Supporting Information

On-the-Fly Phase Transition and Density Changes of Aqueous Two-Phase Systems on a Centrifugal Microfluidic Platform

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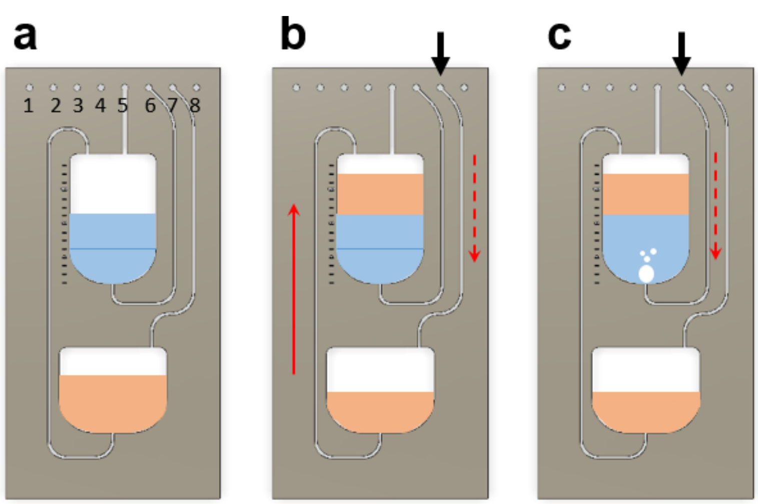

Figure S1. Schematic drawing of the fluidic control and liquid transfer process. (a) The ATPS solution and DI water are first introduced manually onto the chip via the channels, to the top and bottom chambers, respectively. (b) The DI water (or another ATPS) in the lower chamber is transferred to the upper chamber with an applied pressure by activating pneumatic port #7. Red and dashed red arrows indicate the channels used for the delivery of liquid and air, respectively. (c) The two solutions are now mixing by air bubbles, which is introduced by pneumatic port #6.

Table S1. Microfluidic implementation of the two-phase-to-single-phase protocol using the centrifugal microfluidic platform

Step No.	Precedure	Sample volume (μL)		Active port	Pressure (psi)	Rotation speed (rpm)	Duration	Indication in Fig. 2
		Main	Lower					
1	ATPS sample loading	600	530	-	-	-	-	**a**
2	Phase separation 1	600	530	-	-	600	10 min	**b**
3	Water transfer	960	170	7	2	400	30 sec	**c**
4	Air bubble mixing	960	170	6	1.5	400	10 sec	**d**
5	Phase separation 2	960	170	-	-	600	10 min	**e**

Table S2. Microfluidic implementation of the single-phase-to-two-phase protocol using the centrifugal microfluidic platform

Step No.	Precedure	Sample volume (μL)		Active port	Pressure (psi)	Rotation speed (rpm)	Duration	Indication in Fig. 3
		Main	Lower					
1	ATPS sample loading	300	600	-	-	-	-	**a**
2	Phase separation 1	300	600	-	-	600	10 min	**b**
3	Air bubble mixing	300	600	5,6	2	600	10 sec	**c**
4	ATPS transfer	900	0	7	2.5	400	30 sec	**d**
5	Air bubble mixing	900	0	6	2	600	10 sec	**e**
6	Phase separation 2	900	0	-	-	600	10 min	**f**

Table S3. Microfluidic implementation of the density-matched color bead fractionation protocol using the centrifugal microfluidic platform

Step No.	**Precedure**	**Main chamber volume (μL)**	**Active port**	**Pressure (psi)**	**Rotation speed (rpm)**	**Duration**	**Indication in Fig. 4**
1	ATPS sample loading	500	-	-	-	-	-
2	Phase separation 1	500	-	-	600	10 min	**c (1)**
3	Metering	500	8	4.0-5.3	600	10 sec	**b (1)**
4	Water transfer	545	2,3,8	2.0-2.3	600	3 sec	**b (2)**
			Repeat No. 3-4 process				
5	Bubble mixing	590	7	3.0	600	2 sec	**b (3)**
6	Phase separation 2	590	-	-	600	10 min	**c (2)**
			Repeat No. 3-4 process × 4				
7	Bubble mixing	770	7	3.0	600	2 sec	-
8	Phase separation 3	770	-	-	600	10 min	**c (3)**
			Repeat No. 3-4 process × 4				
9	Bubble mixing	950	7	3.0	600	2 sec	-
10	Phase separation 4	950	-	-	600	10 min	**c (4)**
			Repeat No. 3-4 process × 5				
11	Bubble mixing	1175	7	3.3	600	2 sec	-
12	Phase separation 5	1175	-	-	600	10 min	**c (5)**
			Repeat No. 3-4 process × 4				
13	Bubble mixing	1355	7	3.3	600	2 sec	-
14	Phase separation 6	1355	-	-	600	10 min	**c (6)**

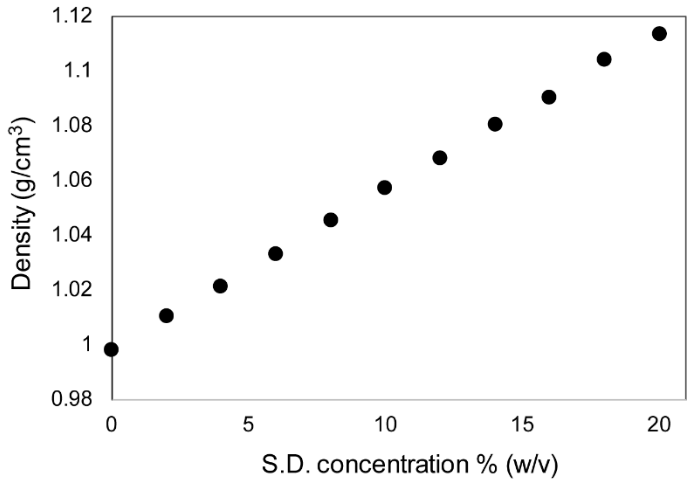

Figure S2. Plot of density change of water as a function of sodium diatrizoate concentration. As the sodium diatrizoate concentration is increased, the density of the solution increases linearly (R^2=0.9996). Note that for this experiment, we use only sodium diatrizoate without the addition of PEG or DEX. The examined density ranges are between 1.00 and 1.11 g/cm^3.

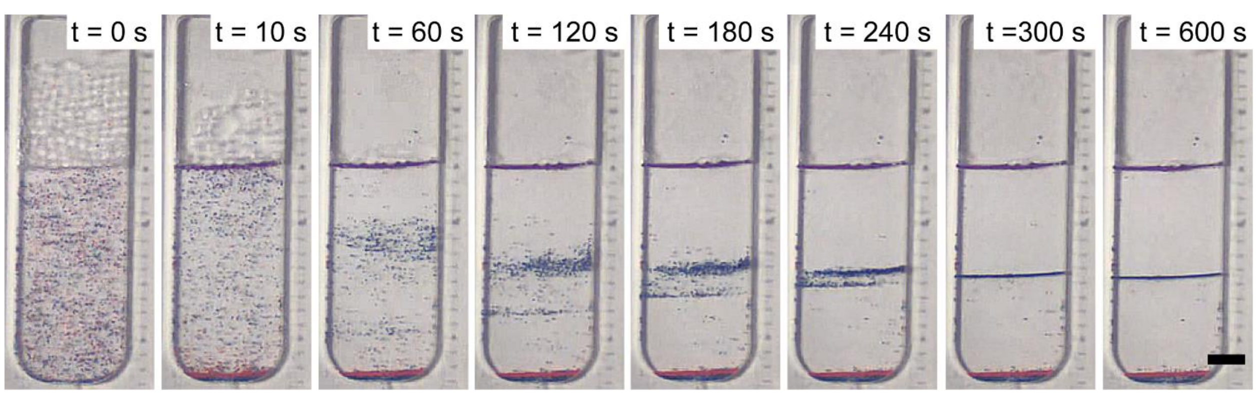

Figure S3. A time series images of the bead fractionation process. After air bubble mixing (t = 0 s), different densities of color beads are suspended together with the ATPS mixture. As the ATPS solution is phase-separated over time, the density-matched dark blue beads are falling onto the PEG-DEX interface. The fractionation procees is completed within 10 min at a rotation speed of 600 rpm. Scale bar = 5 mm.

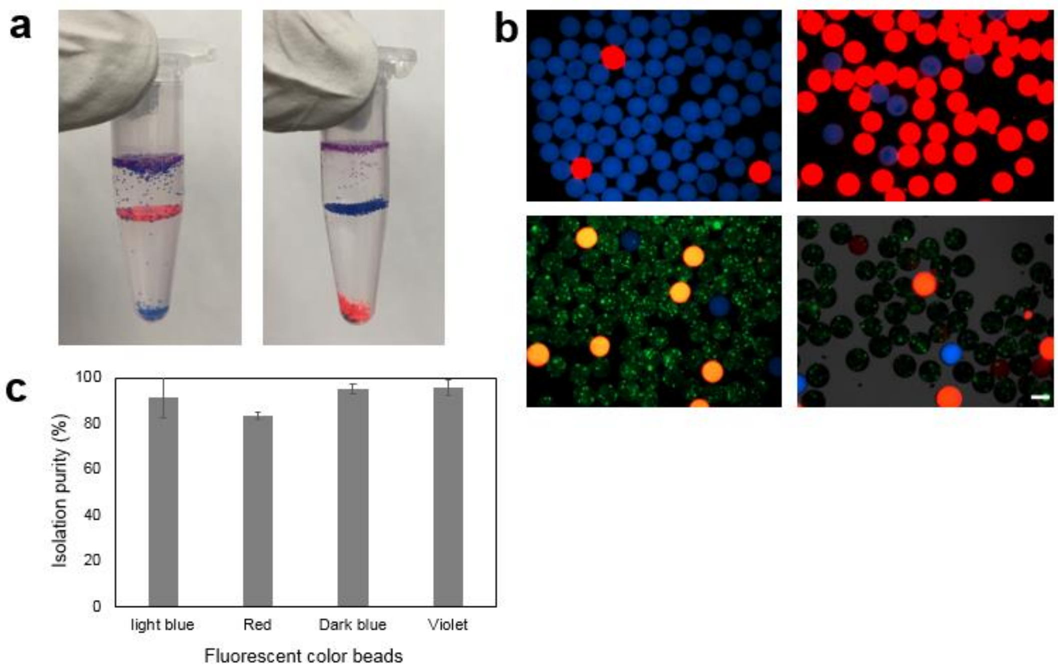

Figure S4. (a) Effect of ATPS density changes in the Eppendorf tube. The four different (fluorescence) color beads are separated according to the density-matched ATPS solutions. The left and right ATPS solutions are diluted with water to 35% and 47% from the initial solution of the ATPS #3, respectively. The beads are manually extracted after centrifugation by using a pipette tip. (b) Representative images of fluorescent color beads. The purity of the collected beads are validated using ImageJ. Scale bar = 200 µm. (d) A graph of isolation purity. The purity of the light blue, red, dark blue and violet beads are 91, 84, 95 and 96%, respectively. Of note, the isolation purity may be affected by manual sample handling and bead density variations.

Supplementary Information Movie 1:

Video shows two-phase-to-single-phase change dynamics. Initially, the main chamber has a two-phase solution. The solution is turned into a single-phase solution upon the additional water solution from the lower chamber to the main chamber *via* the mixing and centrifugation processes. As indicated in the video, the playback speed of each steps has been modified for better visualization.

Supplementary Information Movie 2:

Video shows single-phase-to-two-phase transition dynamics. The single phase ATPS #1 solution is changed to the two-phase solution with the mixture of a higher concentration of ATPS #2 in the lower chamber. As indicated in the video, the playback speed of each steps has been modified for better visualization.

Supplementary Information Movie 3:

Video shows on-demand ATPS density changes. Four differnet density markers of violet, dark blue, red and light blue color beads are added to the ATPS solution to visualize density-matching layers. After each stage separation, water solution is supplied by means of multiple processes of metering, liquid transportation and mixing. As indicated in the video, the playback speed of each steps has been modified for better visualization.