A magnetic trap for living cells suspended in a paramagnetic buffer

Adam Winkleman, Katherine L. Gudiksen, Declan Ryan, and George M. Whitesides[a)]
Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

Derek Greenfield and Mara Prentiss
Department of Physics, Harvard University, 17 Oxford Street, Cambridge, Massachusetts 02138

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This manuscript describes the fabrication and use of a three-dimensional magnetic trap for diamagnetic objects in an aqueous solution of paramagnetic ions; this trap uses permanent magnets. It demonstrates trapping of polystyrene spheres, and of various types of living cells: mouse fibroblast (NIH-3T3), yeast (*Saccharomyces cerevisiae*), and algae (*Chlamydomonas reinhardtii*). For a 40 mM solution of gadolinium (III) diethylenetriaminepentaacetic acid (Gd·DTPA) in aqueous buffer, the smallest cell (particle) that could be trapped had a radius of ~2.5 μm. The trapped particle and location of the magnetic trap can be translated in three dimensions by independent manipulation of the permanent magnets. This letter a1so characterizes the biocompatibility of the trapping solution. © *2004 American Institute of Physics*. [DOI: 10.1063/1.1794372]

The ability to position cells, on surfaces and in suspension, is broadly useful in cellular biology. Microcontact printing of self-assembled monolayers[1–3] and other techniques of surface engineering[4,5] are used for confining and controlling the mobility of cells on surfaces. Optical traps can confine and manipulate cells and microspheres in suspension, and have been used to determine the elasticity of the cell membrane,[6–8] to observe cell division,[9] to measure inhibition of cell adhesion,[10] and to strain cells to induce activity in signaling pathways.[11]

While optical traps have enabled many experiments in biophysics, they also have limitations. The laser power required to trap a micron-sized particle (for example, a cell) is proportional to the ratio of the refractive index of the particle to that of the medium.[12] Since the ratio of the refractive indices for most biological materials to biocompatible fluid media is near unity, trapping requires lasers having powers up to ~100 mW. This high laser power can raise the local temperature in the liquid by several degrees,[13] and this heating can damage or kill a trapped cell. In addition, traditional optical tweezers cannot trap objects with ratios of refractive indices of the object to the environment of less than unity (e.g., a gas-filled glass sphere in water[14] or a water drop in liquid parafilm[15]) or greater than 1.5 (e.g., diamond particles in water).[16] Optical tweezers are restrictive in the size of the particle that can be trapped; particles must be $\leqslant 10$ μm in diameter. The trapping force of optical tweezers is minimal outside the focus of the laser beam, requiring objects to enter the focus of the laser before they can be trapped. The small capture volume for many particles (only a few cubic microns) results in significant waiting periods before objects are trapped in dilute samples. The short working distance of the objective lens used to focus the light restricts the trap to regions near (<200 μm) the surface. The high-powered lasers and infinity-corrected, high numerical aperture objectives used to construct an optical trap are expensive.

Although the use of magnetism to manipulate objects was described by Thales (c.500 B.C.), the trapping of objects in a stable magnetic equilibrium was considered for many years to be impossible. Materials having magnetic susceptibility greater than that of their environment are drawn into regions of high magnetic field and, conversely, materials having magnetic susceptibility smaller than that of their environment are drawn into regions of low magnetic field. Magnetic field *maxima* can exist only at the source of the field and, therefore, stable trapping of materials having greater magnetic susceptibility than their environment is impossible except at the source of the field.[17] Magnetic field *minima* can, however, be achieved outside a magnetic field source. These magnetic minima have been used to levitate and confine biological and other diamagnetic materials.[18–21] Diamagnetic materials (or paramagnetic materials in a fluid with a higher magnetic susceptibility) can therefore be held in a stable magnetic trap.

For a material whose magnetic moment is proportional to the applied field, a linear material, the magnetic force acting on a particle in a medium of higher magnetic susceptibility is given by Eq. (1), where χ_P and χ_m are the magnetic

$$\boldsymbol{F} = \frac{(\chi_p - \chi_m)V}{\mu_0}(\boldsymbol{B}\cdot\nabla)\boldsymbol{B} \tag{1}$$

susceptibilities of the particle and of the medium, respectively, V is the volume of the particle, μ_0 is the magnetic permeability of free space, and $\boldsymbol{B}$ is the magnetic field vector. The volumetric magnetic susceptibilities (χ_p and χ_m) and the volume of the particle (V) can be adjusted by changing the medium or the material (i.e., permeability) of the object to be trapped. For a given set of materials, the adjustable parameter is the term $(\boldsymbol{B}\cdot\nabla)\boldsymbol{B}$, which is determined by the strength and configuration of the magnets.

Our experimental setup consists of a paramagnetic aqueous solution, containing a suspension of diamagnetic particles or cells, placed between two cone-shaped, rare earth magnets with opposing fields [Fig. 1(a)]. We used a gadolinium (III) salt solution as the trapping medium, so that the

a)Electronic mail: gwhitesides@gmwgroup.harvard.edu

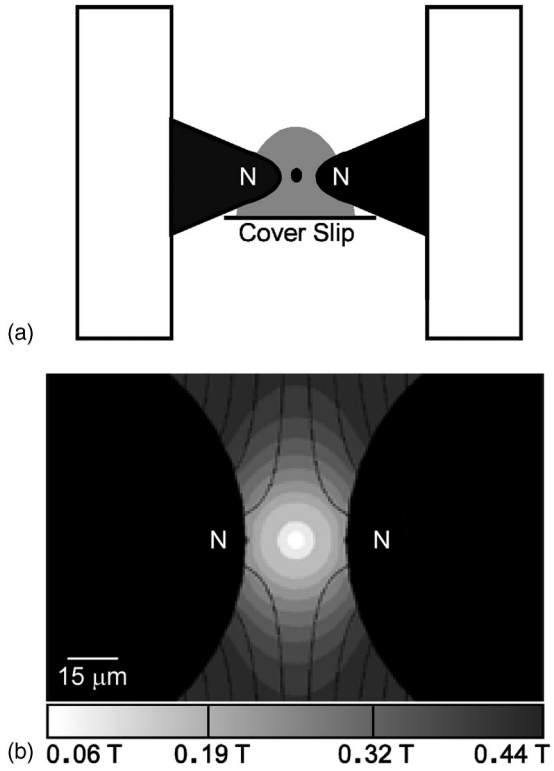

FIG. 1. (a) A schematic illustration of the experimental apparatus is shown. In order to trap a diamagnetic particle, the poles of the two magnets are arranged such that they oppose each other. A suspension of diamagnetic particles in an aqueous solution of paramagnetic ions is placed between the two magnetic tips. (b) The calculated magnetic field intensity and field lines of the magnets are shown. The magnetic field intensity ($|\vec{B}|$) is indicated by the shading in the plot; the darker regions correspond to higher field intensities. The bright point located between the two magnets is the region of lowest field strength—the location of the trap. The calculation is based on a distance of 30 μm between the two magnets, a radius of curvature of each magnet of 76 μm, and a magnetic field of 0.4 T at the surface of each magnet.

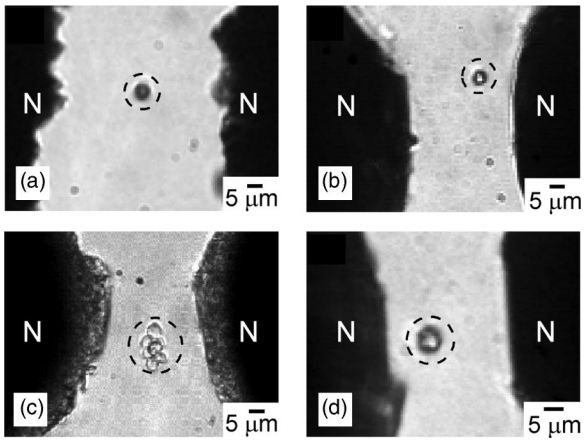

FIG. 2. Trapping of particles or cells between two magnet tips with a radius of curvature of ~76 μm. The trapped particle in each figure is indicated by a dashed circle. (a) A single 6.5 μm bead in a 50-mM-$GdCl_3$ solution; (b) a single 3T3 fibroblast cell with 40-mM-Gd·DTPA solution in 0.5 ×DMEM culture media (Invitrogen); (c) multiple yeast cells with 40-mM-Gd·DTPA×DMEM solution (pH 7.2) in 0.5× YPD broth (1% yeast extract, 2% peptone, 2% dextrose); (d) a single *Chlamydomonas* cell trapped with 40-mM-GdDTPA solution (pH 7.2) in sporulation media (ATCC No. 5).

magnetic susceptibility of the solution was higher than any object we wished to trap. This paramagnetic salt has a high magnetic susceptibility (2.8×10^{-2} cm^3/mol)[22] and is benign biologically.[23] Figure 1(b) shows the field intensity profile of our system. The figure was created using finite element analysis (Finite Element Method Magnetics).[24] We calculated the trapping force on a diamagnetic sphere with a radius of 2.5 μm to be 5.5 pN in a 40 mM solution of gadolinium (III) ions.[25] This calculated trapping force is comparable to those used in optical traps for biological species (0.01–44 pN).[26,27] The maximum force is located 25 μm from the center of the trap; we calculate that our trap has a capture volume of ~62,500 μm^3.

We trapped polystyrene spheres, mouse fibroblasts (NIH-3T3), yeast (*Saccharomyces cerevisiae*), and algae (*Chlamydomonas reinhardtii*) (Fig. 2). We were unable to trap bacterial cells in this trap. For an *E. coli* cell with a volume of ~1 μm^3 the trapping force is only ~2% of that on a mammalian cell with a volume of ~65 μm^3. In a 40-mM-gadolinium (III) salt solution, the smallest particle we have trapped is a polystyrene sphere with radius ~2.5 μm.

The magnetic trap can also be used to manipulate the position of the cell (or particle). Both magnets can be moved in three dimensions; the cell (or particle) follows the position of the magnetic minimum (i.e., the position of the trap). One of the magnets is controlled relative to the second magnet using three independent micromanipulators. The ability to move each magnet independently allows both the strength and location of the trap to be changed. The trapped particle could be manipulated over distances up to ~50 μm in all three dimensions (Fig. 3).

The biocompatibility of a concentrated solution of paramagnetic ions is a concern. The solution is benign biologically provided the Gd(III) is chelated with diethylenetriaminepentaacetic acid (DTPA); the association equilibrium constant is 7.2×10^{17} M^{-1} at 25°C at pH 7.2.[28] Gd·DTPA has Food and Drug Administration approval as a MRI contrast agent.[29] In addition to any biological effects, osmotic pressure, due to high concentrations of salt, can also damage a cell. In order to minimize any detrimental effects from osmotic pressure, we used concentrations of Gd·DTPA that had an osmotic pressure similar to that inside a cell. The

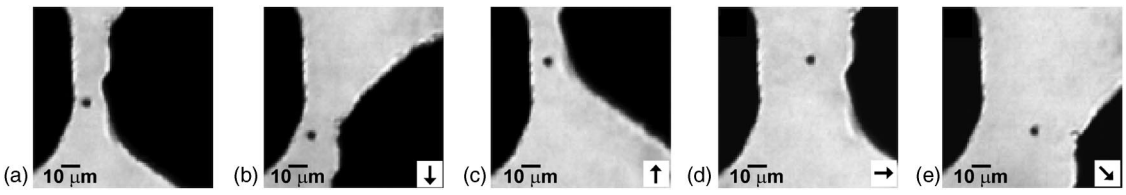

FIG. 3. Trapping of a polystyrene bead in a 40-mM-GdDTPA solution. The left magnet was held stationary and the right magnet was adjusted with a three-dimensional micromanipulator: (a) the initial trapping position is shown. The right magnet was moved (b) down, (c) up, (d) right, and (e) right and down with respect to the left magnet.

trapping medium was the appropriate cell culture medium containing 40 mM Gd·DTPA (pH 7.2). The osmotic pressure of a mammalian cell is 300 mOsm; the measured osmolarity of this trapping medium was 278 mOsm. We confirmed that Gd·DTPA does not kill NIH 3T3 fibroblast cells over a period of 48 h, at concentrations that are used for trapping. We also verified that concentrations of Gd·DTPA between 0 and 40 mM (in Dulbecco's Modified Eagle Medium, DMEM) did not inhibit the growth of fibroblast cells over a period of two days.

In growth medium containing 200 mM Gd·DTPA, fibroblasts did not attach to polystyrene substrates. We presume the osmotic pressure arising from the high salt concentration was lethal to the cells. We passaged fibroblast cells from wells containing 0, 4, and 40 mM Gd·DTPA in media. At concentrations of 0 and 4 mM Gd·DTPA, fibroblast cells attached to polystyrene and spread; at a concentration of 40 mM Gd·DTPA, they did not.

We also investigated the effect of different concentrations of Gd·DTPA on the viability of yeast cells (*Saccharomyces cerevisiae*). At concentrations greater than 4 mM, we observed a reduction in the number of cells produced for a typical life cycle: for 40 and 200 mM Gd·DTPA, a 6- and 24-fold reduction. We observed that the ratio of living to dead cells (measured using the Molecular Probes Live/Dead Yeast Assay) for all concentrations of Gd·DTPA studied was similar. This result suggests that the additional Gd·DTPA did not kill the cells, but did inhibit normal cell division.

This work demonstrates the first successful manipulation of living cells using a magnetic trap whose trapping volume can be positioned in three dimensions. The advantages of a magnetic trap include the ability to trap a wide range of materials, including most biomaterials. In addition, magnetic traps can contain larger objects, and be positioned further into a medium, than optical traps. Magnetic traps can have capture volumes much greater than traditional optical tweezers. The phenomenon of magnetic trapping requires only a magnetic susceptibility of the particle lower than that of the medium. Magnetic traps can, therefore, be used to control a wider range of materials (e.g., gas-filled particles and metallic particles with low magnetic susceptibility) than optical traps. In contrast to optical traps, magnetic traps permit extended periods of observation and manipulation without causing cell damage from local temperature increases, and are relatively inexpensive because no laser source or high numerical aperture objective lens is required.

The magnetic trap demonstrated in this paper is limited to particles larger than 2.5 μm in radius; this limitation reflects the curvature of the permanent magnets (and, thus, the magnitude of the field gradient) we have used, and the concentration of paramagnetic salt compatible with cell viability. The use of microfabricated electromagnets[30] should reduce the minimum size of an object that can be trapped by increasing both the field and its gradient. Furthermore, microfabricated electromagnets in conjunction with external fields and micropositioning would allow multiple traps in numerous geometries (e.g., rings, lines, and U-shapes).[31]

Magnetic traps are orthogonal to traditional optical traps and can, therefore, be used in conjunction with optical traps. The major disadvantage of these magnetic traps is the need for a liquid with high concentrations of nonphysiological salts, and the unknown biological consequences of this medium.

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