Electronic Supplementary Material (ESI) for Nanoscale Horizons.

SUPPORTING INFORMATION

Trapping, Manipulation, and Crystallization of Live Cells Using Magnetofluidic Tweezers

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Section 1. Experimental details.

1.1. Synthesis of biocompatible ferrofluid stock dispersion.

Biocompatible ferrofluid consisting of dextran-stabilized iron oxide nanoparticles was synthesized as described in literature [1] with small modifications. Briefly, 15 g of dextran from *Leuconostoc spp.* (Sigma-Aldrich, M_r = 15-25 kDa) was dispersed in 20 mL of water in 50 mL polypropylene centrifuge tube (VWR 37001-468) to form a thick white slurry. Subsequently, 990 mg of $FeCl_3 \cdot 6H_2O$ (Riedel-de Haën, 99%) and 360 mg of $FeCl_2 \cdot 4H_2O$ (Sigma-Aldrich, 98%) were added and thoroughly mixed, resulting in a thick yellowish slurry. 3 mL of 28-30% NH_4OH was added and the tube was closed and shaken vigorously to mix the reagents for a minute (occasionally opening the cap to release the pressure building from the evolved NH_3). The tube was left open overnight inside a fume hood to allow NH_3 to be released completely. On the following morning, the contents of the tube (brown thick slurry) were mixed with 30 mL of water and centrifuged for 2.5 hours at 5000 g (Sorvall Biofuge Primo), resulting in sedimentation of excess dextran. The supernatant (ca. 50 mL) containing the nanoparticles was collected and mixed with 4 mL of ultrapure 10x PBS free of magnesium and calcium (Trevigen 4870). Subsequently, the mixture was centrifuged again at 5000 g overnight. Supernatant (ca. 40 mL) was collected, discarding any nanoparticles that had aggregated upon addition of PBS. Finally, ca. 2 mL of 10x PBS was added to adjust the salt concentration close to 1x PBS.

1.2. MDA-MB-231 cell culturing and staining.

Human breast cancer cells (MDA-MB-231, ATCC) were cultured in Leibovitz's L-15 Medium (Life Technologies Gibco, 21083-027) supplemented with 10% fetal bovine serum (Atlanta biological, S11150), 0.1% gentamicin sulfate (Corning Cellgro, 30-005-CR) at 0% CO_2 at 37 °C. For staining, cells were plated in low-attachment 24-well plates (Corning, 3473) and incubated in PBS (Corning Cellgro, 21-040-CV) for 1 hour at 37 °C. Cells were stained using cell viability/cytotoxicity kit (Life Technologies, L3224) or Cell Mask Green plasma membrane stain

(Life Technologies, C37608) according to the manufacturer protocols. Nuclei were stained with Hoechst (Life Technologies, H1399) according to manufacturer protocols.

1.3. Magnetofluidic tweezing of MDA-MB-231 cells.

Non-adhesive glass coverslips were prepared by pipetting 200 μL of 10% ethanol solution of Lipidure (Amsbio CM5206) on round glass coverslip (30 mm diameter) and allowing to dry overnight. A glass cylinder (ca. 1 cm in diameter and height) was glued onto the coverslip to form a container for the cells.

1 mL of the biocompatible ferrofluid stock dispersion was washed three times with 5 mL of 10 mg/mL dextran sulfate sodium salt (Sigma-Aldrich 68841, M_R ~ 20,000) in 1x PBS by centrifuging for 30 min at 3000g with centrifuge filter tubes (Amicon Ultra-15, 10 kDa cut-off). Finally, the remaining sediment (ca. 200 μL) was dispersed in 1.5 mL of PBS. Stained cells were centrifuged and re-suspended in PBS, and mixed together with the washed ferrofluid (typically: 300 μL of cells in PBS + 150 μL of washed ferrofluid) and pipetted into the glass container with coverslip bottom.

Magnetofluidic tweezing was done as described in the main text and our previous work.[2] Briefly, magnetic pen was fabricated by electrodepositing nickel-iron-molybdenum alloy (supermalloy) on metallic tungsten wire (100 μm diameter). After deposition, the coated core-shell rod was cut and polished to reveal a clean cross-section. Finally, the pen was glued to the end of a 1.0 mm thick and 50 mm long tungsten rod attached to a motorized three-axis micromanipulator. The micromanipulator was mounted on top of the motorized stage of an inverted Nikon A1 confocal microscope and the attached magnetic pen was immersed into the cell dispersion. External magnetic field was applied with a small home-built electromagnet.[2]

1.4. MCF-10A culturing and staining.

MCF-10A (Homo sapiens, epithelial mammalian cells, ATCC® CRT-10317™) were cultured employing as growth medium a mixture of DMEM/F12 (Invitrogen 1330-032, 500mL), horse serum (Invitrogen 16050-122, 25 mL), human epidermal growth factor (Sigma E9644, 100 μg/mL stock in dH2O, 100μL), hydrocortisone (Sigma H-0888, 1mg/mL stock in EtOH, 250 μL), cholera toxin (Sigma C-8052, 1mg/mL stock in dH2O, 50μL), and insulin (Sigma I-1882, 10mg/mL stock in dH2O with 1% glacial acetic acid, 500 μL).

MCF-10A cells were cultured in tissue culture flasks (25 mL) until 90% confluency. The growth solution was then extracted and discharged, 2mL PBS (Corning, 21-030) solution was added, the flask was shacked and the solution extracted and discharged. 1mL of trypsin to induce proteolyzation (Corning, 25-052) was then added; the flask was shacked and moved into an incubator (37 ° C, 5% CO_2) for 1 minute. 2mL of growth media were added to the cells to inhibit trypsin. The solution was immediately transferred to a centrifuge tube, centrifuged (1216 G) and the supernatant solution extracted and discharged. 1 mL of PBS solution was added and shacked, the solution was centrifuged and the supernatant extracted and discharged two times. A fluorescent dye (LIVE/DEAD Viability/Cytotoxicity Kit, Invitrogen) was then added to the cells, letting it to react in the incubator for 30 minutes.

Section 2. Iron oxide uptake experiments.

2.1. MCF-10A cell counting using confocal microscopy.

MCF-10A cells were plated in a twelve-well plate and cultured to reach 90% confluence. Cells were washed with PBS, followed by the addition of 2 mL of PBS and 100µL of LIVE/DEAD dye solution (consisting of 1.2 mL of PBS, 10.8 µL of ethidium homodimer-1, and 3.2 µL of calcein AM) and imaging on the Nikon A1 confocal microscope (**Fig. S1a**). Subsequently, varying amounts of iron oxide nanoparticle stock dispersion and fresh PBS were added to the wells (**Fig. S1b**). All samples with different nanoparticle concentrations were prepared in triplicate. The cells were then incubated at 37°C with 5% CO_2 for 3h. Incubated cells were gently washed three times with PBS and imaged again (**Fig. S1b**). No significant toxicity was observed in cells incubated with ferrofluid in comparison to cells incubated in PBS. However, we note that the concentration of cells (estimated from confocal images) was lower in all samples after incubation (including those incubated without ferrofluid) (**Fig. S1b**).

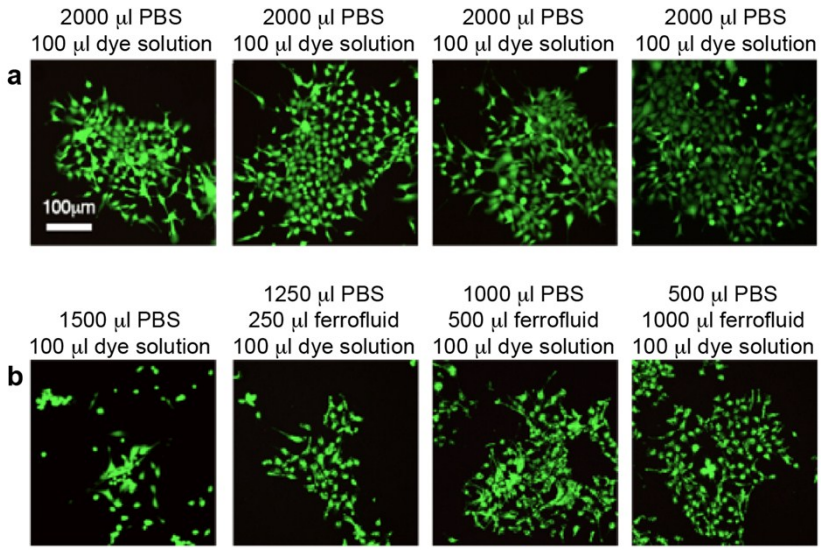

Figure S1. Confocal imaging and phenotype determination for MCF-10A cells before and after incubating with biocompatible ferrofluid. **a)** Confocal images of cells in PBS before adding biocompatible ferrofluid. **b)** Confocal images of cells after incubation in different amounts of the biocompatible ferrofluids.

2.2. MCF-10A uptake quantified by ICP-AES.

The quantitative determination of nanoparticle uptake was performed by culturing MCF-10A cells in six-well plates until reaching ~ 80 % confluence. Six-well plates were employed to assure high enough concentration of nanoparticles to reach the detection limit of ICP-AES (0.3 ppm). 500 µL of 0.05 % trypsin was added to each well, followed by incubation for 10 minutes to detach the cells. After that, 500 µL of cell culturing media was added to inhibit the trypsin. Contents of the wells were transferred to 1.5 mL centrifuge tubes and centrifuged and washed three times with PBS. After that, different amounts of PBS and biocompatible iron oxide nanoparticle dispersion or non-biocompatible ferrofluid (citrate-coated iron oxide nanoparticles) were added to adjust iron concentration between 0 mg/mL and 18 mg/mL. The cells were incubated for 3h at room temperature and centrifuged and washed three times with PBS to

remove non-internalized nanoparticles. 5% of HCl was then added to the centrifuge tubes to digest the cells and particles for 30 min. ICP-AES measurements of iron concentration in the digested cell solutions were performed on the Varian Vista MPX instrument (**Fig. S2**)

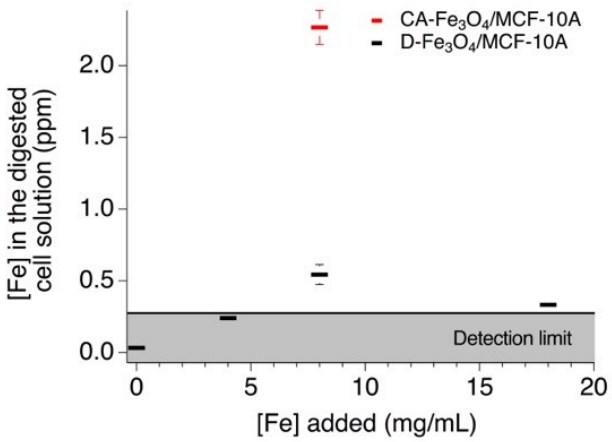

Figure S2. ICP-AES uptake results for MCF-10A cells in the biocompatible (D-Fe_3O_4) and non-biocompatible (CA-Fe_3O_4) ferrofluid. Statistic over three samples are shown.

Section 3. Extension to other cell types: Magnetofluidic tweezing of MCF-10A cells.

To demonstrate generality of magnetofluidic tweezing, we also applied the technique to MCF-10A cells. In these experiments, MCF-10A cells in PBS were mixed with the biocompatible iron oxide nanoparticle stock dispersion in a 1:1 ratio. Mixtures were deposited in similar containers as in the experiments with MDA-MB-231 cells, with the exception that no non-adhesive coating was used. The cells could be magnetofluidically tweezed if tweezing was carried out before cells started adhering to the glass surface (**Fig. S3**).

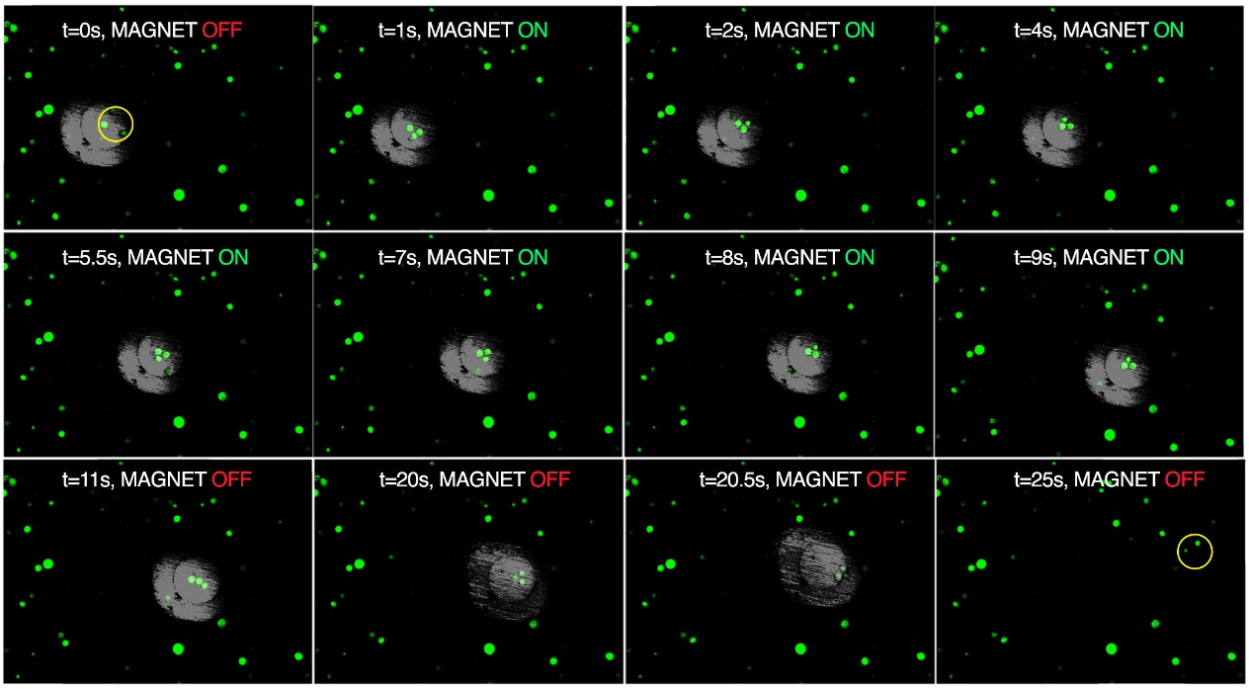

Figure S3. Magnetofluidic tweezing of MCF-10A cells.

Section 4. Simulation of magnetic forces

Magnetic field under and around a magnetized micropen (of similar dimensions as used in experiments) was simulated using finite element method with COMSOL 5.2. Simulations were carried out in 2D axisymmetric geometry and 10 mT external magnetic field. The supermalloy shell was allowed to magnetize in non-linear fashion (using the M-H loop of COMSOL 5.2 default material "Supermalloy"). See ref [2] for full details of the simulations.

The contour map of the calculated field distribution is shown in Figure **Fig. S4a** while magnetic fields along the pen's symmetry axis and perpendicular to this axis are shown, respectively, in **Figs. S4b** and **S4c**. The magnetic force corresponding to the field distribution is given by

$$\mathbf{F}_{\mathrm{M}} \approx -\frac{1}{2}\chi_{\mathrm{S}}\mu_0 V \nabla H^2 \quad \text{(Eq. S1)}$$

We calculated the force for a typical cell (approximated as a non-magnetic sphere, diameter 20 μm) in a magnetic fluid containing 2 μM of 6.5 nm iron oxide nanoparticles ($\chi_S = M/H$ = 0.00057, linear approximation of Eq. (1) in the main text). The vertical lifting force acting on the cell along the symmetry axis of the micropen is plotted in **Fig. S4d**, and the lateral force in **Fig. S4e**. We note that the lift force peaks at ca. 40 pN (**Fig. S4d**). Compared to the estimated 4 pN force required to lift a single cell (see main text), this shows that the nanoparticle concentration may be reduced even to one tenth of the original concentration while maintaining ability to lift cells.

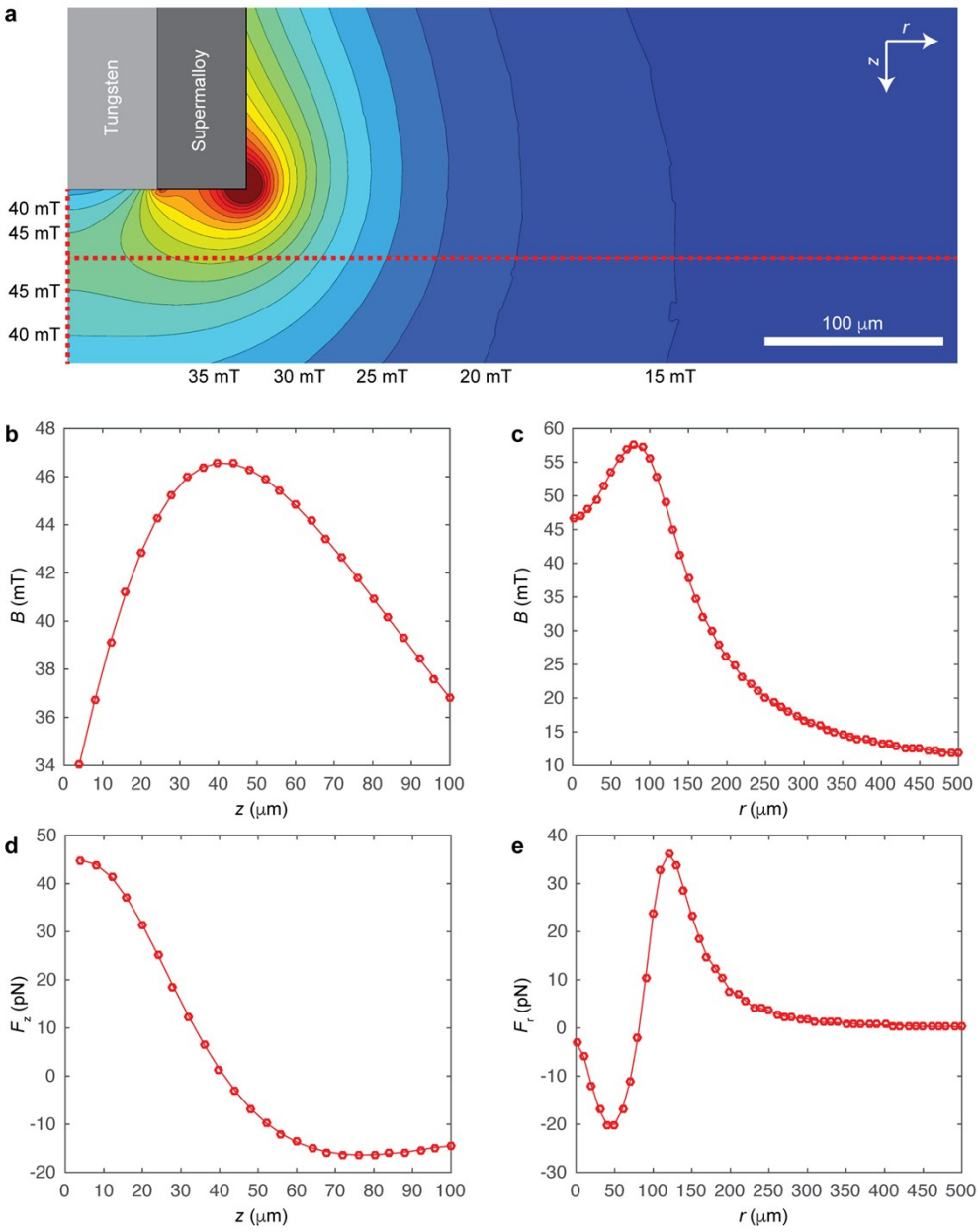

Figure S4. Simulation of magnetic forces. **a)** Side-view of the axisymmetric magnetic field under a magnetic pen (tungsten core diameter 100 μm, supermalloy shell thickness 50 μm, length 2000 μm) in 10 mT external field. Dashed red lines indicate sections of data plotted in subfigures b-e. **b)** Magnetic field as a function of distance (z) measured from the bottom surface of the micropen (along symmetry axis). **c)** Magnetic field as a function of distance (r) from the symmetry axis (41 μm below the bottom surface of the micropen). **d)** Lifting magnetic force and **e)** lateral magnetic force calculated as described in the text above.

List of Supplementary Movies:

Movie S1. 3D reconstruction of single-cell tweezing and lifting up a cell from lipidure surface (reconstruction size 212x212x62μm^3)

Movie S2. Single-cell trapping and translation along an arbitrary path (movie size 422x422μm^2, length 494s)

Movie S3. Tweezing of four cells into a symmetric cluster (318x318μm^2, 245s)

Movie S4. 3D reconstruction of tweezing of four cells into a symmetric cluster (212x212x79μm^3)

Movie S5. Tweezing of clusters of 2, 5, and 7 cells (212x212μm^2, 84s, 363s, 188s)

Movie S6. Translation of a large cell cluster along an arbitrary path (318x248μm^2, 394s)

Movie S7. Stretching of a large cell cluster (446x261μm^2, 193s)

Movie S8. Separation of one live cell from a population of dead cells (317x317μm^2, 43s)

Supplementary References:

1. T. Shen, R. Weissleder, M. Papisov, A. Bogdanov and T. J. Brady, *Magn. Reson. Med.* , 1993, **29**, 599-604.
2. J. V. I. Timonen, A. F. Demirörs and B. A. Grzybowski, *Adv. Mater.*, 2016, **28**, 3453-3459.