## **Supplementary materials**

## Inertial co-focusing of heterogeneous particles in hybrid microfluidic channels with constantly variable cross-sections

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## The design of HMC B, C, and D

**HMC B (Fig. S1A):** The outer wall of HMC B was a three-quarter circle arc with a curvature radius of 5,100  $\mu$ m ( $R_1$ ), and the inner wall was a three-quarter helix with radii of 4,500  $\mu$ m ( $R_2$ ) and 4,800  $\mu$ m ( $R_4$ ). This caused the main channel width of HMC B gradually decreasing from 600  $\mu$ m (inlet,  $W_1$ ) to 300  $\mu$ m (outlet,  $W_2$ ). Twenty semi-circular obstacles were distributed on the inner wall of HMC B with an identical angular distance between adjacent obstacles of  $\alpha = 13^{\circ}$ . The radii of the obstacles ( $r_2$ ) were determined by a three-quarter helix with radii of 4,800  $\mu$ m ( $R_3$ ) and 4,950  $\mu$ m ( $R_5$ ), and thus the height of obstacles gradually decreased from 300  $\mu$ m (first obstacle near the inlet) to 150  $\mu$ m ( $r_1$ ) and 1,000  $\mu$ m ( $r_3$ ).

**HMC C (Fig. S1B)**: Both outer and inner walls of HMC C were three-quarter circle arcs with curvature radii of 5,100 µm ( $R_1$ ) and 4,900 µm ( $R_2=R_4$ ), respectively. This induced an uniform main channel width of 200 µm ( $W_1=W_2$ ) from inlet to outlet in HMC C. Twenty semi-circular obstacles were distributed on the inner wall with an identical angular distance adjacent obstacles of  $\alpha = 13^{\circ}$ . The radii of the obstacles ( $r_2$ ) were determined by a three-quarter helix with radii of 5,000 µm ( $R_3$ ) and 5,050 µm ( $R_5$ ), and thus the height of obstacles gradually increased from 100 µm (first obstacle near the inlet) to 150 µm ( $r_1$ ) and 1,000 µm ( $r_3$ ).

**HMC D (Fig. S1C)**: Both outer and inner walls of HMC D were three-quarter circle arcs with curvature radius of 5,100 µm ( $R_1$ ) and 4,900 µm ( $R_2=R_4$ ), respectively. This induced an uniform main channel width of 200 µm ( $W_1=W_2$ ) from inlet to outlet in HMC D. Twenty semi-circular obstacles with radii of 100 µm ( $R_3 = R_5 = 5,000$  µm) were distributed on the inner wall with an identical angular distance between adjacent obstacles of  $\alpha = 13^\circ$ . The obstacles were smoothly filtered by 300 µm ( $r_1$ ) and 1,000 µm ( $r_3$ ).



Fig. S1 The design of HMC B, C, and D.



Fig. S2 The fabricated chips of HMC A, B, C, and D using standard soft lithography technique and PDMS molding processes. The coin of one Chinese RMB is used for comparison. The scale bar is 5 mm.



Fig. S3 The color contours indicating main flow velocity magnitude in HMC B, C, and D.



Fig. S4 The maximum secondary flow velocity as functions of cross-section number and flow rate (Re) in HMC B, C, and D. The flow rates vary from 138 to 1,380  $\mu$ L·min<sup>-1</sup> in HMC B, from 66 to 660  $\mu$ L·min<sup>-1</sup> in HMC C, and from 79 to 790  $\mu$ L·min<sup>-1</sup> in HMC D, resulting in identical Re ranging from 10.5 to 105.



Fig. S5 The fluorescent streaks at the observation area in HMC A at different flow rates of 200  $\mu$ L·min<sup>-1</sup>, 400  $\mu$ L·min<sup>-1</sup>, 600  $\mu$ L·min<sup>-1</sup>, 800  $\mu$ L·min<sup>-1</sup>, and 1,000  $\mu$ L·min<sup>-1</sup>. The particle diameters are 10  $\mu$ m, 12  $\mu$ m, 15  $\mu$ m, and 20  $\mu$ m. The yellow arrow shows the fluid flow direction. The red dashed lines represent the channel walls. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.



Fig. S6 The images of focused particles captured by a high-speed CCD camera at flow rates of 200  $\mu$ L·min<sup>-1</sup>, 400  $\mu$ L·min<sup>-1</sup>, 600  $\mu$ L·min<sup>-1</sup>, 800  $\mu$ L·min<sup>-1</sup>, and 1,000  $\mu$ L·min<sup>-1</sup>. The particle diameters are 10  $\mu$ m, 12  $\mu$ m, 15  $\mu$ m, and 20  $\mu$ m. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.



Fig. S7 The fluorescent intensity profiles of fluorescent streak images captured at the observation areas in HMC A with particle diameters of 10  $\mu$ m (A), 12  $\mu$ m (B), 15  $\mu$ m (C), and 20  $\mu$ m (D). The flow rates vary from 200 to 1,000  $\mu$ L·min<sup>-1</sup> (Re: 21 ~ 105).



Fig. S8 (A) The fluorescent streak of particle focusing. The yellow arrow shows the fluid flow direction. The red dashed lines represent the channel walls. The purple arrow indicates the position and direction of image processing. (B) The illustration of full width at half maximum and Ds (the distance between the fluorescent streak center and the inner wall) for the determination of focusing width and focusing position.



Fig. S9 (A) The images of focused multi-sized particles captured by a high-speed CCD camera at flow rates of 100  $\mu$ L·min<sup>-1</sup>, 800  $\mu$ L·min<sup>-1</sup>, 900  $\mu$ L·min<sup>-1</sup> and 1,000  $\mu$ L·min<sup>-1</sup> in HMC A. A particle mixture with a ratio of 1:1:1:1 of 10  $\mu$ m, 12  $\mu$ m, 15  $\mu$ m, and 20  $\mu$ m particles are prepared for the co-focusing experiments. The yellow arrow shows the fluid flow direction. (B) The composite images of focused multi-sized particles using 50 single images at flow rates of 100  $\mu$ L·min<sup>-1</sup>, 800  $\mu$ L·min<sup>-1</sup> and 1,000  $\mu$ L·min<sup>-1</sup> in HMC A. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.



Fig. S10 (A) The images of focused multi-sized particles captured by a high-speed CCD camera at flow rates of 138  $\mu$ L·min<sup>-1</sup> to 1,380  $\mu$ L·min<sup>-1</sup> (Re: 10.5 ~ 105) in HMC B. A particle mixture with a ratio of 1:1:1:1 of 10  $\mu$ m, 12  $\mu$ m, 15  $\mu$ m, and 20  $\mu$ m particles are prepared for the co-focusing experiments. The yellow arrow shows the fluid flow direction. (B) The composite images of focused multi-sized particles using 50 single images at flow rates of 138  $\mu$ L·min<sup>-1</sup> to 1,380  $\mu$ L·min<sup>-1</sup> (Re: 10.5 ~ 105) in HMC B. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.



Fig. S11 (A) The images of focused multi-sized particles captured by a high-speed CCD camera at flow rates of 66  $\mu$ L·min<sup>-1</sup> to 660  $\mu$ L·min<sup>-1</sup> (Re: 10.5 ~ 105) in HMC C. A particle mixture with a ratio of 1:1:1:1 of 10  $\mu$ m, 12  $\mu$ m, 15  $\mu$ m, and 20  $\mu$ m particles are prepared for the co-focusing experiments. The yellow arrow shows the fluid flow direction. (B) The composite images of focused multi-sized particles using 50 single images at flow rates of 66  $\mu$ L·min<sup>-1</sup> to 660  $\mu$ L·min<sup>-1</sup> (Re: 10.5 ~ 105) in HMC C. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.



Fig. S12 (A) The images of focused multi-sized particles captured by a high-speed CCD camera at flow rates of 79  $\mu$ L·min<sup>-1</sup> to 790  $\mu$ L·min<sup>-1</sup> (Re: 10.5 ~ 105) in HMC D. A particle mixture with a ratio of 1:1:1:1 of 10  $\mu$ m, 12  $\mu$ m, 15  $\mu$ m, and 20  $\mu$ m particles are prepared for the co-focusing experiments. The yellow arrow shows the fluid flow direction. (B) The composite images of focused multi-sized particles using 50 single images at flow rates of 79  $\mu$ L·min<sup>-1</sup> to 790  $\mu$ L·min<sup>-1</sup> (Re: 10.5 ~ 105) in HMC D. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.



Fig. S13 (A) The images of focused WBCs captured by a high-speed CCD camera at flow rates of 100  $\mu$ L·min<sup>-1</sup>, 200  $\mu$ L·min<sup>-1</sup>, 300  $\mu$ L·min<sup>-1</sup>, 900  $\mu$ L·min<sup>-1</sup> and 1,000  $\mu$ L·min<sup>-1</sup> in HMC A. The WBCs are indicated using red dots, since they were transparent and can hardly be seen in processed figures. Original images showing WBCs focusing are provided in Supplementary materials. The yellow arrow shows the fluid flow direction. (B) The composite images of focused WBCs using 50 single images at flow rates of 100  $\mu$ L·min<sup>-1</sup>, 200  $\mu$ L·min<sup>-1</sup>, 300  $\mu$ L·min<sup>-1</sup>, 900  $\mu$ L·min<sup>-1</sup> and 1,000  $\mu$ L·min<sup>-1</sup> in HMC A. The scale bar is 100  $\mu$ m. More images can be provided by reasonable requirements.