Integrated microfluidic isolation platform for magnetic particle manipulation in biological systems

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We have developed a micromachined fluid-cell platform that consists of patterned magnetic thin-film elements supported on a thin silicon–nitride membrane. In the presence of an external magnetic field, the field gradients near the magnetic elements are sufficiently large to trap magnetic particles that are separated from the patterned films by a 200 nm thick nitride membrane. The two main applications of this fluid-cell platform are to provide a means to control and position magnetic microparticles, which can be tethered to biological molecules, and also to sort superparamagnetic microparticles based on their size and magnetic susceptibility. We determine the characteristic trapping forces of each trap in the array by measuring the Brownian motion of the microparticle as a function of applied external field. Typical force constants and forces on the superparamagnetic particles are $4.8\times10^{-4}\pm0.7\times10^{-4}$ N/m and 97 ± 15 pN, respectively.
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The motivation behind developing single molecule measurement techniques is to probe information about the statistical distributions of biological systems that would be otherwise obscured by ensemble measurements.[1] These techniques also provide a means by which individual molecules can be sorted and manipulated in order to alter the physical or chemical reaction pathways that occur in biological organisms at the most fundamental level.[2,3] There are many different approaches to single molecule measurement and manipulation: atomic force microscopy, micropipettes, electrophoretic translocation, and optical and magnetic tweezers.[4–9] The geometry that is characteristic to each technique limits the throughput capabilities of the technique as well as the type of biological system which can be studied. Among the many techniques, tweezers technologies have proven amenable to studying a variety of systems ranging from DNA elasticity to molecular motor dynamics while preserving throughput capabilities, thereby making it one of the more powerful single molecule techniques currently available.[8–12]

There are two main types of tweezers: optical and magnetic. Optical tweezers involve tethering biological molecules to dielectric spheres and then capturing the spheres at the focal point of an electric field gradient. These tweezers can selectively manipulate a single molecule and manipulate each end of a molecule independently.[13–16] Despite these strengths, manipulation is limited to lateral displacement with a low throughput, and force measurements are limited by the laser power, the object dimensions, and the difference between the refractive indices of the object and its surrounding medium. Alternatively, magnetic tweezers trap magnetic microparticles in tailored magnetic field gradients. Due to the magnetic anisotropy inherent in the particles, rotation of the magnetic poles generating the magnetic field gradients that capture the particles imparts torque to the microparticle and, consequently, to a biological molecule attached to the particle. This torsional motion can be used to stretch, twist, or uncoil the biological molecule with a smaller force than that resulting from lateral displacement of the particle.[17,18] With this type of tweezers, one end of the biological molecule must be attached to a fixed point, typically a microscope slide.

To address some of the limitations of the tweezers techniques, our goal is to construct magnetic tweezers based on a chip-scale microfluidic platform that can be placed in a magnetic force microscope to trap, measure, manipulate, and sort biological molecules in an array. The platform consists of an array of magnetic elements, which function as traps, separated from the biological sample by an optically transparent thin membrane so that the platform may be implemented in an optical fluorescence microscope. Solitary superparamagnetic microparticles that can be tethered to biological molecules are trapped in the local field gradients produced by the array of magnetic traps. In this letter, we provide a demonstration of the general concept of using the platform as a trap for superparamagnetic microparticles. In addition, we apply the equipartition theorem to the Brownian motion of the trapped particles to characterize the spring constants and forces exerted on the particles by the magnetic traps as a function of distance away from the trap.

Figure 1 illustrates the microfluidic platform we developed using bulk micromachining techniques developed for silicon. We start with a double-side polished 350 μm thick Si(100) wafer and deposit a 0.2–1 μm thick layer of low stress silicon nitride on the front and back side surfaces. We etch an array of 0.014 mm^2 squares in the nitride film on the

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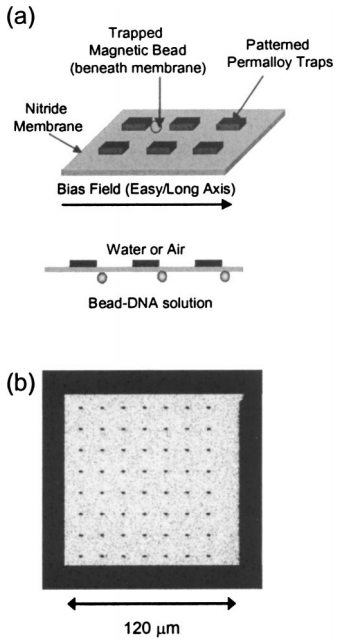

FIG. 1. (a) Illustration of the micromachined magnetic trap platform and (b) photomicrograph plane view of a single well in the microfabricated chip. The magnetic field is applied along the long axis of the traps.

back side of the wafer and then etch wells in the silicon to the nitride film on the opposite side (front side) of the wafer using an aqueous potassium hydroxide anisotropic etch. This process yields an array of suspended nitride membranes supported by a silicon frame. The nitride membrane acts as a barrier between the magnetic traps and the fluid, a superparamagnetic microparticle/biological solution, that fills the wells. It also provides a means by which external manipulation of the magnetic microparticle solution can be achieved without removing particles from the solution. After etching the wells, we fabricate the magnetic traps by patterning a two-layer liftoff photoresist on the front side nitride membrane and then sputter deposit a tantalum adhesion layer of 5 and a 30 nm film of Permalloy ($Ni_{80}Fe_{20}$) on the nitride membrane in a 6×10^4 Pa background pressure of argon using a magnetron sputtering source. The excess Permalloy is removed by a liftoff process, thereby leaving a patterned array of Permalloy traps. The number of traps in an array is typically between 50 and 200. The dimensions of each trap are 1.2 μm$\times$3.6 μm, which is equivalent to the size of the superparamagnetic particles used in these experiments (2–3 μm). We note that the traps are not perfect rectangles. Instead, they are somewhat rounded around the edges. This feature is desirable since it decreases the influence of closure domains that would be present at the edges of the rectangles. To improve adhesion of the traps to the nitride membrane, we sputter deposit a tantalum seed layer of 5 nm on the nitride membrane before depositing the Permalloy. To align the domains in the trap and produce a relatively high local field gradient near the trap, we apply an external magnetic field, produced by either rare earth magnets that are placed on the inner ring of the microscope stage insert plate or by a Helmholtz coil that is placed on the exterior sides of the sample stage of the microscope. The direction of the field is along the easy axis (long axis) of the magnetic traps. For these experiments, the superparamagnetic particles, which

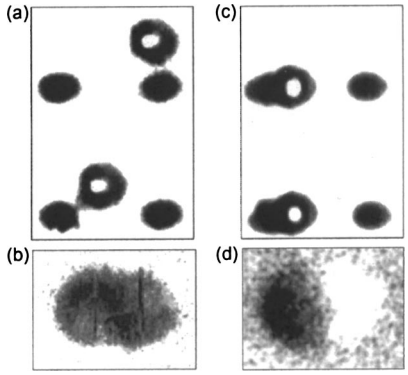

FIG. 2. (a) Image from the video sequence of the Brownian motion of the superparamagnetic particles without an external applied magnetic field. The traps are the solid black ovals, and the magnetic microparticles are the white circles with black edges; (b) magnetic force microscope image of a single trap without an applied magnetic field; (c) image with an external applied magnetic field of 6.4×10^{-3} A/m; and (d) the corresponding magnetic force microscope image of a trap.

are polystyrene spheres embedded with iron oxide particles, are suspended in a solution of deionized water and injected into the wells of the microfluidic platform. The platform is placed onto a coverslip that rests on an inverted optical microscope with fluorescence imaging capability. The microscope is equipped with a CCD camera to record the motion of the particles and a scanning probe microscope to measure magnetic forces

Figure 2 shows two video sequences of the microparticles before a magnetic field is applied and after an external magnetic field of 6.4×10^{-3} A/m (80 Oe) is applied along the long axis of the traps. In the absence of an applied magnetic field, the Permalloy traps exhibit a multiple domain magnetic structure as seen in the magnetic force microscope images taken in air; there is no preferential magnetization axis in the trap and hence, no distinct location of the magnetic field gradient. In general, we observe Brownian motion movement of the particles in the solution. This reveals that the field gradients due to the individual domains are not sufficient in strength to trap the particle at distances far away from the trap. If the particle is placed or finds its way to the end of the trap along its long axis, then it will remain there; however, the particle will show significant Brownian motion in that location, indicating that the adhesion force between the microparticle and the surface of the nitride is relatively small in comparison to forces due to magnetic field gradients in the trap.

With the application of a 6.4×10^{-3} A/m field along the long axis of the trap, the magnetic domains align with the direction of the applied field. The magnetic force microscope image, taken in air, shows a dipole structure indicative of a single-domain configuration in the trap. The applied field is large enough to saturate the magnetization of the trap and generate sufficient magnetic field gradients at the ends of the traps. In this situation, the microparticles react to the magnetic field from far away, up to 5 μm, and once trapped will show significantly reduced Brownian motion. If the applied magnetic field is removed, the particles will once again exhibit significant motion, indicating the return of a multiple domain structure in the traps.

From the Brownian motion of the particles, we can de-

termine the spring constant associated with the traps. Here, we implement the equipartition theorem, which relates the energy of the system to the energy of the particles by the fact that they are in thermal equilibrium with one another.[18] By solving for the spring constant we obtain the following relationship:

$$k_{\text{trap}} = \frac{k_B T}{\langle x^2 \rangle}, \tag{1}$$

where k_{trap} is the spring constant of the trap, k_B is the Boltzmann constant, T is the temperature of the system, and x is the displacement of the particle from its energy minimum and is also known as the Brownian motion of the particle. This relationship assumes the fluctuations of the particle to be harmonic. The displacement of the particles is measured using a time sequence of CCD images. For a 200 nm nitride membrane thickness, which establishes the minimum particle-to-trap separation, and an applied field of 6.4×10^{-3} A/m, the spring constant is $4.8 \times 10^{-4} \pm 0.7 \times 10^{-4}$ N/m and the associated force is 97 ± 15 pN.[11] As a comparison to physical and chemical processes occurring in biological organisms, molecular motors exert forces of ~ 10 pN, cohesion forces due to hydrogen bonding in biomolecules are on the order of 100 pN, and the force required to break a covalent chemical bond is on the order of 1 nN. While the force that the trap imparts on the particle is not large enough to dissociate a covalent bond, it is possible to manipulate molecular motors and change the physical conformation of twisted biomolecules.

The present magnetic tweezers device is capable of trapping magnetic particles with high location specificity. By using a facile microfabrication process, we are able to create arrays of traps so that many particles can be manipulated simultaneously. The forces exerted on the particles by the traps match and exceed those of current tweezers techniques and can be adjusted by changing the applied magnetic field. It is possible that this platform can be used to sort magnetic particles based on their size and magnetic susceptibility more rapidly than conventional magnetic separations, which require long distances and hence lengthy times.

This platform will be used to sort and homogenize magnetic particle samples. A possible extension of this technique includes incorporation of spin valve structures as magnetic traps, which would act as on/off switches by switching from parallel (ferromagnetic) to antiparallel (antiferromagnetic) configurations to allow for rotational manipulation of the magnetic particles. The tweezers platform may be used in conjunction with a magnetic force microscope tip to apply forces to biological molecules using lateral and torsional displacements of magnetic particles attached to the biological molecules. Ultimately, the magnetic tweezers platform will be amenable to large scale reproduction and integration with other biological measurement devices.

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