Electronic Supplementary Material (ESI) for Analyst.
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Supplementary information

A portable immunomagnetic cell capture system to accelerate culture diagnosis of bacterial infections

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1. Magnetic capture simulation

Magnetic nanoparticles are enriched in two stages in iMC[2] setup, first a vertical capture of MNPs to bottom of the chip and second, by sweeping of settled particles to the recovery chamber. Magentic force experience by a magnetic nano particle in external magnetic field[1, 2] can be written as equation (1) below, where μ_0, V and $\Delta\chi_{bead}$ are the magnetic permeability constant, volume and effective magnetic susceptibility of the MNPs respectively. Other than force due to the magnetic field, particles experience a viscous drag and gravitational force. Gravitational force on the nanoparticles are very small in comparison to magnetic and drag forces and has been omitted in this study.

$$\overrightarrow{F_m} = \frac{\Delta\chi_{bead}V}{\mu_0}(\vec{B}.\nabla)\vec{B} = \frac{\Delta\chi_{bead}V}{\mu_0}\begin{pmatrix} B_x\frac{\partial B_x}{\partial x} & B_y\frac{\partial B_x}{\partial x} & B_z\frac{\partial B_x}{\partial x} \\ B_x\frac{\partial B_y}{\partial x} & B_y\frac{\partial B_y}{\partial y} & B_z\frac{\partial B_y}{\partial z} \\ B_x\frac{\partial B_z}{\partial x} & B_y\frac{\partial B_z}{\partial y} & B_z\frac{\partial B_z}{\partial z} \end{pmatrix} \quad \text{Eq 1}$$

Vertical capture of magnetic particles and cells happen for 5 minutes in iMC[2] system after the capture chip is placed on top of magnetic checkerboard. The distance between the bottom layer of capture chip and magnets surface is ~0.5 mm. Magnetic flux's (B_x, B_y, B_z) along x,y,z axes as function of x,y is shown in Figure S1.A and Figure S2. All three magnetic flux's decrease exponentially along the z axis. For simplicity, we have evaluated only the the $\frac{\partial B_z}{\partial z}$ component for calculation force on nanoparticle (F_{mz}) along z axis. A single nanoparticle experience an attractive force (toward the magnets surface) that decrease exponentially as function of height of the particle in z axis (circular points, Figure S1.B). Time of settlment can be calculated using an equation of motion written as equation (2). Figre S1.B square points shows the time taken for settlement of single 100 nm magnetic nanoparticle from different z heights. For 5 ml sample, the height of liquid will be ~2.5 and take 45 seconds to settle to the bottom of the chip. This is

very simple scheme in comparision to the actual situation, where cells bound to 10-12 magnetic nanoparticles get captured in much denser medium. Thus the time for vertical capture has been set to 300 seconds to maximize the vertical capture efficiency. Parameters used in the calculation are: $\Delta\chi_{bead}$ = 5 (SI units), μ_0=4π x 10^{-7} *T.m.A*$^{-1}$ and η = *0.001 N.s.m*$^{-2}$.

$$F_{mz} = F_d = 6\pi\eta r \frac{dz}{dt} = \frac{\Delta\chi_{bead}V}{\mu_0} B_z \frac{\partial B_z}{\partial z} \qquad \text{Eq- 2}$$

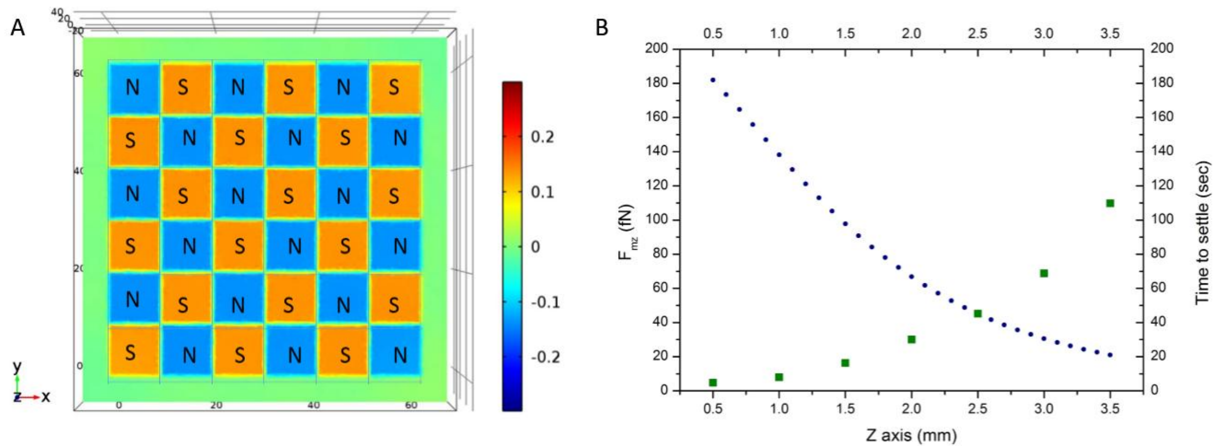

Figure S1: A. Magnetic flux density B_z at z=0.5 mm as function of x and y. Gradient is between ±0.3 Tesla. B. Force experienced by a single 100 nm particle as function of z height (circular points). Time for settlement of a single nanoparticle from different z height (square points).

Lateral sweeping

The magnetic flux B_x and B_y as function of x, y axis is show in figure S2. All the values are calculated at z = 0.5 mm, as the lateral sweeping happens only after particles settled at the bottom of the chip. Though at the beginning the particles can settle down at any point, as the linear positioner moves the particle get trapped at the maximum gradient points (i.e., midpoint between two opposite polarity magnets). At z= 0.5 mm, a single 100 nm MNP experience a maximum force of ~0.2 pN at the peak gradient points. Also the particles accumulate along the gradient points as the linear positioner moves the magnetic checkerboard, the accumulated particles clump together to act as larger magnetic particle and experience much larger force. Though there is frictional force between the MNP and bottom layer of the capture, the magnetic force is greater and the particles remain trapped at the gradient points and get swept to the recovery chamber.

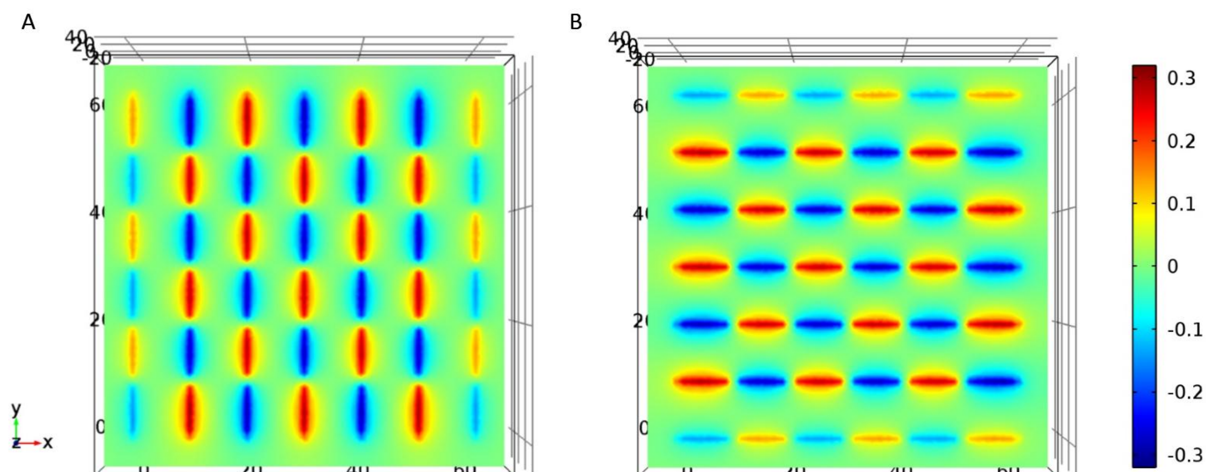

Figure S2: Magnetic flux density B_x (A) and B_y (B) as function of x,y. Flux densities are calculated at z =0.5 and gradient is between ±0.3 Tesla.

2. Comparison between various high through put magnetic cell capture methods

Literature is abundant with many immuno-magnetic cell sorting methods, particularly developed towards capture of low abundance cells. We have compared some of the large volume capture methods recently published in Table S1 below. The capture efficiency, flow rate of sample or time taken for 5 ml processing and size of the magnetic particles are used to compare these methods with iMC^2. We could not compare the enrichment factors directly as these methods have used in chip detection using fluorescent microscopy and final enriched sample volume is not available.

Flow rate (µl/min)	Capture Efficiency (%)	Particle Size (nm)	Time for 5 ml sample processing	Schematic arrangement and Reference
166	>90%	50	30 min	3
166	86%	100	30 min	

				4
83	90%	50	60 min	5
NA	72%	100	15 min	iMC^2 system

Table S1.

3. Performance of media enrichment step and immuno-magnetic enrichment steps

Number of bacteria available for detection is increased by two steps: by incubating in TSB media for 6-8 hours and by immuno-magnetic separation. Figure 4 and Figure 5 show enrichment data by media and immuno capture for spiked *S. typhi* cells in buffer and blood samples. Here we discuss the statistical variation in performance of enrichment between samples to sample. Media enrichment performance can be quantified by calculating number of doubling cycles achieved in given duration of incubation using formula: $X_t = X_0 2^n$, where n is number of doubling cycle and X_0 and X_t are cell concentrations before and after incubation. Number of doubling cycles achieved for 8 hour incubation is on average ~14 (Figure S3A) and for 6 hour incubation on average ~11 (Figure S3C). Immuno-magnetic enrichment performance is directly correlated by calculating % of cells collected. The capture efficiency also showed a clustered peak centered at 70% for both buffer and blood spiked data (Figure S3B & D).

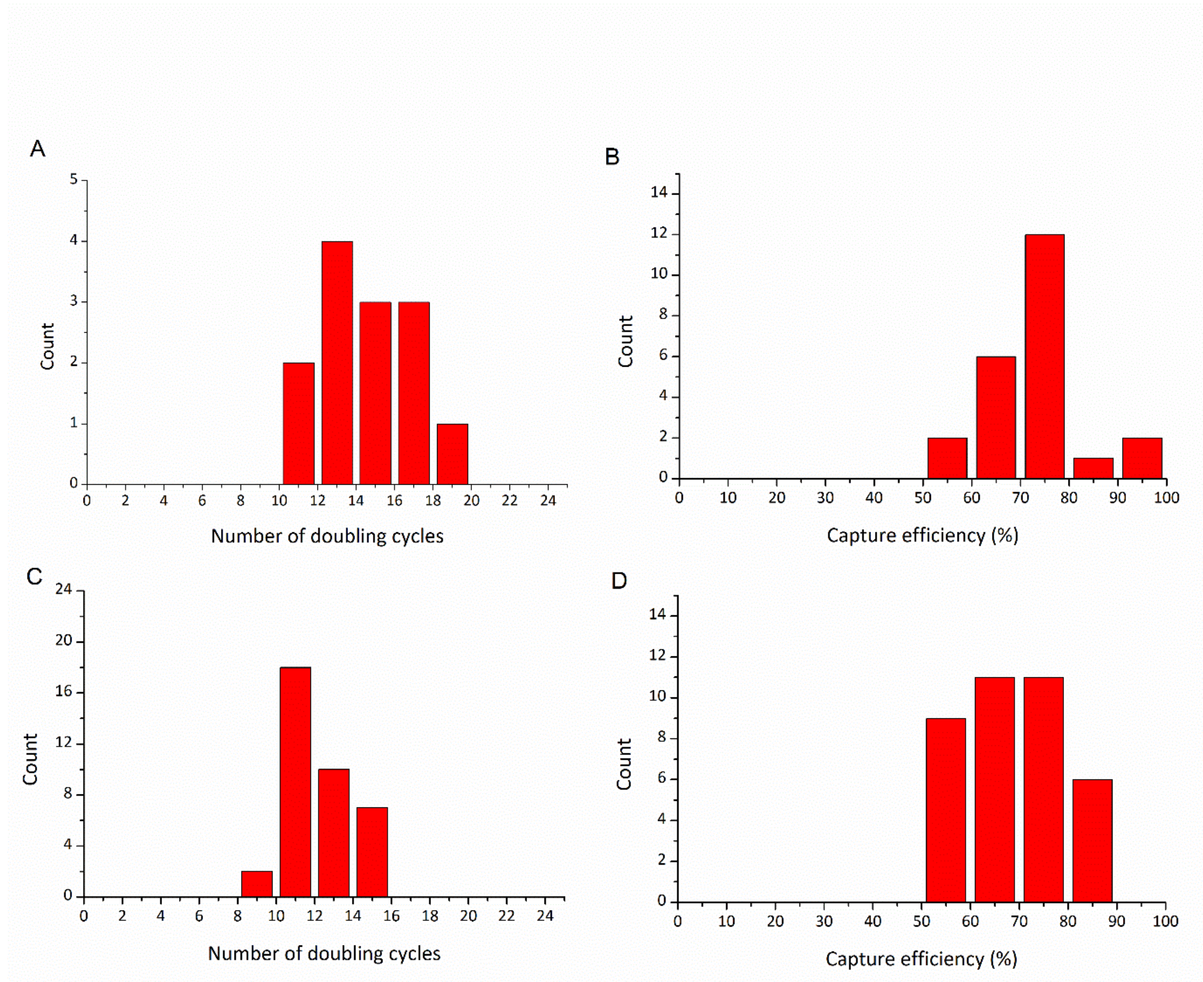

Figure S3: A. Variation in number of doubling cycles achieved during 8 hour incubation for buffer spiked samples is shown as histogram. Bin size is 2 cycles. B. Variation in capture efficiency for buffer spiked samples is shown as histogram. Bin size is 10%. C. Variation in number of doubling cycles achieved during 6 hour incubation for blood spiked samples is shown as histogram. Bin size is 2 cycles. B. Variation in capture efficiency for various blood spiked samples is shown as histogram. Bin size is 10%.

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