Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Velocity Valleys Enable Efficient Capture and Spatial Sorting of Nanoparticle-Bound Cancer Cells

Justin D. Besant,[1] Reza M. Mohamadi,[2] Peter M. Aldridge,[1] Yi Li,[2] Edward H. Sargent,[3]

Shana O. Kelley[1,2,4]

[1]Institute for Biomaterials and Biomedical Engineering, University of Toronto, Toronto, M5S 3M2, Canada, [2]Department of Pharmaceutical Science, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, M5S 3M2, Canada, [3]Department of Electrical and Computer Engineering, Faculty of Engineering, University of Toronto, Toronto, M5S 3G4, Canada, [4]Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, M5S 1A8, Canada.

1. Simulations:

Magnetic field simulations. For ease of calculation, a simplified 2D magnetic geometry was used for the simulations. The magnetic field was calculated using COMSOL Multiphysics with four alternating NdFeB magnets (1/4"x1/16") with magnetization of 0.75 T (K&J Magnetics).

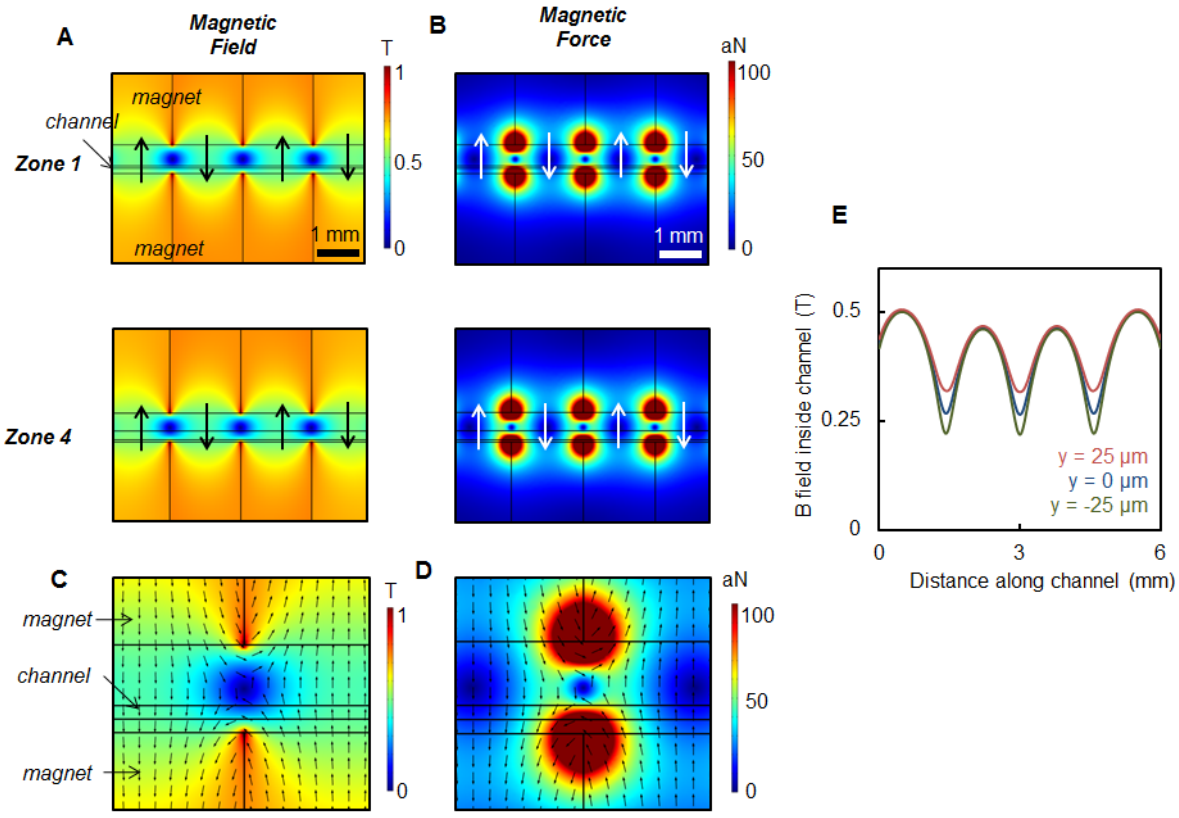

Fig. S1: Simulations of the magnetic fields (A) and magnetic forces (B) on a single nanoparticle in Zones 1 and 4. Close up simulations of the magnetic fields (C) and magnetic forces (D) with overlaid normalized field vectors. (E) The magnetic field inside the channel as a function of height above the channel center (y=0) and distance along the channel.

Fluid flow simulations. Using COMSOL Multiphysics, we simulated the distribution of linear velocities inside the chip for the five different trapping structure designs using an average linear speed of 600 µm/s which corresponds to a 1 mL/hour flow rate for a 50 µm channel height.

Parameter	Description	Value
$\boldsymbol{\eta}$	Dynamic viscosity of the medium	0.001 $Pa\times s$
$\boldsymbol{V_m\Delta\chi_{bead}}$	Magnetic nanobead parameter (V_m is the nanobead volume and $\Delta\chi_{bead}$ is the difference of the magnetic susceptibility of the beads and the surrounding medium)	2.5 x 10^{-16} mm^3
$\boldsymbol{\mu_0}$	Permeability of free space	$4\pi\times10^{-7}$ H/m
$\boldsymbol{r}$	Cell radius	10 μm

Table S1 Simulation parameters

2. Model of capture efficiency

In each chip there are 17 rows of capture structures (Figure 1C). On a path from the inlet to the outlet, each cell will pass by one structure, per row, for each of the 17 rows. Thus, there are 17 opportunities for a cell to be captured. The probability of escaping all 17 rows is $\left(1-\mathrm{P_{capture}}\right)^{17}$. Therefore the capture efficiency, E, can be calculated as:

$$\mathrm{E} = 100\%\left(1-\left(1-\mathrm{P_{capture}}\right)^{\mathrm{N}}\right) \quad \text{(S1)}$$

where N, the number of structures in each cell's path, is 17, $\mathrm{P_{capture}} = \alpha\frac{\mathrm{A_{v<v_t}}}{\dot{\mathrm{Q}}}$, $\dot{\mathrm{Q}}$ is the flow rate (mL/hr), $\mathrm{A_{v<v_t}}$ is the average percentage of area surrounding a capture structure in which the linear velocity is less than the threshold, and α is an experimentally determined proportionality constant with units set to ensure $\mathrm{P_{capture}}$ is unitless (units are hr/mL).

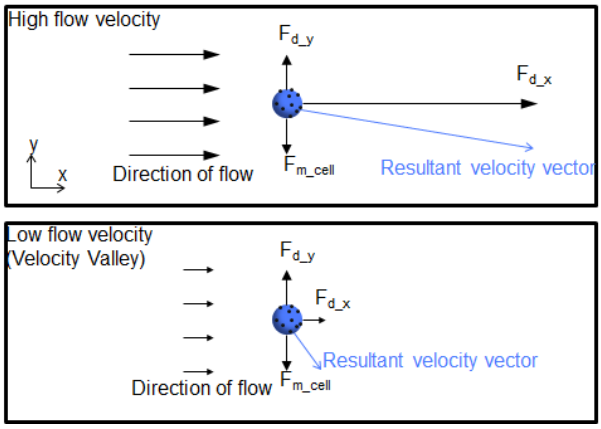

Figure S2 Forces acting on a cell in the channel under various flow conditions.

Calculation of $\mathbf{v_t}$. First we measured the average number of nanoparticles per cell using the method described below. Using the magnetic simulations, we calculated the maximum magnetic force acting on a cell in the channel. The threshold velocity, $\mathbf{v_t}$, is defined as the required linear velocity such that the drag force acting on the cell is equal to the maximum magnetic force.

Calculation of $\mathbf{A_{v<v_t}}$. Using the fluid flow simulations, $A_{v<v_t}$, the average percentage of area surrounding a capture structure in which the linear velocity is less than the threshold, was calculated for each structure design. We simulated the spatial distributions of linear velocity and used COMSOL to calculate the percentage area of the chip in which the linear velocity was less than the threshold linear velocity for capture.

Fitting the model to the experimental data (α). The data was fit to the SKBR3 capture efficiency data, and we found the model best fit the data using a proportionality constant of 0.1. For VCaP we found the model best fit the data using a proportionality constant of 0.4.

Chip geometry	$A_{v<v_t}$ Fraction of area with linear velocity less than the threshold velocity for capture	N Number of structures	α Experimentally determined proportionality constant	E_{model} Predicted capture efficiency [%]	$E_{experimental}$ Experimentally measured capture efficiency [%]
'x'	0.17	17	0.4	91.5	100
'+'	0.078	17	0.4	66.8	59
'o'	0.018	17	0.4	21.8	22

Table S2 Simulation parameters for different chip geometries using VCaP cells.

Flow rate [mL/hr]	$A_{v<v_t}$ Fraction of area with linear velocity less than the threshold velocity for capture	N Number of structures	α Experimentally determined proportionality constant	E_{model} Predicted capture efficiency [%]	$E_{experimental}$ Experimentally measured capture efficiency [%]
0.25	0.19	17	0.1	74.7	81
1	0.13	17	0.1	20.3	25
4	0.063	17	0.1	2.76	6

Table S3 Simulation parameters used to validate the model as a function of flow rate using SKBR3 cells.

Cell line	Q Flow rate [mL/hr]	$A_{v<v_t}$ Fraction of area with linear velocity less than the threshold velocity for capture	N Number of structures	α Experimentally determined proportionality constant	E_{model} Predicted capture efficiency [%]	$E_{experimental}$ Experimentally measured capture efficiency [%]
VCaP	0.25	0.19	17	0.4	99.8	90
SKBR3	0.125	0.22	17	0.1	96.1	93
MDA-MB-231	0.0625	0.15	17	0.1	99.1	100

Table S4 Simulation parameters used to validate capture efficiency as a function of cell line.

3. Measurement of nanoparticles per cell

Overview of method. To estimate the number of magnetic nanobeads bound to a cell, we used a previously described method.[1] Cells labelled with magnetic nanobeads were flowed in a straight channel adjacent to a magnet (Fig. S3). In the presence of the magnetic force, labelled cells flow on a diagonal towards the magnet.

The magnetic force was calculated according to Eq. 2. Neglecting wall effects, the transverse drag force acting on a cell (modelled as a spherical particle) at low Reynolds numbers is given by Stokes' law:[2]

$$\vec{\mathbf{F}}_{\mathrm{D_y}} = 6\pi\eta r\overrightarrow{\mathrm{v_y}} \tag{S2}$$

where $\eta\ [\mathrm{Pa.s}]$ is the fluid viscosity, $\overrightarrow{\mathrm{v_y}}\ [\mathrm{m/s}]$ is the transverse component of cell velocity, and $\mathrm{r}\ [\mathrm{m}]$ is the cell radius. Neglecting inertia and assuming no other forces are acting on the cells, the magnetic and drag forces acting on the cells should be equal and opposite:

$$\mathrm{F_{D_y}} = -\mathrm{F_m} \tag{S3}$$

Substituting in for the drag and magnetic forces and rearranging, we arrive at the following equation for the number of beads per cell:

$$\mathrm{N_b} = \frac{6\pi\eta r\mathrm{v_y}}{\frac{\mathrm{V_m}\Delta\chi_{\mathrm{bead}}}{\mu_0}(\vec{\mathrm{B}}\cdot\nabla)\vec{\mathrm{B}}} \tag{S4}$$

Validation of Stokes' Drag. In order to confirm that the drag force on the cells was accurately described by Stokes' law, a series of validation experiments were performed using fluorescently labelled paramagnetic microbeads. Microbeads having diameters of 8 μm (COMPEL, Bangs Laboratories) and 30 μm (PLA-M-greenF, micromod) were tested. A NdFeB permanent magnet provided the magnetic field, and the magnetic flux density & magnetic field strength were calculated via computer simulations carried out in COMSOL Multiphysics. Magnetization curves provided by the microbead suppliers were used to calculate the magnetic susceptibility of the paramagnetic beads, which was a function of the local magnetic field strength.

The 8 μm and 30 μm microbeads had densities of 1100 kg/m^3 and 1400 kg/m^3, respectively. Initial experiments were carried out in PBS, however the high density of the beads relative to PBS led to a significant portion of the microbeads settling out of the solution. Particle settling velocity, known as Stokes' settling velocity, is governed by:[3]

$$\mathrm{V_s} = \frac{\mathrm{D}^2(\rho_\mathrm{p} - \rho)\mathrm{g}}{18\eta} \tag{1}$$

where $\mathrm{V_s}\ [\mathrm{m/s}]$ is the particle settling velocity, $\mathrm{D}\ [\mathrm{m}]$ is the particle diameter, $\eta\ [\mathrm{Pa.s}]$ is the fluid viscosity, $\rho_\mathrm{p}\ [\mathrm{kg/m^3}]$ & $\rho\ [\mathrm{kg/m^3}]$ are the particle and fluid densities, respectively, and $\mathrm{g}\ [\mathrm{m/s^2}]$

is the gravitational acceleration. Clearly, the particle settling velocity is minimized when the difference between the particle and fluid densities is minimized and when the fluid viscosity is maximized. The viscosity and density of the test solutions were increased by adding glycerol, which is much more viscous and dense ($\eta_{\mathrm{glycerol}} = 1.412\ \mathrm{Pa.s},\ \rho_{\mathrm{glycerol}} = 1261\ \mathrm{kg/m^3}$) than PBS ($\eta_{\mathrm{PBS}} = 0.001\ \mathrm{Pa.s},\ \rho_{\mathrm{PBS}} = 1000\ \mathrm{kg/m^3}$). Experiments were carried out with 75% and 85% glycerol solutions. The viscosity of the test solutions was characterized at laboratory temperature (22°C) using a shear rheometer fitted with a 40 mm, 0.5° cone (TA Instruments, AR2000).

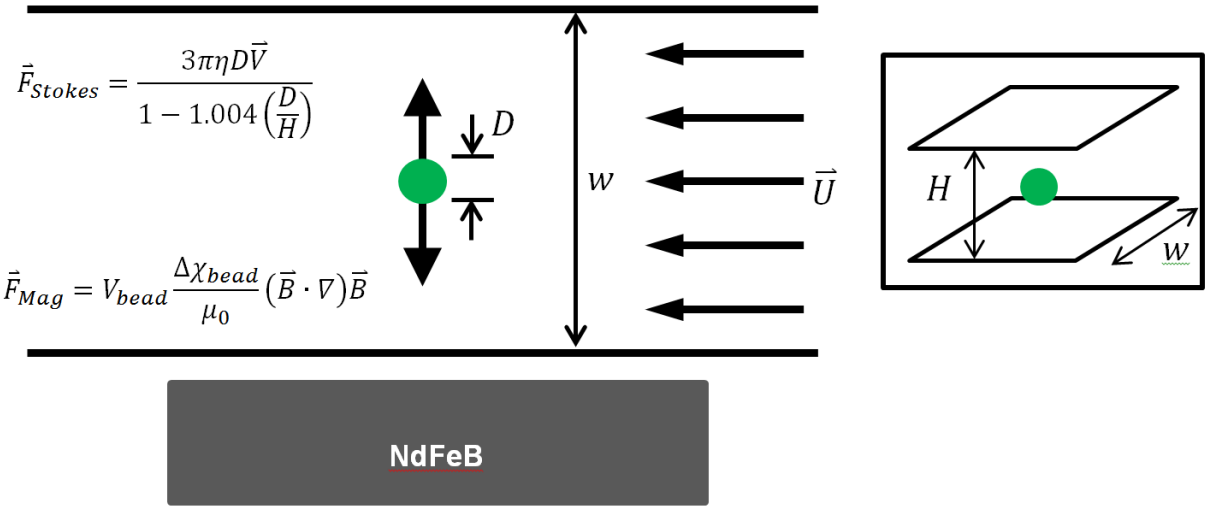

Fig. S3 Schematic of the experimental setup used for measuring the number of nanoparticles per cell.

For each experiment, a small amount of the microbeads was added to the test solution, which was then pumped at a constant flow rate ranging from 100 µl/hr to 1000 µl/hr in a straight walled channel having a width of 1000 µm and a height of 105 µm (Fig. S3). A sequence of images was captured using fluorescent microscopy at a frame rate of 100 FPS. After each experiment, the positons and velocities of the particles in each image were calculated using a suite of MATLAB functions developed at Yale University.[4]

Supporting information

Fig. 3A shows that the ratio of the magnetic force to the measured Stokes drag force for both the 8 μm and 30 μm microbeads was consistent with the expected value of unity, confirming the validity of Stokes' law for this experimental setup.

Zone	Width (mm)	Height (μm)	Length (mm)	Device Surface Area (mm^2)
1	4.5	100	4.5	42.3
2	4.5	200	4.5	44.1
3	4.5	400	4.5	47.7
4	9	400	4.5	83.7

Table S5: Device surface area across four zones of the device.

References

1. K. S. Kim and J.-K. Park, *Lab Chip*, 2005, **5**, 657–64.
2. H. Brenner, *J. Fluid Mech.*, 1962, **12**, 35–48.
3. M. Blanchard, *Geol. Soc. Am. Bull.*, 1967, **78**, 385–404.
4. D. H. Kelley and N. T. Ouellette, *Am. J. Phys.*, 2011, **79**, 267.