Manipulation and sorting of magnetic particles by a magnetic force microscope on a microfluidic magnetic trap platform

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ABSTRACT: We have integrated a microfluidic magnetic trap platform with an external magnetic force microscope (MFM) cantilever. The MFM cantilever tip serves as a magnetorobotic arm that provides a translatable local magnetic field gradient to capture and move magnetic particles with nanometer precision. The MFM electronics have been programmed to sort an initially random distribution of particles by moving them within an array of magnetic trapping elements. We measured the maximum velocity at which the particles can be translated to be 2.2 mm/s ± 0.1 mm/s, which can potentially permit a sorting rate of approximately 5500 particles/minute. We determined a magnetic force of 35.3 ± 2.0 pN acting on a 1 μm diameter particle by measuring the hydrodynamic drag force necessary to free the particle. Release of the particles from the MFM tip is made possible by a nitride membrane that separates the arm and magnetic trap elements from the particle solution. This platform has potential applications for magnetic based sorting, manipulation, and probing of biological molecules in a constant-displacement or a constant-force mode.

Magnetic particles have a variety of applications in biology, ranging from *invitro* sample sorting, measurement and manipulation to *invivo* magnetic resonance imaging contrast enhancement.[1] While *invivo* treatments are essential to improving current efforts in drug screening and diagnosis of patients, *invitro* applications prove useful in gaining an essential understanding of the process by which biological systems function and the manner in which they can be changed on a single molecule level.

Typical magnetic particle sorting applications include separation of biological analytes such as cells, proteins, and DNA. The premise of this sorting is to attach a chemically functionalized magnetic particle to a desired biological specimen and then apply a magnetic field gradient to pull the magnetic particles away from the solution, thereby leaving the unwanted molecules behind. In this case, sorting is done as an ensemble, and single-particle location specificity as an end result is not achievable. Single-particle sorting techniques have recently been demonstrated based on magnetic wires or domain wall tips.[2-6] The limitations of this technique are power consumption and that the particles cannot be sorted into an array for long periods of time without causing local heating and hence, possibly damaging the samples.

The application of lateral and torsional forces to biomolecules by tethered magnetic particles remains an essential method for revealing information about molecular motors, protein-DNA interactions, and the forces associated with folding and unfolding dynamics of DNA.[7-9] In these experiments, one end of the biological molecule is immobilized onto a microscope slide while the other is attached to a magnetic particle that follows the field gradients generated by macroscopic rare-earth magnets. These experiments are generally limited by the fact that the sample must be immobilized and the information obtained is via constant force on the magnetic particle. In this paper, we describe a novel platform for both sorting of

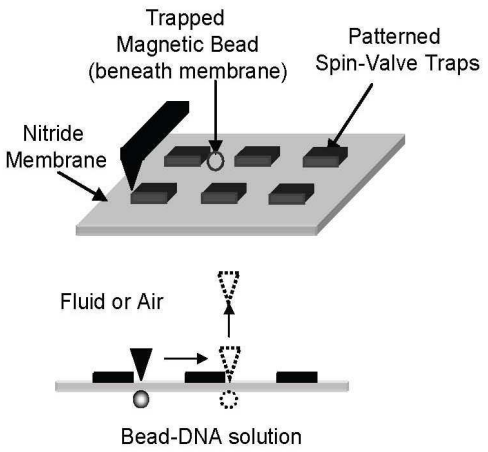

Fig. 1. An illustration of the micromachined magnetic trap platform and the location of the magnetic force microscope (MFM) tip.

magnetic particles that can be attached to biological samples and 'tweezing' of individual particles. The platform provides the characteristics of high throughput, a high degree of location specificity, and the ability to selectively immobilize individual samples for measurement and/or manipulation. The possibility of releasing the particles for transport or further examination by alternative single molecule diagnostic techniques can provide the necessary versatility for classification of the vast quantity of biological processes occurring in the body.

Figure 1 shows an illustration of the platform. A summary description of the platform is given below, but details of the fabrication process can be found in previous work.[10] The key elements of this platform include an array of Permalloy elements that are patterned onto a transparent membrane. In the presence of an externally applied magnetic field, these elements provide local magnetic field gradients that act as magnetic particle traps. The transparent membrane consists of a 200 nm silicon nitride layer. This membrane separates the magnetic trapping and sorting elements from the magnetic microparticle or biological sample solution and allows for selective translation using a permanently magnetized magnetic force microscope (MFM) tip. The transparency of the membrane allows for placement of the device in an inverted optical microscope for observation of translocation events. The optical microscope is equipped with a charge-coupled device (CCD) camera and imaging software. The images obtained using the CCD camera provide information on the location of the magnetic particles with respect to the traps. By interfacing the CCD image with the MFM software, we can implement a program to sort particles, based on size, color, chemical functionality, and magnetic susceptibility, into their respective positions in an array.

In these experiments, we used a commercial cobalt coated MFM tip with a radius of 90 nm, a height of 15 μm, and width at the top of the tip of 10 μm. Initial scans of the tip show that the particles are not strongly attracted to the tip field gradients and do not translate with the tip. We attribute this to the fact that the tip slope is sufficiently large to allow for a diminished interaction between the magnetic material on the sidewall and the particle. To increase the area of interaction between the tip and the particle, we sanded the tip by scanning it rapidly on a hard surface such as vicinal yttria-stabilized cubic zirconia and observed the change in area of the tip in-situ with a CCD camera. Scanning electron microscopy images show the tip with a 0.8 μm wide sanded plateau. With this geometry, the magnetic material that produces the required field gradient to capture the particles consists of a ring of 60 nm width and a radius of approximately 400 nm.

For the size sorting experiments, we inject a solution of 2.8 μm and 5 μm diameter polystyrene spheres, which are embedded with iron and iron oxide particles, into the wells of the chip. Figure 2 (a) shows the initially random distribution of

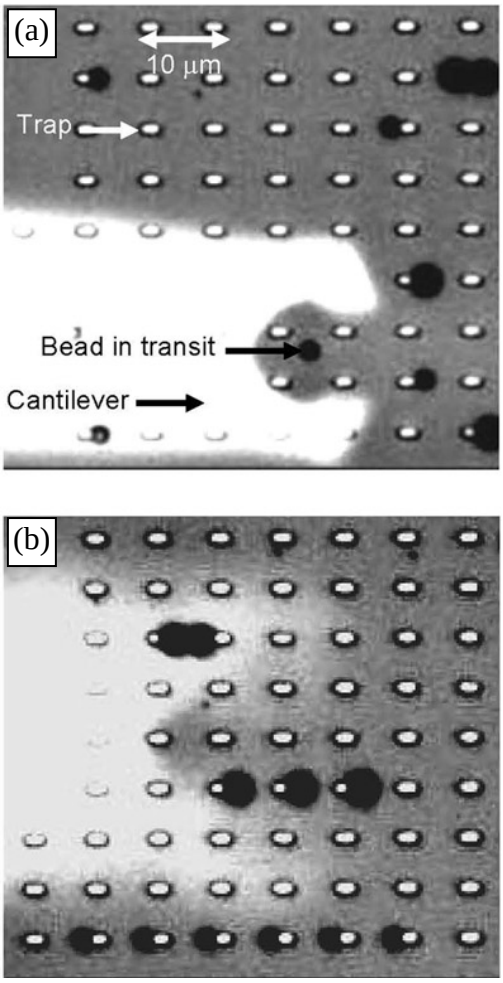

Fig. 2 Images of the magnetic Permalloy traps (white rectangles) on the silicon nitride membrane and the magnetic particles (dark circles) beneath the membrane. The MFM cantilever is the solid white area. (a) The initially random distribution of the 2.8 μm and 5 μm particles with a bead in transit under the cantilever tip and (b) the sorted particles.

particles and (b) the sorted particles. The tip provides the translatable magnetic field gradient, while the Permalloy elements are used to spatially confine the sorted particles. The particles are placed into each position by approaching the surface where the particle to be moved is located. Since the tip field gradients diminish with distance as r^{-3}, it is necessary to bring the tip as close to the particle as possible. Our minimum distance is limited by the thickness of the nitride membrane, which is currently 200 nm; however, we can to decrease the thickness of the membrane to 100 nm without damaging the resilience of the membrane. Once the tip contacts the surface, the particle is moved to a predetermined trapping element. To release the particle from the tip field gradient, we retract the tip from the surface to distances of 9 μm. At this height, the tip then moves to the next particle to be sorted. Once the particles are placed into the array, each particle can be annotated for future manipulation and analysis. The rates of sorting for the larger particles were not measured, for two main reasons. The first was that the tip geometry was not optimized to accommodate large particles and the second was that the larger particles typically lack the homogeneity in magnetic moment that is present in the smaller particles. However, both sorting of larger particles and tailoring of the tip geometry for specific size ranges are possible.

Since the tip size for these experiments is 800 nm, the geometry is optimized for a 1 μm particle, and hence we can sort 1 micrometer particles at a rate faster than that for larger particles. The maximum velocity at which the particles are translated is measured by rastering the tip in incremental velocities and recording the point at which the magnetic microparticle no longer follows the tip. We measured a maximum translation velocity of 2.2 mm/s ± 0.1 mm/s for a 1 μm particle. To determine the maximum sorting rate, we assume that with an average translation distance of 20 μm, a tip repositioning time of 2 ms, and a computer interface time of 1 ms, a maximum of approximately 5500 individual particles can be sorted per minute.

The magnetic homogeneity and smaller size of the 1 micrometer magnetic particles makes them a suitable choice for magnetic tweezers experiments. To implement the current magnetic tweezers platform, we need to compare the forces acting on the particle to those obtained with conventional tweezers instruments. To determine the

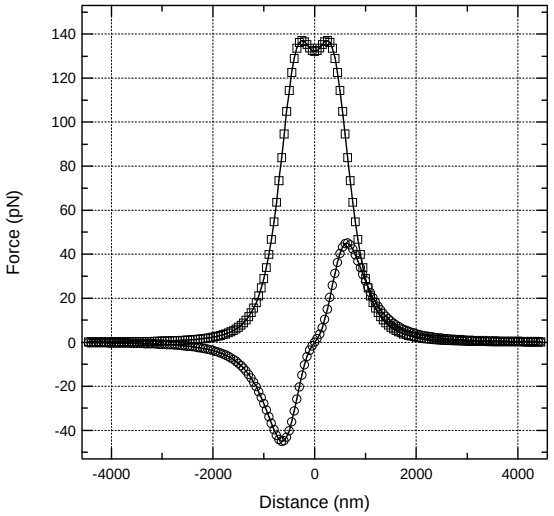

Fig. 3. Simulated results showing the force-distance relationship. A truncated tip F_x (circles) is the force along the x axis and F_z (squares) is the normal force as a function of x-displacement at Z=200 nm. The Z axis is along the axis of the tip. The maximum force trapping the particle is 45 pN. We attribute differences between experimental and theoretical values to frictional force between the particle and nitride membrane resulting from the force along the Z axis.

force acting on the particle we use the measured velocity. However, since the particle is near the surface, a simple treatment using the Stoke's Law for viscous drag is not appropriate in determining the force acting on the particle. Here we implement the relationship determined by Goldman, Cox and Brenner for hydrodynamic drag on a particle positioned at a surface. The force is expressed as[11] $F=1.7005 \times 6\pi\eta r^2 G$, where η is the viscosity of the medium (which in this case is water) r is the radius of the sphere and G is the shear rate of the fluid flow. For this equation to be valid, we must prove that our system is under laminar flow conditions. For laminar flow, the Reynold's number (Re) for the system must be less than 1, and, for our system, we calculated $Re = 2.3 \times 10^{-6} \pm 0.1 \times 10^{-6}$ from the velocity measurements made by scanning the tip. Therefore, the shear rate can be calculated under the condition of a uniform velocity gradient by using the velocity at the center of the sphere, which in this case corresponds to the distance from the surface to the center of the sphere. Under these conditions, we obtained a shear rate of $4.6 \pm 0.1 \times 10^3$ s^{-1}, which corresponds to a force of 35.3 ± 2.0 pN.

To confirm the experimental force measurements, we used micromagnetic simulations to calculate the total force acting on the particles. Figure 3 shows a simulation of the force versus distance for a truncated tip with an 800 nm diameter and a 1 μm diameter magnetic particle. As a note, simulations confirm that a truncated tip provides a stronger trapping force than the original conical tip, which confirms our experimental observations. For the truncated tip, the maximum lateral force acting on the particles is 45 pN. This value is slightly larger than the experimental value we measure. Deviations from the experimental values obtained using the hydrodynamic drag equation are most likely due to the frictional force resulting from the normal force F_z pulling the bead into the silicon nitride surface.[12] The force as a function of displacement from the center of the tip indicates that the range of the field gradient is comparable to the size of the particle, and the field outside the particle decreases rapidly. This localization of the magnetic trapping field allows for these tweezers to perform constant displacement and constant force measurements, which is in contrast to typical magnetic tweezers that function solely as force clamps. While the current set-up produces forces comparable to optical tweezers, we can tailor the tip-particle geometry and the magnetic material used to increase the force acting on the particle to a magnitude typical of current magnetic tweezers apparatus ($\sim 10^2$ pN).

The magnetic material coating the side walls of the cone comprising the tip produces magnetic field gradients sufficient in strength to attract more than one particle at a time. This is an undesirable attribute that can be resolved by implementing the

traps to separate the particles. Particles less than 5 μm in diameter that are stuck together may be separated by dragging the particles over the center of a Permalloy element, where the particle furthest from the tip will remain with the Permalloy element, while the other continues to track the field gradient of the tip, as shown in Fig. 4. Ultimately, to eliminate this characteristic and improve upon the maximum trapping force of the field gradients, we must construct the tip with a magnetic structure of size equivalent to the particles being sorted in the microfabrication process.

We have demonstrated the ability to use a permanently magnetized MFM tip to sort magnetic microparticles based on size differences with possible translation rates of up to 5500 particles/minute. We are able to separate particles using stationary magnetic elements, which we can implement in the future to confirm attachment of a biological molecule between two magnetic microparticles. In contrast to conventional magnetic tweezers, simulations of the tip fields show that the magnetic tweezers platform can act as a force clamp tweezer. With the appropriate force feedback control the platform can also function in a constant force mode. While the tip-particle geometry in this work favors a 1 μm diameter particle, we can tailor our experiments to specific force ranges and sizes of particles by fabricating specialized tips. Ultimately, we will implement this apparatus to manipulate and measure force-induced phenomena of biopolymers.

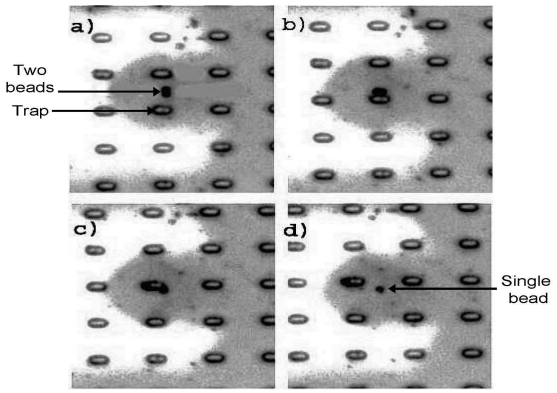

Fig. 4. Two 1 μm diameter particles that are stuck together can be separated by dragging the particles over a trap, where one particle remains in the field gradients produced by the Permalloy trap and the other continues to follow the tip field gradients**.**

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