

Supplementary Information

S1. Synthesis of low magnetization beads. A 250 µl volume of neat M270 Dynabeads beads was placed in a centrifuge tube and washed twice with deionized water before resuspending them in 500 µl DI water. A 500 µl volume of concentrated hydrochloric acid was added to the beads and suspension was placed on a rotating wheel for 3 hours, after which the beads had changed from an orange/brown to yellow colour. A black color is characteristic of Fe₃O₄ nanoparticles while Fe₂O₃ nanoparticles have a yellow/red color. The beads were collected with a permanent magnet and washed three times with DI water to stop the reaction. They were then coated with 1 ml of 5% (w/w) polyethyleneimine solution in a sodium carbonate buffer for 2 hours, followed by three DI water washes. A 1 ml volume of 5% polyacrylic acid-maleic acid solution was then added to the beads at room temperature for 2 hours, before being washed three times with DI water and then suspended in PBST.

S2. Measurement of the magnetophoretic velocity of magnetic beads. Magnetic properties of the **L**, **M**, and **H** beads were characterized by measuring the velocity of the beads under constant magnetic field²⁹. This technique is based on the fact that at equilibrium, the magnetic force, F_m , applied to a bead is equal to the viscous drag force, F_h . The magnetic force acting on a SPM bead under an external field is

$$F_m = (\mathbf{m} \cdot \nabla) \mathbf{B} = \frac{V \cdot \chi}{\mu_o} (\mathbf{B} \cdot \nabla \mathbf{B}) ,$$

where, m is magnetic dipole of the bead, B is external magnetic field, V is the bead volume, χ is the bead susceptibility, and μ_o is the magnetic permeability of a vacuum. The hydrodynamic force acting on a bead is

$$F_h = -6\pi\eta a v f_D ,$$

where η is kinematic viscosity of the fluid, a is the bead radius, v is the bead speed, and f_D is drag factor of the bead. Thus, for a known size of bead and hydrodynamic factors, the magnetic forces acting on the bead and the magnetic moment of the bead can be calculated. The magnetic susceptibility of the bead is

$$\chi \propto \frac{v}{r^2}$$

for the same magnetic field and fluidic properties. Since the **L** and **M** beads have the same diameter and are subjected to the same magnetic field and field gradients, the magnetic susceptibility is proportional to drag velocity. The average size of **H** beads was slightly larger than that of **L** and **M** beads. To determine the susceptibility differences between the **H** and **M** beads the measured velocities of were corrected by a size factor.

The magnetic field was imposed by two pairs of NdFeB magnets 12.7×12.7×6.35 mm in size with a 1 mm air gap separating the two like poles²⁰. An aluminium magnet holder was attached to a micromanipulator (Eppendorf, Hamburg, Germany) to bring the magnets over the imaging field of an inverted microscope (Aviovert, Zeiss, Hertfordshire, UK) at desired distances from the beads. Magnetic beads and fluorescent microspheres (Polysciences, Warrington, PA) were mixed in a 55% (w/w) sucrose solution and a magnetic force was applied in the plane of the flow cell by bringing the magnet assembly at 3 mm distance from the imaging field. The strong optical activity of the magnetic beads made it easy to identify them with transmitted light using a 40× objective. The bead movement was recorded using an EMCCD camera (Hamamatsu, Hertfordshire, UK) at 3.3 fps and the drag velocity of beads were measured by tracking beads using the NIH ImageJ software. The relative average lateral velocity was calculated by using the position of fluorescent beads as reference.