Electronic Supplementary Information for:

Magnetic Separation of Acoustically Focused Cancer Cells from Blood for Magnetographic Templating and Analysis

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1. Numerical analysis of magnetic cell separation

Using a simple dipole model, we determined the magnetophoresis of magnetically labeled cells through the deflection portion of the microfluidic device towards the magnetic outlet (see Fig. 2A in the main text). We made several simple assumptions to inform our model (see Table S1 below for a list of parameters), which allowed us to estimate the magnetic force acting on each magnetically labeled cell. Using this information, we then calculated the counteracting force from Stokes' drag to elucidate the position of magnetically labeled cells in the device as a function of lateral position across the microchannel, flow rate and number of magnetic beads bound per cell, N_b (see Fig. 2C-D in the main text).

Table S1. Model Parameters. Model parameters used for the analysis shown in Figure 2C-D of the manuscript.

Variable	Value
$\boldsymbol{\mu_0}$	4π x 10^{-7} N/A^2
$\boldsymbol{V}$	5.315 x 10^{-19} m^3
$\boldsymbol{M_s}$	18,000 A/m
$\boldsymbol{\eta}$	1 x 10^{-3} (N·s)/m
$\boldsymbol{R_{eff}}$	9.015 x 10^{-6} m
$\frac{\partial H_x}{\partial x}$	2.67 x 10^8 A/m^2

2. Bead Sorting

To ensure the sorting module did not possess any intrinsic bias, we performed control experiments without acoustic or magnetic stimulation (Fig. S1). Similar to the experiments described in the main text, we prepared a 1:1 mixture of magnetic polystyrene particles (yellow fluorescent, 8.4 μm; Spherotech, Inc.) to non-magnetic polystyrene particles (red fluorescent, 10.2 μm; Spherotech) at a concentration of 60 particles/μL. We passed the sample through the device using "withdraw mode" on two syringe pumps (NE-300; New Era Pump Systems, Inc.), connected to either of the two outlets. We found there was statistically no difference between the concentration of particles in the inlets and either of the two outlets.

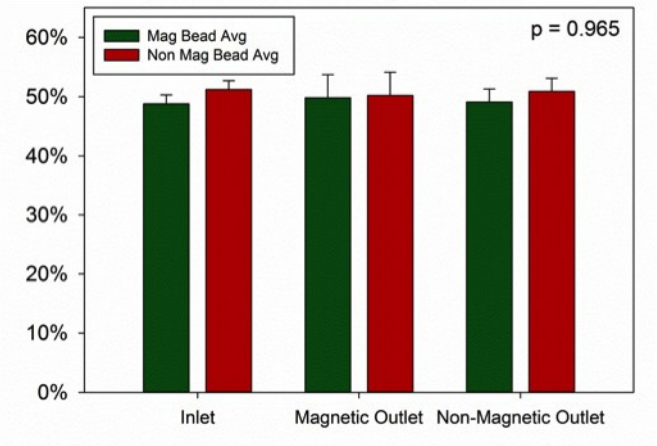

Figure S1. Control tests of the acoustically enhanced magnetic sorting microfluidic device. A mixture containing approximately a 1:1 ratio of magnetic to non-magnetic particles was passed through the device without forces from an acoustic standing wave or magnetic field. Analysis from a one-way ANOVA shows no significant difference in the ratio of magnetic to non-magnetic particles across the inlet or either outlet (alpha = 0.05, p = 0.965 for magnetic bead and non-magnetic bead population; n = 5).

For our sorting experiments with particles, we analyzed the outputs of our device with a flow cytometer (Accuri C6; BD Biosciences, Co.). For each condition, we generated scatter plots of the particles collected from each of the two outlets, and we gated the populations to generate the bar graphs shown in Figure 3D of the main text. We colorized the data points green and red to correspond to magnetic and non-magnetic particles, respectively (Fig. S2).

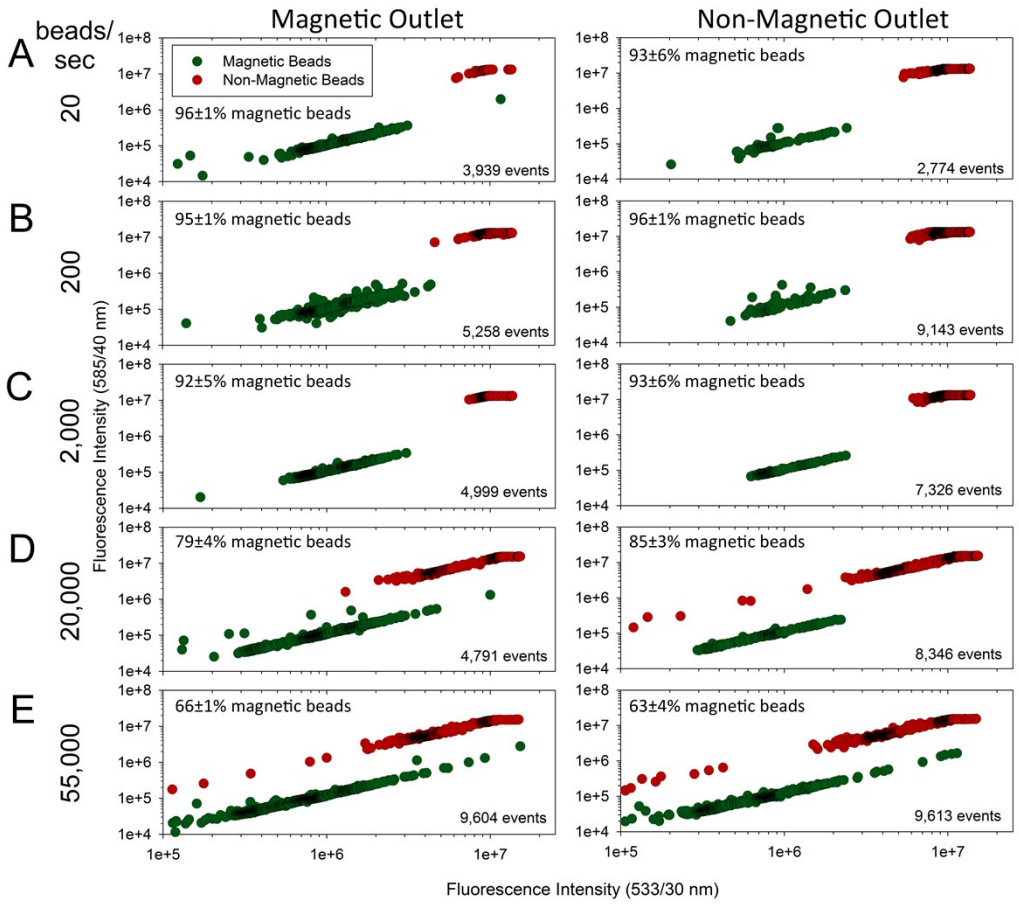

Figure S2. Performance of the acoustically enhanced magnetic sorter. The left and right columns represent the outputs from the magnetic and non-magnetic outlets, respectively. A mixture containing a 1:1 ratio of magnetic particles to non-magnetic particles at increasing concentrations was passed through the device at A) 20, B) 200, C) 2,000, D) 20,000 and E) 55,000 particles/sec. Each condition (A-E) indicates the fluorescence intensity of the 533/30 nm laser versus the 585/40 nm laser from a flow cytometer (n = 5).

3. Modeling of local field strength of micromagnets in magnetographic array

We used the electromagnetics (AC/DC) module of COMSOL to estimate the force generated on a single magnetic bead (MyONE^{TM} Dynabeads®, SA T1) as well as a collection of magnetic beads from a 30 x 10 μm micromagnet comprised of 10 nm Cr, 200 nm Co, 10 nm Cr, and 50 nm Au. For the sake of simplicity, we modeled the Co film without the other metallic layers, and we assigned it a relative permeability (μ_r) of 250 compared to air. We estimated the magnetic saturation (M_s) using the relation (1):

$$M_S = 1.2\mu_B N = 1{,}011.65\ kA/m$$

where μ_b and N represent the Bohr magnetron (i.e., 9.27x10^{-24} A/m^2) and the number of atoms (which was calculated via the relation $N = \rho * N_A / MW$), respectively.

Next, we modeled the magnetic properties of the magnetic beads. Using the specifications provided by the manufacturer, we estimated the beads were magnetized (M) to 15 emu/g (or 30,600 A/m) by assuming a bead density of 2,040 kg/m^3. Next, we calculated the magnetic susceptibility, χ_v, of the magnetic beads by following the relation (1):

$$\chi_V = \frac{M}{H}$$

whereby H represents the field, which we assumed was 800 G (H = 0.08 T / μ_0 = 63,662 A/m). Finally, we calculated that the magnetic permeability of the magnetic beads in our system was 1.481 by the relation $\mu_r = 1 + \chi_v$.

Using these parameters in COMSOL, we calculated the field strength around a single micromagnet as well as a magnetic bead at various distances from the attractive end of the magnet. We also calculated the magnetic force acting on a magnetic bead at various distances from the attractive end of a magnet, and assuming the contact area of one bead on a cell is roughly proportional to its cross sectional area, we estimated the pressure exerted on a cell by a

single bead ($P = F/A$). Finally, we estimated the force from multiple (i.e., 15 and 30) beads acting on a single cell by the simplifying equivalent volume approximation. In this case, we modeled 15 and 30 beads as a single bead with a radius of 1.23 and 1.55 μm, respectively.

4. References

1. Bozorth R. Ferromagnetism. Piscataway, NJ: IEEE Press; 1993. p. 968.