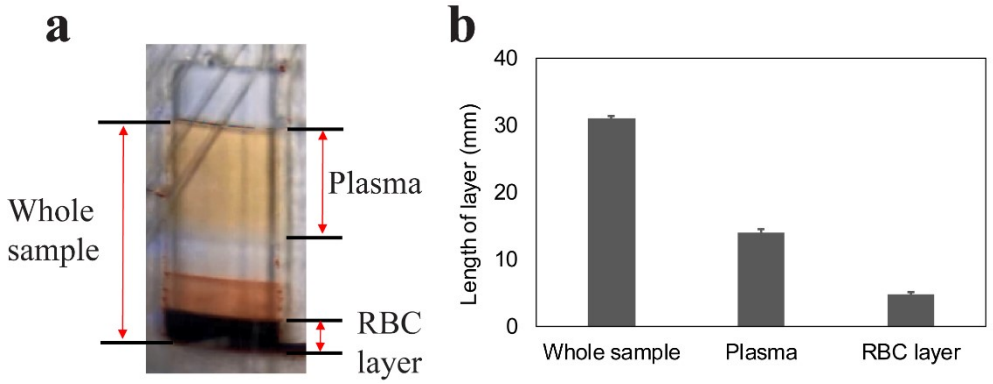

Figure S5. Image analysis for the reproducibility of the system. (a) Fractionated blood sample on the PB. We measured the length of layers of the fractionated whole sample, plasma and packed RBCs using ImageJ. (b) A graph of the length of the layer versus fractionated samples (*n=4* devices from two different donors). We evaluated the overall length of the whole sample layer which indicates reproducible processes as also shown in Table S1."

Table S1. Coefficient of variation of the fractionated layers of whole blood, plasma and RBCs.

	Whole sample	Plasma	RBC layer
CV (%)	1.09	4.35	9.72

Table S2. Whole blood fractionation and extraction protocol using the PB system

Step No.	Operation	Active port	Pressure (psi)	Rotation speed (rpm)	Duration	Indication in Fig. 5
1	ATPS separation	-	-	800	10 min	**a**
2	Transferring blood to the microfluidic device	5	2	300	10 sec	**b**
3	Whole blood fractionation process	-	-	800	20 min	**c** and **d**
4	Transferring plasma to collection chamber	4,5,6,7,8	2.5	400	1 sec	**e**
5	Transferring plasma to the tube (No.1a)	1	-2.5	400	5 sec	-
6	Transferring plasma to collection chamber	4,5,6,7,8	2.5	400	3 sec	**e**
7	Transferring plasma to the tube (No.1b)	1	-2.5	400	5 sec	-
8	Pushing the high density gradient medium (HDGM)	7	4	400	110 sec	**f**
9	Transferring the PBMC layer to collection chamber	4,5,6,7,8	2.5	400	4 sec	**g**
10	Transferring the PBMC layer to the tube (No.2)	2	-2.5	400	5 sec	-
11	Pushing the HDGM	7	4	400	130 sec	**h**
12	Transferring the neutrophil layer to collection chamber	4,5,6,7,8	2.5	400	10sec	**i**
13	Transferring the neutrophil layer to the tube (No.3)	3	-2.5	400	6 sec	-