Supplementary Information for

Digital Microfluidic Magnetic Separation for Particle-Based Immunoassays

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Device fabrication and operation

Clean room reagents and supplies included MF-321 photoresist developer from Rohm and Haas (Marlborough, MA), CR-4 chromium etchant from Cyantek (Fremont, CA), AZ-300T photoresist stripper from AZ Electronic Materials (Somerville, NJ), Teflon-AF from DuPont (Wilmington, DE), acetic acid from Caledon (Georgetown, Ontario), Parylene C dimer and Silane A174 from Specialty Coating Systems (Indianapolis, IN).

Digital microfluidic devices, each comprising a bottom-plate and top-plate (**Figure 1B** in the main text), were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) cleanroom facility, using transparent photomasks printed at 20,000 DPI (Pacific Arts and Designs Inc., Markham, ON). The bottom-plates of DMF devices bearing an array of electrodes were formed by standard photolithography and wet etching. Briefly, chromium- (200 nm thick) and photoresist-coated glass substrates (2" × 3" ×1.1 mm) (Telic Co., Santa Clarita, CA) were exposed to UV light through a photomask using a Suss MicroTec mask aligner (29.8 mW/cm^2, 10 seconds). The exposed substrates were developed in MF-321 (3 min) and post-baked on a hot plate (125^{o}C, 1 min). The developed substrates were etched in CR-4 (3 min) and the remaining photoresist was stripped in AZ300T (5 min). After forming electrodes, the substrates were primed for parylene coating by immersing in silane solution (2-propanol, DI water, A-174, and acetic acid 50:50:1:2 v/v/v/v, 10 min) and curing on a hot-plate (80^{o}C, 10 min). After rinsing and drying, devices were coated with ~7 μm of Parylene C (vapor deposition) and ~200 nm of Teflon-AF (spin-coating, 1% w/w in Fluorinert FC-40, 2000 rpm, 60 s), and post-baked on a hot-plate (165°C, 10 min). The polymer coatings were removed from contact pads by gentle scraping with a scalpel. The top-plates of DMF devices were formed by coating Teflon-AF (~200 nm, as above) on unpatterned indium-tin oxide (ITO) coated glass substrates

(Delta Technologies Ltd, Stillwater, MN). Devices were assembled with an unpatterned ITO glass top-plate and a patterned bottom-plate separated by a spacer formed from two pieces of Scotch double-sided tape (3M, St. Paul, MN) with total spacer thickness of 180 µm.

The device design featured an array of 116 actuation electrodes (2.25 × 2.25 mm ea.) connected to 10 reservoir electrodes (4.5 × 4.5 mm ea.) (**Figure 1C** in the main text). The actuation electrodes were roughly square with interdigitated borders (140 μm peak to peak sinusoids) and inter-electrode gaps of 30-80 µm. The pitch of the actuation electrodes (2.25 mm) enables the alignment of electrodes to standard micro-plate readers (96, 384, or 1536 well-plate format). Unit droplet and reservoir droplet volumes on these devices were ~800 nL and ~3.5 µL, respectively.

To drive droplet movement, AC potentials (~150 V_{RMS}, 10 KHz) were generated by a function generator (Agilent Technologies, Santa Clara, CA) and a high-voltage amplifier (TREK, Inc., Medina, NY). A sine wave output was applied between the top-plate (ground) and sequential electrodes on the bottom-plate via the exposed contact pads. Reagents were delivered to their respective reservoirs by simultaneously applying driving potential to a reservoir electrode and pipetting the reagent adjacent to the gap between the bottom and top plates (**Figure 1C** in the main text). Waste and unused reservoir fluids were removed with KimWipes (Kimberly-Clark, Irving, TX). Unit droplets were dispensed from reservoirs by actuating a series of adjacent electrodes as described previously.[1] To perform a mixing operation, a unit droplet was shuttled in a circular motion across four electrodes; more electrodes were used for larger droplets. During sample or reagent incubation, droplets were continuously mixed to minimize non-specific adsorption on device surface. Droplet actuation was monitored and recorded by a CCD camera mounted with a lens.

Reagent formulations used on-chip

Immunoassay reagents used on-chip included analyte standards, antibody coated paramagnetic microparticles, enzyme conjugated reporters, chemiluminescent substrate, and human serum matrix. The analyte standards and particles were adapted from ARCHITECT immunoanalyzer reagent kits obtained from Abbott Laboratories (Abbott Park, IL). TSH analyte standards in TRIS buffer with protein stabilizer and mouse monoclonal Anti-β TSH coated particles in ARCHITECT particle diluent (ARCHITECT TSH reagent kit 7K62) were used for TSH immunoassays. E2 analyte standards in TRIS buffer with protein stabilizers and rabbit monoclonal Anti-E2 coated particles in ARCHITECT particle diluent (ARCHITECT E2 reagent kit 7K72) were used for E2 assays. Horse-radish peroxidase (HRP) conjugated goat polyclonal Anti-TSH, was purchased from Fitzgerald Industries (Acton, MA). E2-HRP (conjugated via 6-CMO), was purchased from BiosPacific (Emeryville, CA). SuperSignal ELISA Femto chemiluminescent substrate, comprising stable peroxide (H_2O_2) and luminol/enhancer solutions, was purchased from Thermo Fisher Scientific (Rockford, IL).

Most reagents and solutions were from kits (as listed above). Prior to use, these reagents were supplemented with Pluronic L64[2] (0.05% v/v). Three custom reagents (not from kits) were also used, including wash buffer, particle diluent, and conjugate diluent. DMF-compatible wash buffer (pH 7.7) was formed from Tris-base (0.35 g/L), Tris-HCl (1.10 g/L), NaCl (8.367 g/L), and L64 (0.05% v/v). DMF-compatible particle diluent (pH 10) was formed from Tris-base (6.1 g/L), NaCl (5.8 g/L), Sucrose (136 g/L), BSA (1% w/v), Thimerosal (0.05% w/v), and L64 (0.05% v/v). DMF-compatible conjugate diluent (pH 7.4) was formed from Tris-Base (1.9 g/L), Tris-HCl (13.2 g/L), NaCl (17.5 g/L), BSA (1% w/v), cold fish gelatin (0.1% w/v), Thimerosal

(0.05% w/v), and L64 (0.05% v/v). TSH and E2 conjugate solutions were formed by dissolving Anti-TSH-HRP (2 µg/mL) and E2-HRP (1 µg/mL), respectively, in conjugate diluent.

Microparticle preparation

Prior to introducing onto digital microfluidic devices, magnetic particles were processed off-chip in three steps: (1) washing (for TSH and E2 particles), (2) blocking (for TSH particles only), and (3) suspending in DMF-compatible buffer (for TSH and E2 particles). In step (1), particles in ARCHITECT particle diluent were immobilized in an Eppendorf tube using a permanent magnet, the diluent was removed, and the particles were washed twice with DI water. For step (2), particles were suspended in blocking buffer (pH 7.8), formed from Tris-base (0.30 g/L), Tris-HCl (1.2 g/L), NaCl (8.76 g/L), 0.01% (w/v) Tween-20, and 3% (w/v) Sigma-brand non-fat dry milk (NFDM), incubated for 1 hour, and then washed twice in DI water. For step (3), particles were suspended in DMF-compatible particle diluent (recipe above) at ~3.0×10^8 particles/mL.

Estimation of magnetic force

The force on a magnetic particle inside a magnetic field is determined by the volume of the particle (V), the magnetic susceptibility of the particle (χ), and the magnetic flux density (B).[3] Assuming that the particle is in a non-magnetic medium with negligible susceptibility (e.g., air), the force on the particle is given by:

$$F = \frac{V \cdot \chi}{\mu_0}(B \cdot \nabla)B \qquad (1)$$

where μ_0 is the permeability of free space.

During magnetic separation in the work reported here, a neodymium (NdFeB) magnet disc (5/8" diameter × 1/4" thick, relative permeability $\mu_r = 1.05$, remnant field strength $B_r = 1.38$ T) is positioned 1.40 mm below an 800 nL droplet of particle suspension (1.40 mm is the thickness of the microfluidic device). When magnetic force is applied, the particles in the droplet focus into a pellet on the surface of the device, the volume of which is estimated to be the volume of one particle multiplied by the number of particles in the droplet. This configuration can be represented by a geometric model comprising a disc (magnet) and sphere (pellet), where the bottom-edge of the magnet is the x-y-z origin and the pellet is 7.75 mm above the origin (**Figure S1A**).The magnetic flux density and its gradient were numerically calculated using finite element analysis software, COMSOL (Burlington, MA; Model: 3D, Magnetic Fields, No Currents) (**Figure S1B**).

Assuming that the microparticles have magnetic susceptibility $\chi = 1.05$,[4] the magnetic force on the pellet (z-component, directed towards the magnet) was calculated as a function of pellet position (horizontal and vertical) and particle density.[5] In the first calculation, the horizontal position of the pellet was varied from the center of the disc to a distance 15 mm away (**Figure S1C**). Here, the vertical distance of the particles from the magnet was fixed at 1.40 mm, and the particle density was fixed at 3.0×10^8 particles/mL (the minimum density observed experimentally for successful separation of a pellet from a moving droplet). As expected, the maximum force is experienced for particles positioned at the edge of the magnetic disc, ~7.9 mm from the center. This force, 470 μN, represents the minimum force required to overcome the interfacial force of the droplet. In the second calculation, the vertical distance of the pellet was varied from the magnet surface to a distance 3 mm away (**Figure S1D**). As expected, the force decreases as the pellet was moved farther away from the magnet. In the final calculation, the

particle density of the solution was varied (**Figure S1E**). Since volume is proportional to force, as indicated by Eq. (1), the force on the pellet increases linearly with higher particle density.

Measuring droplet volume change

Droplet volume change was evaluated on-chip using an impedance-based measurement circuit attached to the device top-plate (i.e., the ITO-coated slide) as described previously.[6] Before assembling the digital microfluidic device, a 1 µL droplet of wash buffer was pipetted on the assay region of the device. Immediately afterward, the device was assembled by as described above, sandwiching the droplet between two plates. The capacitance of the droplet was measured every minute for 30 minutes and normalized to the initial capacitance (at 0 minutes). Three normalized capacitance measurements were averaged for each point in time and plotted as a function of time (**Figure S2**).

References

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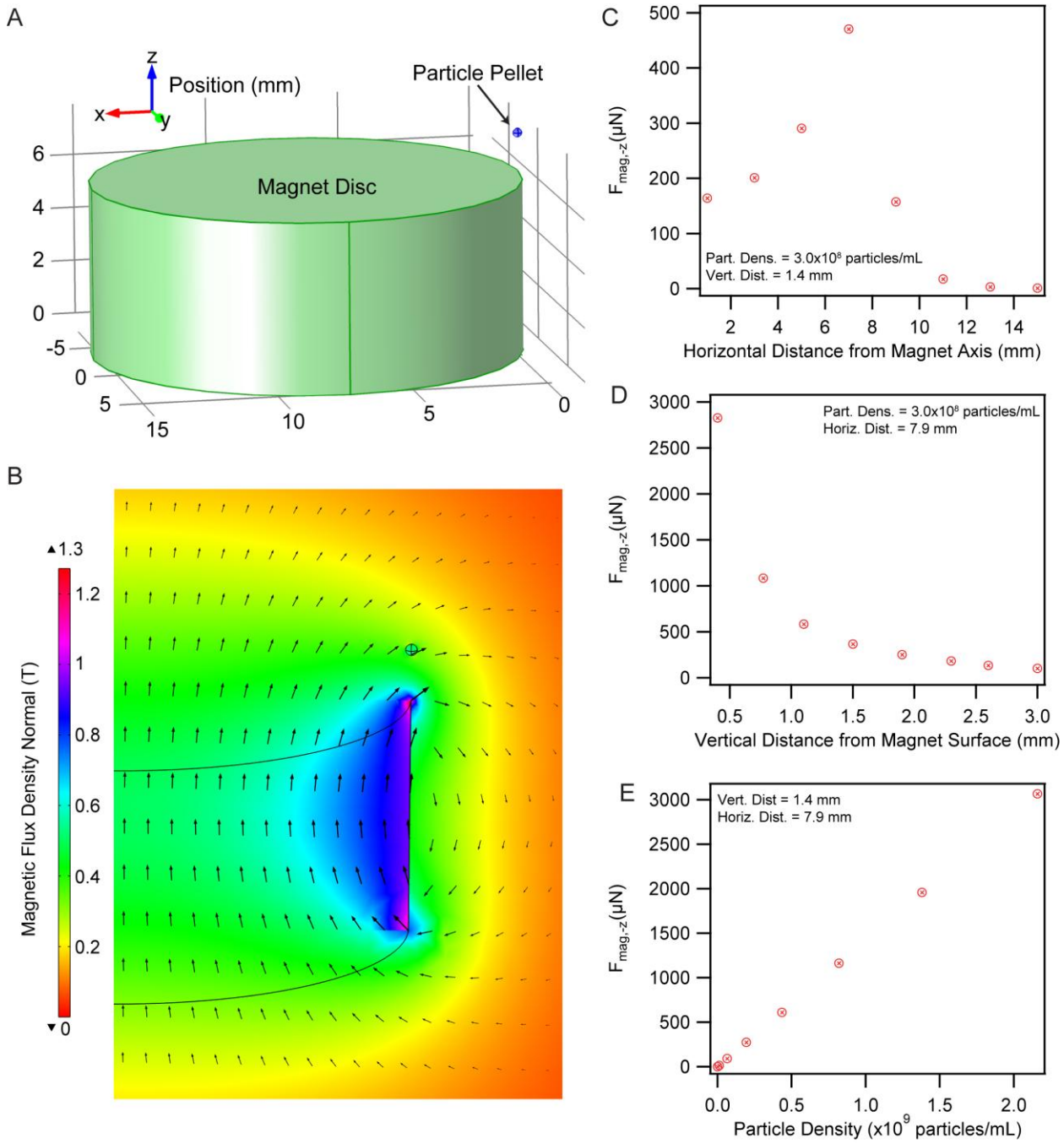

Figure S1: Numerical simulation of magnetic flux density of magnet disc and z-component force on pellet of paramagnetic particles. (**A**) Geometric model of magnet and pellet. (**B**) Intensity of magnetic flux density normal (heat map) and direction of field lines (black arrows). (**C**) Magnitude of z-component force as a function of horizontal distance from magnet center. (**D**) Magnitude of z-component force as a function of vertical distance from magnet surface. (**E**) Magnitude of z-component force as a function of particle density.

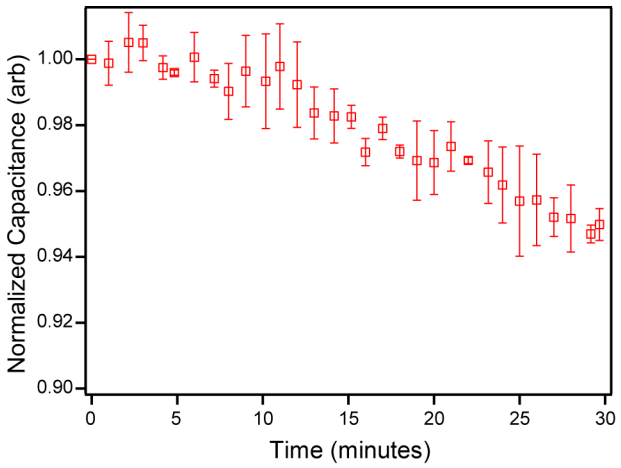

Figure S2: Normalized droplet capacitance over time (which correlates with droplet volume). Error bars are ±1 S.D from three replicates.