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Supporting Information

for Adv. Funct. Mater., DOI: 10.1002/adfm.201503838

Label-Free and Continuous-Flow Ferrohydrodynamic Separation of HeLa Cells and Blood Cells in Biocompatible Ferrofluids

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Control experiments

 2×10^6 HeLa cells and 2×10^6 mouse blood cells suspended in 1 mL ferrofluid were pumped into microchannel respectively while magnetic fields were present using same conditions as separation experiments. The same concentration of HeLa cells, mouse blood cells and mixtures were also introduced into microchannel respectively at same conditions while magnetic fields were not applied. Unseparated HeLa cells, blood cells and mixtures either in 1 mL ferrofluid or 1 mL Hank's Balanced Salt Solution (HBSS, without calcium and magnesium; Sigma-Aldrich, St. Louis, MO, USA) were prepared at room temperature as cytospins and stained by Hematoxylin and Eosin (H&E) for controls (**Figure S2**).

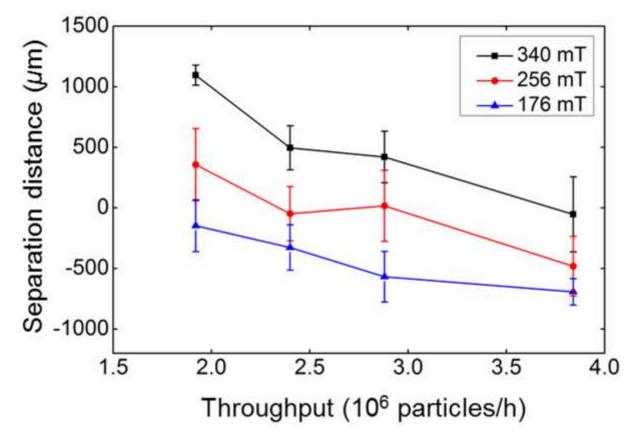


Figure S1. Separation distance as a function of throughput and magnetic fields when the magnet was placed 1, 2 and 3 mm away from the microchannel, respectively. Error bars are from 3 repeats of experiments. A flow rate of 8 μ L·min⁻¹ at Inlet A corresponded to a throughput of 1.92×10^6 particles·h⁻¹; 16 μ L·min⁻¹ corresponded to 3.84×10^6 particles·h⁻¹. Mean magnetic field strengths were 340, 256 and 176 mT, and mean magnetic field gradient were 97, 82 and 56 T·m⁻¹, when the magnet was placed 1, 2 and 3 mm away from the microchannel, respectively. Closer distance between the magnet and channel resulted in higher magnetic field strength and gradient, which in turn led to larger separation distance between the two microparticle streams at the end of channel. Meanwhile, the lower throughput also resulted in larger separation distance due to the longer experiencing time of microparticle separation in the channel. At a flow rate of 8 μ L·min⁻¹ and a magnetic field flux density of 140 mT, separation distance was approximately 1100 μ m. On the contrary, at a flow rate of 16 μ L·min⁻¹ and a magnetic field flux density of 176 mT, separation distance was approximately 600 μ m, indicating an overlap between two microparticle streams at the end of channel.

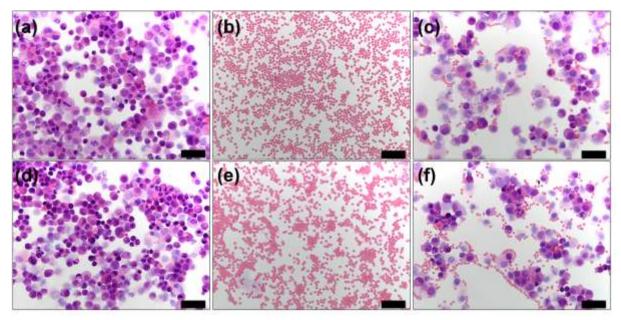


Figure S2. Hematoxylin and Eosin (H&E) stain of cytospins of unseparated HeLa, blood cells and mixtures in ferrofluid and Hank's Balanced Salt Solution (HBSS). (a)-(c) HeLa cells, blood cells and mixtures in ferrofluid, respectively. (d)-(f) HeLa cells, blood cells and mixtures in HBSS, respectively. Scale bars represent 50 μ m.

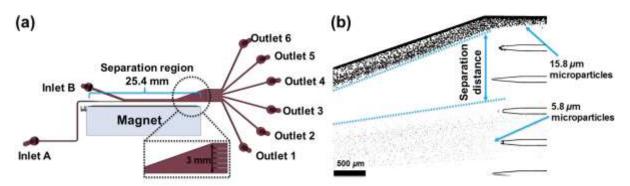


Figure S3. (a) Configuration of the microfluidic device. The vertical separation distance was measured at the 3 mm wide section of the channel. (b) Separation distance between 15.8 μ m and 5.8 μ m microparticle streams.