

SUPPLEMENTARY INFORMATION

Microfluidic Droplet-Array Liquid-Liquid Chromatography Based on Droplet Trapping Technique

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Chemicals and Reagents.

All reagents were of analytical reagent grade and used as received unless otherwise stated, and demineralized water was used throughout. Dimethyl sulfoxide (DMSO) and cyclohexane functioning as stationary phase and mobile phase were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Two polycyclic aromatic hydrocarbons (fluoranthene and benzo[b]fluoranthene) were used as model analytes and purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solution of each analytes (1 mg/mL) was prepared in cyclohexane and stored in a refrigerator at 4°C. Working sample solution (0.25 mg/mL) was prepared by mixing the stock solutions and diluting with cyclohexane. A room-temperature ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF₄), was purchased from Chemer Chemicals (Hangzhou, China). Polyethylene glycol (PEG) 1000 and anhydrous dibasic potassium phosphate (K₂HPO₃) were obtained from Aladdin-Reagent (Shanghai, China). The

aqueous two phase system (ATPS) was prepared by dissolving 15 g of PEG 1000 and 15 g of K_2HPO_3 in 90 mL of distilled water and allowing the mixture to completely separate into two phases before use.

Fabrication of the microchip.

The microchannels were fabricated on a glass substrate using standard photolithographic and wet chemical etching techniques.¹ The channel design of the chip is shown in Figure S1. After an access hole was drilled at the outlet of the central channel, the etched substrate was bonded with a cover plate using the room-temperature prebonding and high-temperature (560 °C) bonding methods.² The appearance of the microchip is as shown in Figure S1a. The central channel was 20 μm in depth, 60 μm in width, and total 40 cm in length. Two arrays of micro-recesses were fabricated on both sides of the central channel. Each micro-recess was 20 μm in depth, 50 μm in width, and 50 μm in length (Figure S1b and S1c). The micro-recess spacing between the edges of the adjacent recesses was 10 μm unless mentioned otherwise. A monolithic sampling probe with a tip size of 50 μm was produced at the inlet of the central channel for introducing sample and reagents (Figure S1d).³

Detection system.

The chromatographic separation was monitored using an inverted fluorescence microscope (ECLIPSE TE-2000-S, Nikon Co., Japan) equipped with a 10 \times objective and a UV filter cube (EX 330-380 nm, DM 400 nm, BA 420 nm). The fluorescence images of the chip microchannel were recorded with a sensitive CCD camera (SPOT RT-SE6 Monochrome, Diagnostic Instruments, Sterling Heights, MI, USA). Since the total length of the LC column on the chip was 40 cm, the detection point was changed by adjusting the imaging position of the fluorescent microscope according to the actual effective length of the LC column. For an effective column length of 4 cm, the detection position was the droplet with a distance of 4 cm to the inlet of the column. During the separation process, the fluorescence images of the droplet were continuously recorded with an exposure time of 500 ms and an acquisition speed of 12 frames per min. Then, a self-made program written with LabView (National Instruments, Austin, TX, USA) was used to read the grayscale values of a fixed region of 20 \times 20 pixel in the center of the droplet in each

fluorescence image. The chromatogram was assembled using these values corresponding to their time points.

Procedure

The operation procedures are illustrated in Figure 2 (Also see movies in the Supporting Information). Before use, the slotted vials were filled with rinsing solvent (acetone), stationary phase (DMSO), mobile phase (cyclohexane), sample solution, and mobile phase (cyclohexane), respectively. The waste reservoir was placed at a lower position than the chip plane to generate a liquid level difference for liquid driving. The sample injection and separation were performed by linearly moving the SVA stage, allowing the sampling probe to sequentially enter the vials through the slot to introduce different reagents and sample into the chip channel. First, acetone was introduced to rinse the channel (Figure 2a). Second, DMSO was introduced into and fully filled the central channel and the micro-recesses (Figure 2b). Third, the mobile phase cyclohexane was introduced into the channel, and excluded DMSO out from the central channel, leaving two arrays of DMSO droplets trapped in the micro-recesses served as the stationary phase (Figure 2c). Fourth, a sample plug was injected into the channel with a definite sampling time (Figure 2d). Finally, cyclohexane was continuously introduced into the channel to accomplish the sample separation (Figure 2e). The whole process could be automatically achieved with the SVA system under control of a program written in LabVIEW.

To measure the velocity of the mobile phase in LC separation, we injected a plug of non-retained compound, perfluorodecalin (Sigma-Aldrich, St. Louis, USA) in the LC column, because it is immiscible with both the stationary-phase DMSO and mobile-phase cyclohexane, and its phase interface with cyclohexane could be clearly observed with the fluorescent microscope. The velocity was obtained by measuring the required time when the plug of perfluorodecalin passed through a certain distance.

Safety Considerations.

Wet etching of the glass chips should be carried out in a well-ventilated hood, while wearing protective gloves and goggles. UV protection glasses should be worn to protect eyes from UV light of the high mercury lamp in assembling the fluorescence detection system.

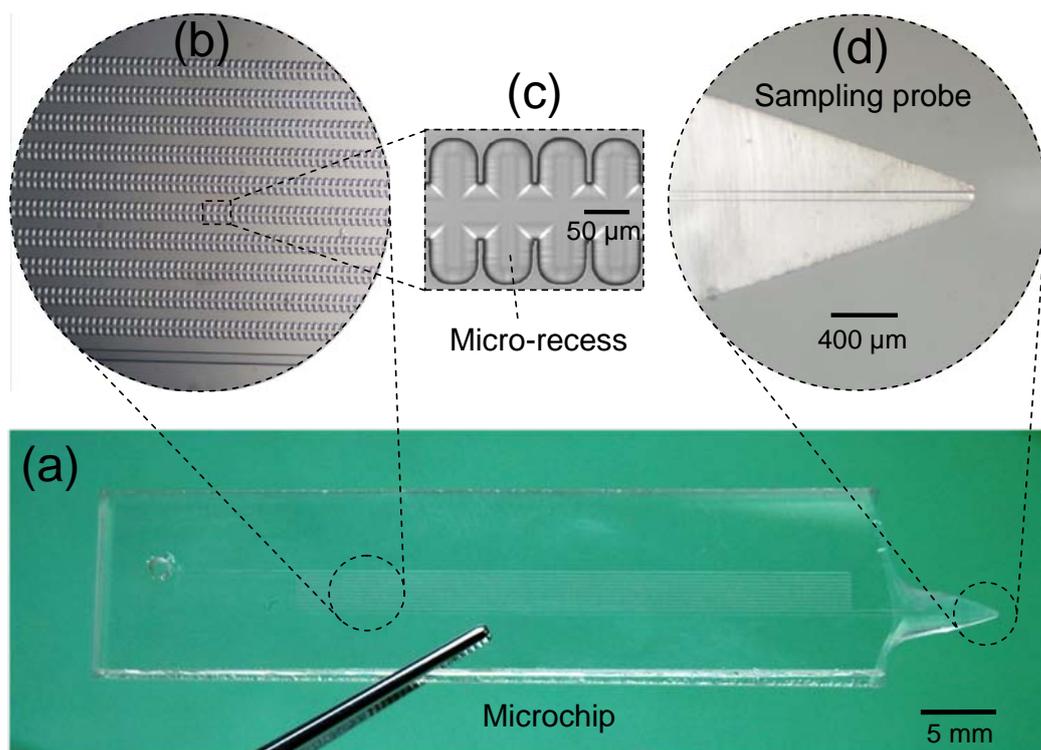


Figure S1. (a) Appearance of the droplet-array chromatography chip; (b) The enlarged view from (a) showing the micro-recess array; (c) The enlarged view from (b) showing the size of each micro-recess; (d) The enlarged view from (a) showing the on-chip monolithic sampling probe.

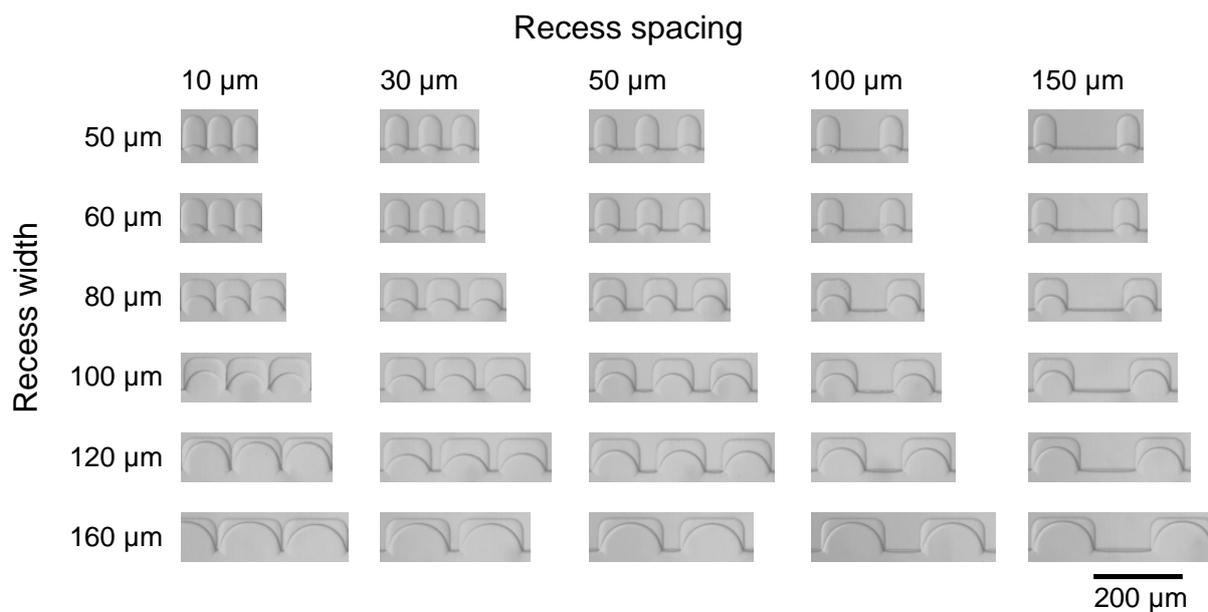


Figure S2. The effect of the recess spacing (the distance between the edges of the adjacent droplets) on the performance of droplet trapping for the micro-recesses with widths of 50 μm , 60 μm , 80 μm , 100 μm , 120 μm , and 160 μm . The depth and the length of the micro-recesses were 20 μm and 80 μm , respectively.

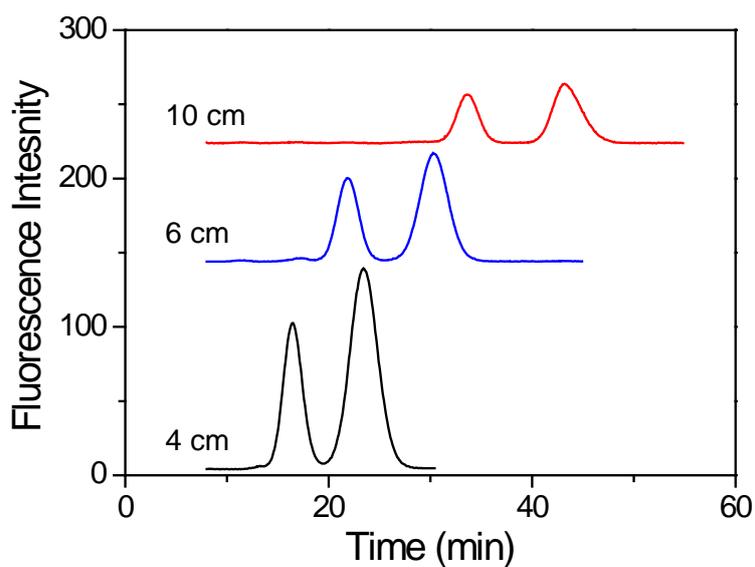


Figure S3. The effect of the effective column length on the separation performance.

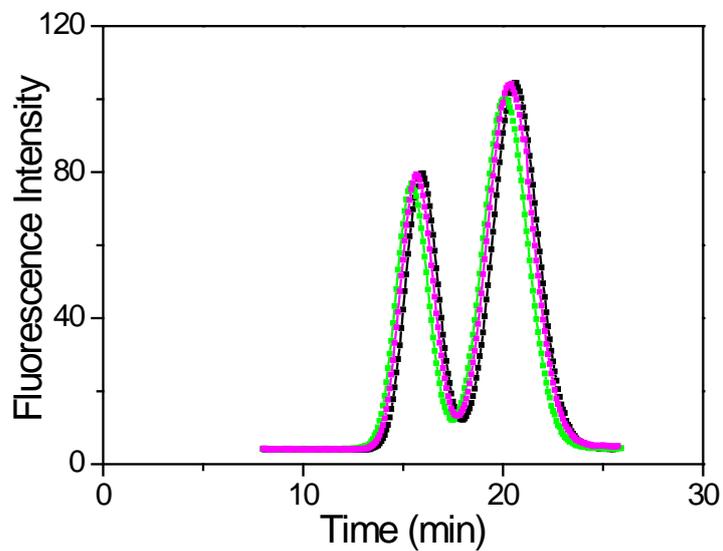


Figure S4. Typical chromatograms of repetitive separation of fluoranthene and benzo[b]fluoranthene showing the repeatability of the system. Conditions: sample injection volume: 3.3 nL; velocity: 120 $\mu\text{m/s}$; effective column length: 4 cm; stationary phase: DMSO; mobile phase: cyclohexane.

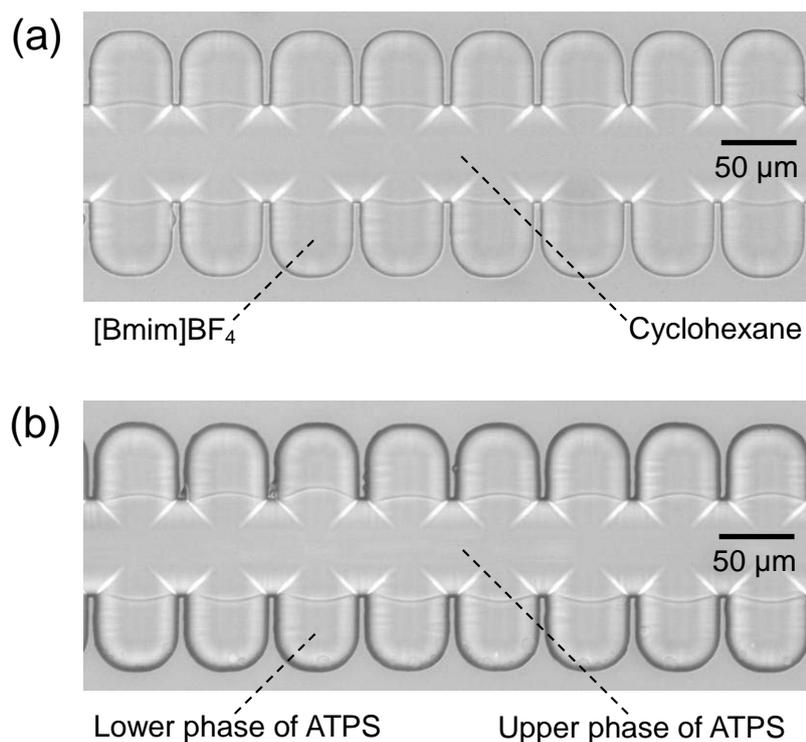


Figure S5. (a) Droplet array formed using ionic liquid system. Stationary phase: [Bmim]BF₄; mobile phase: cyclohexane. (b) Droplet array formed using aqueous two phase system (ATPS). ATPS: 12.5% PEG1000- 12.5%K₂HPO₃ in water; stationary phase: lower phase; mobile phase: upper phase.

REFERENCES

- (1) Fang, Q.; Xu, G. M.; Fang, Z. L. *Anal. Chem.* **2002**, *74*, 1223–1231.
- (2) Jia, Z. J.; Fang, Q.; Fang, Z. L. *Anal. Chem.* **2004**, *76*, 5597–5602.
- (3) Zhu, Y.; Pan, J. Z.; Su, Y.; He, Q. H.; Fang, Q. *Talanta* **2010**, *81*, 1069–1075.