

## Label-free separation of nanoscale particles by an ultrahigh gradient magnetic field in a microfluidic device

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### Section S1. Details on the geometrical features and fabrication of the microfluidic system

The detailed structural parameters of microfluidic system are illustrated in Fig. S1. The width of the separation channel is 200  $\mu\text{m}$ , widths of the sample inlet and the outlet channel are 50  $\mu\text{m}$  and 200  $\mu\text{m}$ , respectively (Fig. S1 A). The distance between the permalloy and the magnetic pole is 10  $\mu\text{m}$ , and the distance between the magnetic pole and the separation channel is 3  $\mu\text{m}$ .

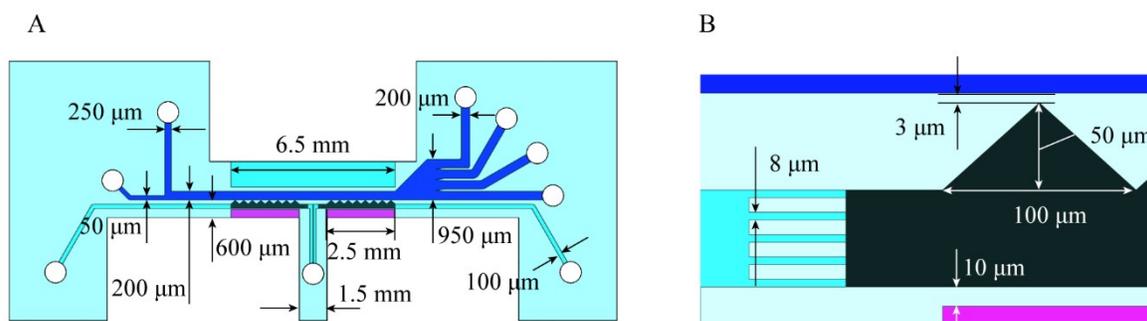


Figure S1. Schematic drawings of the separation region. (A) Design details on the microchannel size, the height of all channels is 40  $\mu\text{m}$ ; (B) Design of the magnetic pole.

The microfluidic system can be fabricated by 6 steps, as shown in Fig. S2: 1) A PDMS microfluidic layer is fabricated by soft lithography and then bonded to a glass substrate; 2) Rectangle shaped permalloy (1J85, the pink regions in Fig. 5) is embedded at the separation channel side; 3) NdFeB magnet (N52) is put on the permalloy; 4)  $\text{Fe}_3\text{O}_4$  powder mixed with pure water

(mass to volume  $m/v = 1:500$ ) is injected into the magnetic pole channel from inlet A and inlet B, then the  $\text{Fe}_3\text{O}_4$  powder is concentrated in the magnetic pole array area under the action of filter structure and the permalloy magnetized by the magnet; 5) Liquid PDMS is then injected into the magnetic pole channels slowly, two rectangle shaped permalloy are embedded at the magnetic pole channel side (the light blue regions in Fig. 5) to hold the  $\text{Fe}_3\text{O}_4$  powder, and then the chip is heated by a thermostat at  $80^\circ\text{C}$  for 2 hour to cure the magnetic pole array structure; 6) The magnet and the permalloy at the separation channel side are removed, and two NdFeB magnet are put on the permalloy at the magnetic pole channel side.

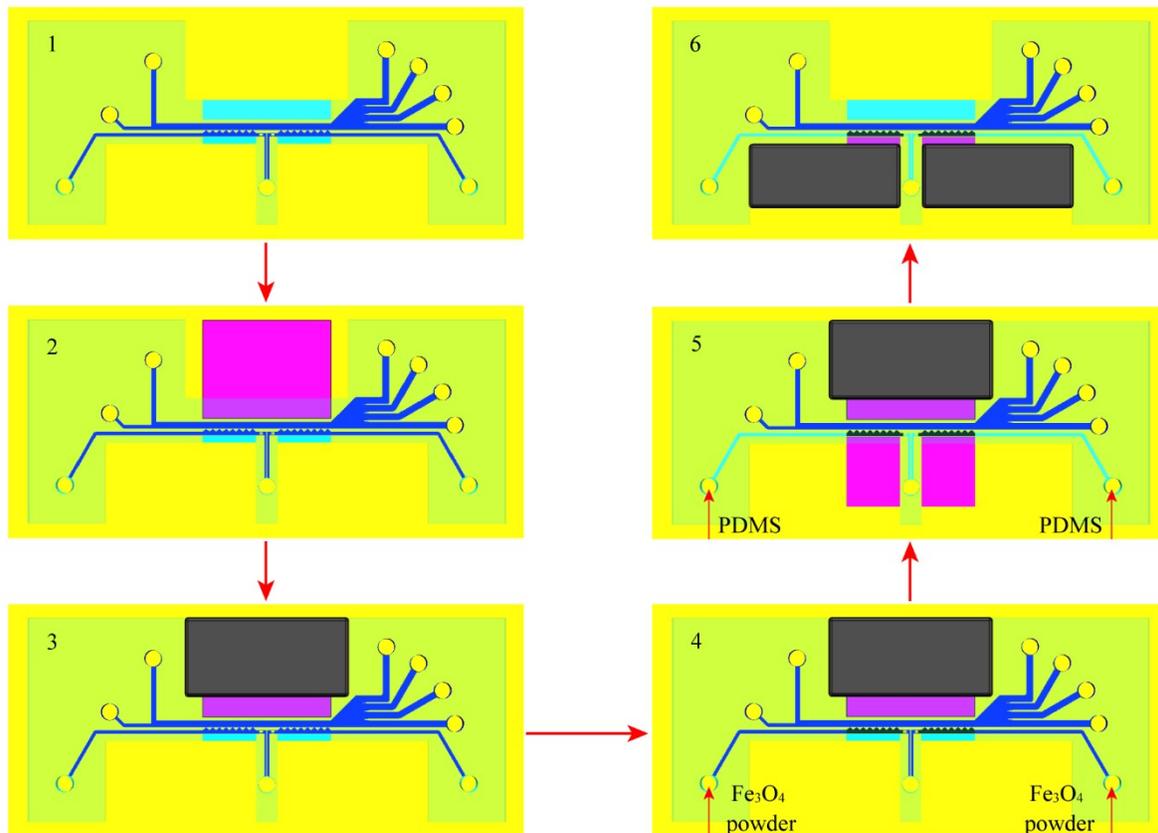


Figure S2. Fabrication process of the microfluidic system.

### Section S2. Details on the numerical simulation

The simulation model was built in COMSOL Multiphysics based on the chip design. In simulation, the “Magnetic fields, No Current” module was used to solve the magnetic field; the “Laminar Flow” module was used to solve the flow field; and the “Particle Tracing for Fluid Flow” module was used to solve the particle moving trajectories in ferrofluid. The used parameters are shown in Table S1.

Table S1 The related parameters of numerical simulation

Parameters	Value
Remanent flux density of the magnets	1.48 T
Relative permeability of ferrofluid after dilution	1.001
Relative permeability of Fe <sub>3</sub> O <sub>4</sub> powder	4
Relative permeability of permalloy	80000
Dynamic viscosity of the ferrofluid after dilution	0.001 Pa · s

### Section S3. Details on system calibration and measurements of flow cytometer

The flow cytometer (FCM) was calibrated firstly by using a set of reference beads, including 0.1  $\mu\text{m}$  fluorescent polystyrene particles (Thermo Fisher Scientific, USA) as well as polystyrene particles with diameters of 0.2  $\mu\text{m}$ , 0.5  $\mu\text{m}$  and 1  $\mu\text{m}$  (Thermo Fisher Scientific, USA), respectively. The beads were diluted in ddH<sub>2</sub>O to a final concentration of 10 nM. After the system calibration procedure, the standard particle samples were detected by FCM. The small standard particles (diameter of 0.2  $\mu\text{m}$ ) were diluted with ferrofluid (0.003X) to a concentration of  $\sim 70000$  particles/ $\mu\text{L}$ ; while the large standard particles sizing 1.0  $\mu\text{m}$  were diluted to a low concentration of  $\sim 7000$  particles/ $\mu\text{L}$ , which ensures detected events of 300 to 3,000 per second on FCM. Using FSC versus SSC as the main size parameter<sup>1</sup>, the standard particles of both sizes gathered in a cluster, respectively. Small particles were detected in the lower left corner, while large particles were gathered in the upper right corner (as shown in Fig. S3). Moreover, the distribution of particles in a diameter of 1  $\mu\text{m}$  represented more condensable than those small ones.

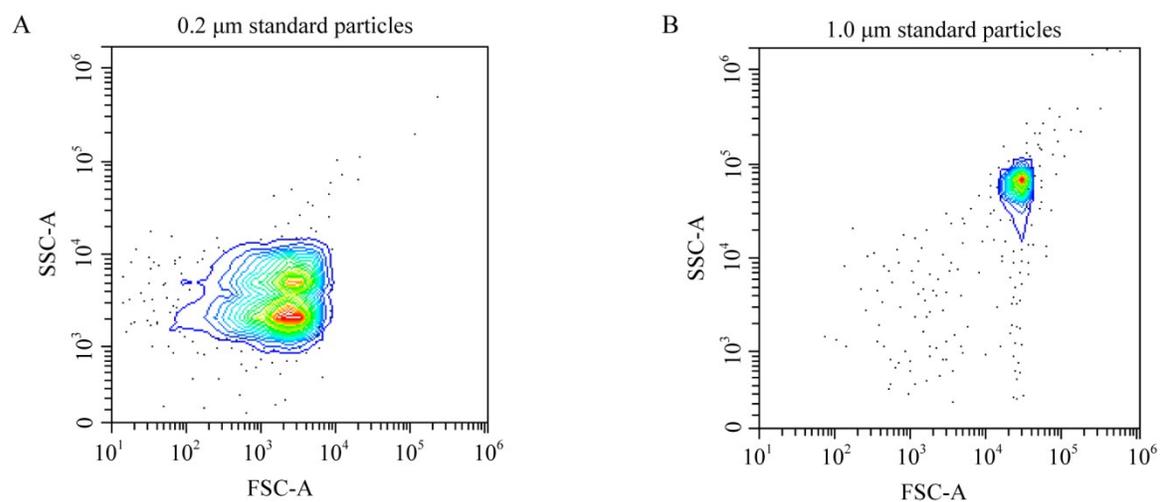


Figure S3. Flow cytometry test results of standard particle samples. (A) Test results of 0.2 μm standard particles. (B) Test results of 1.0 μm standard particles.

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## REFERENCES

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