Design of a microfabricated magnetic cell separator

A new magnetic separation idea utilizing several ideas from microfabrication and nanomagnetics is presented. The basic idea comes from our earlier work using asymmetry in obstacles and Brownian motion to effect separation of objects [10] by moving them in streams whose angle to the hydrodynamic average velocity is a function of the diffusion coefficient of the object. The device we propose here is not technically a Brownian ratchet device but uses the idea of force which acts at angle to the hydrodynamic flow. In our case, the force is generated by a magnetic field gradient which comes from an array of magnetized wires which lie at an angle to a hydrodynamic field flow. The sum of the hydrodynamic force and the magnetic force create a new vector which as in the case of the Brownian ratchet moves the cell out of the main stream direction.

**Keywords:** Microfabrication / Magnetic cell separations

1 Introduction

Blood carries nourishment and oxygen to, and waste products away from, all parts of the body through the arteries, veins, and capillaries. Blood also mediates the immune system, recognizing foreign macromolecules and mounting an attack against them. Humans contain approximately five liters of blood, which accounts for 7% of our body weight. There are three main types of blood cells: erythrocytes (red blood cells), leukocytes (white blood cells), and platelets. However, all three arise from precursor cells in the bone marrow, called hematopoietic stem cells. Erythrocytes are the most common type of blood cell, existing at concentrations around 5 \times 10^{12} cells per liter [2]. They constitute approximately 45% of the total volume of blood. Erythrocytes are very small (7.8 \mu m) and are normally shaped as biconcave disks. Aside from rare exceptions, mature erythrocytes have no nuclei or internal membranes. Their purpose is the first listed above: they are primary components of the circulatory system. Leukocytes exist at nearly one-thousandth the concentration of erythrocytes, and they serve an entirely different purpose. Leukocytes protect the body from infection, in cooperation with the organs of the immune system. They are typically classified into three types: granulocytes, monocytes, and lymphocytes. Granulocytes, so named because of granules in their cytoplasm, make up the majority of leukocytes at 5 \times 10^9 cells per liter. They range from 12 to 15 \mu m in diameter. Monocytes exist at only 4 \times 10^8 cells per liter, but they range from 15 to 18 \mu m across. Lymphocytes, at 3 \times 10^9 cells per liter, are only slightly larger than erythrocytes, though each has a very large nucleus which occupies most of the cell.

All adaptive immune responses are mediated by B-lymphocytes (B-cells) and T-lymphocytes (T-cells). All lymphocytes bear variable cell-surface receptors to detect antigens, or foreign macromolecules and cells. Of special interest to cellular and molecular biologists are the bloodborne proteins called antibodies, for they can be used as highly specific probes to identify and distinguish between different cell populations. Antibodies are Y-shaped proteins of the immunoglobulin (Ig) family. The body produces antibodies as a defense against extracellular materials. For instance, an antibody may bind to a virus or toxin to prevent it from infecting a cell, or it may coat a foreign bacterial cell and mark it for destruction. However, a particular antibody can only bind to select molecules that fit into its antigen-binding site. Although the amino acid sequence among all antibodies is mostly constant, the end of each “arm” of the Y-shaped molecule sports a variable region. These arms form the binding site of an antibody, and it is their variability that accounts for the specificity in what they can bind to. Today, antibodies can be obtained which distinguish between two polypeptides that differ by only a single amino acid [3]. Once families of antibodies have been produced, they can be conjugated to small fluorescent molecules, such as fluorescein or rhodamine, by which they can be detected under a fluorescent microscope.

Cell biologists have isolated families of antibodies that selectively recognize different subpopulations of leukocytes by specific proteins contained in the cells’ outer membranes. Though they are natural components of the leukocytes, these cell-surface molecules are called “antigens” because antibodies can be raised against them. A more fitting name for them is “markers” because they are...
Figure 1. (a) The original thermal ratchet concept. As molecules are moved down in a flow field, the odds of moving to the left or right are not equal. (b) The magnetic force separation idea. High magnetic field gradients provide forces at an angle to the flow of cells.

Figure 2. Labeled antibodies and their attachment to a cell membrane.

characteristic of specific cell populations. Markers can be grouped into multiple categories; some are specific for cells of a particular lineage, while the presence of others may vary according to the stage of differentiation of cells of the same type [4]. Any cell surface marker that identifies a particular lineage or differentiation stage and is recognized by a group of monoclonal antibodies is part of a “cluster of differentiation.” All leukocyte surface markers whose clusters are defined are designated with a CD, followed by a number.

The CD system has allowed immunologists to identify cells participating in various immune responses. For instance, most helper T-cells are CD3+ CD4+ CD8−, while cytotoxic T-cells are CD3+ CD4− CD8+ [4]. Fluorescent molecules can be conjugated to the antibody clusters that recognize their specific markers, and then incubated with the cells in a sample. However, the CD system also enables the fractionation of blood cells according to their specific surface antigens. Clusters of antibodies can be used to selectively bind fluorescent molecules or magnetic beads to certain leukocytes, which are then isolated by flow cytometry techniques. This system is crucial to our method of separating cells as well as to the existing technologies. Figure 2 gives a picture of how antibodies can be labeled and attached to cells.

Fluorescence-activated cell separation provides scientists with one way to isolate cells of a uniform type from a tissue or cell suspension. Specific cells in a sample are labeled with antibodies of an appropriate cluster, coupled to a fluorescent dye. The cells are sent single file in a fine stream through the path of a laser beam. As each cell passes the beam, it is monitored for fluorescence. A nozzle then forms droplets containing single cells and gives each a positive or negative charge, depending on whether the cell it contains is fluorescent. Finally each droplet is deflected by a strong electric field into a collection chamber [2]. This process results in two collection tubes, one containing labeled cells and one containing unlabeled cells. Consequently, the specific cells in the sample that were labeled are isolated from the rest of the sample. A schematic view of a FACS machine and a magnetic sorting device is shown in Fig. 3.

The beads themselves are composed of iron(II) oxide nanocrystals approximately 50 nm in diameter, coated with a polysaccharide which provides functional groups
for the attachment of antibodies [6]. Thus, each bead is smaller than the average leukocyte by factor of more than $10^6$. Their extremely small size makes them very gentle on the cells to which they attach, and they bear apparently no effect on the cells' function or viability. However, although only a few dozen beads are needed to separate a cell, several thousand may be bound to a particular cell after incubation [7]. An interesting physics result of the nanosize of the beads is that they are superparamagnetic, which means that they are single domain but too small to form a stable magnetic moment (ferromagnetic materials have stable magnetic moments in the absence of an external magnetic field $H$. Ferromagnetism comes from the Fermi exchange interaction between two atoms, each of which has a net electronic unpaired spin $S$ and the net odd symmetry that the total wavefunction must have. The sign of the exchange interaction is such that the spins have a parallel alignment energy on the order of 400 K, and if the system is big enough can be ferromagnetic at room temperature. However, for small volumes thermal fluctuations will be sufficient to overcome the anisotropy energy and cause the spontaneous loss of a permanent magnetic moment. Under such conditions the material is classified as superparamagnetic. In zero external field, the net magnetic moment of the superparamagnetic beads is zero. In the presence of an external magnetic field the beads can be highly magnetized.

The high specificity and efficiency of magnetic methods have made them quite useful in obtaining rare cell types. Hematopoietic stem cells, residual tumor cells, and antigen-specific B- and T-cells can be isolated and used in a variety of functional assays. Hematopoietic stem cells can be isolated by their expression of the CD34 antigen [8]. Stem cell purification techniques are of great value for both science and medicine. Pure populations of stem cells will make possible scientific studies of blood cell formation and differentiation. Additionally, they are necessary for successful transplantation procedures. Stem cells from the bone marrow and peripheral blood are transplanted in combination with chemotherapy for the treatment of certain malignant and genetic disorders.

Figure 3. Two conventional ways to sort labeled cells from whole blood.
The success of a transplant procedure depends on the effectiveness of techniques that are used to isolate the cells for the transplant. Any additional cells lingering in the preparation may pose a risk to the recipient. Cancer patients benefit from cell separation techniques by their capacity to remove residual tumor cells from the bone marrow. Since conventional cancer therapy is toxic to bone marrow stem cells, a fraction of bone marrow must be removed from a patient before high dose therapy can be given. The bone marrow can subsequently be reinfused into the patient. But before this occurs, it is desirable to eliminate all tumor cells from it. Tumor cells can be selected by specific antibodies to surface markers and then removed from the sample. Scientists have achieved removal of 99.9% of the malignant cell population using magnetic separation techniques.

We describe an alternative way to isolate biological cells from a larger sample. Our method is similar to magnetic-activated cell separation (MACS) in that specific cells are attached to antibody-coated magnetic beads in a high magnetic field gradient. However, our device is entirely confined to one single microchips. Additionally, it confers the advantages of continuous input and two-dimensional separation. It is believed that our device could potentially yield a higher efficiency and a greater degree of purification than existing cell separation techniques.

2 Magnetic separation

In our device, cell fractionation is made possible by an array of very thin magnetized “wires” which are aligned at an angle to a net hydrodynamic flow direction. There are two advantages to these microfabricated wires: the extremely thin ferromagnetic layer forces the spin system to be single domain, or at least “domain” with resultant very high magnetic fields. Secondly, because of the small length scale of the wires such small structures have large magnetic field gradients at their edges. Since magnetic force depends on field gradients, the path of a paramagnetic object exposed to this array of wires will be altered. Thus, our device can separate paramagnetic objects from diamagnetic ones. Paramagnetic beads attached to cells are attracted to the wires and are deflected away from unlabeled cells.

Since there are no magnetic monopoles, forces result from field gradients acting on magnetic dipole moments. If an object is ferromagnetic with a permanent magnetic moment, \( \mu \), it will feel a force in the presence of a magnetic field gradient given by:

\[
\mathbf{F} = (\mu \mathbf{V}) \mathbf{B}
\]  

(1)

The magnetic moment of a paramagnetic object is induced by an external field. A paramagnetic object with magnetic susceptibility \( \chi \) will feel a force given by:

\[
\mathbf{F} = (\chi \mathbf{B} \cdot \mathbf{V}) \mathbf{B}
\]  

(2)

In our apparatus, beads attached to the cells have an induced magnetic moment \( \chi \mathbf{B} \) aligned parallel to the field produced by the wires. The preferred magnetization direction of the magnetic stripes is to place \( \mathbf{B} \). Wires are magnetized perpendicular to the plane of the wafer, so the field points in the +\( \mathbf{y} \)-direction. Since beads travel through our device at a fixed height \( y_0 \) above the wires, the force that they encounter is:

\[
F = \mu \mathbf{B} \cdot \mathbf{y}/\partial x
\]  

(3)

Our beads are single domain, 50 nm particles of ferrous iron oxide (FeO). They are superparamagnetic, which means that they are too small to sustain a stable dipole moment but that they will exhibit a net moment in an external applied field.

3 Microfabrication

Microfabricated devices are capable of accessing the small length scales of biological cells and providing magnetic field gradients high enough for cell fractionation. Microfabrication was performed at the Princeton Center for Photonics and Optoelectronic Materials (POEM). The facility offers a full range of sophisticated processes and equipment for scientific research, which enables the fabrication of devices that could not have been made several years ago. The fabrication of our device required a series of steps to make it suitable for magnetic cell separation. First, the outer design was exposed onto a silicon wafer by photolithography. This included the channels, inlets, outlets, and central chamber, constituting the framework of the apparatus. Then, this design was etched 16 \( \mu \)m into the wafer and the remnant photoresist was stripped off. A separate pattern for the diagonal magnetic wires was exposed onto the central chamber, again by photolithography. Grooves for these wires were then etched an additional 0.2 \( \mu \)m so that the wires would be countersunk and not impede the flow of cells. A ferromagnetic metal alloy was deposited onto the wafer in a uniform coat. The unexposed photoresist was then removed from the wafer, lifting off the overlapping metal with it. This left behind only the thin wires on the wafer. Finally, a protective layer of SiO2 was deposited on top of all structures. Masks for positive imaging were made commercially by Adtek Photomask of Montreal, Quebec. Our device required two masks: one for the outer channels and outlets, and one for the magnetic wires. Each mask was a square...
quartz plate, 5" × 5" × 0.09". The masks were designed on L-Edit, a computer graphics program, and they were fabricated by electron beam lithography. A pair of alignment marks at opposite ends of each mask ensured that the patterns overlapped each other precisely.

To form magnetic wires, a cobalt-chrome-tantalum (Co-Cr-Ta) alloy was sputtered onto silicon wafers that had been exposed to the diagonal wire pattern. Co-Cr-Ta was used because of its high remanent magnetization. Co-Cr-Ta was chosen as a suitable alloy for our magnetic structures because it has an exceptionally high remanent magnetization of 6000 Gauss [16]. This should be compared to the saturation magnetization of its main component, cobalt (1400 Gauss) [15]. As an alloy, Co-Cr-Ta has the ability to acquire and retain a greater magnetic moment than each of its three components. Co-Cr-Ta occupies a hexagonal close packed (hcp) lattice structure, and, like most sputtered films, it is isotropic [17]. Additionally, as the wires in our device are very thin films, it is expected that they are single domain. Evidently the wires are capable of acquiring a large, uniform magnetization in an applied field and retaining it when removed from the field. As they are isotropic, they may be magnetized either in the plane of the wafer or perpendicular to it. Wires were magnetized perpendicular to the plane of the wafer, in a uniform external field. The magnetic field was provided by a large electromagnet and measured at 5 kG. It is believed that wires achieved their saturation magnetization in this field and retained a nearly uniform internal field of 6000 Gauss as the electromagnet was disabled. The strength of the magnetic remanence of the wires was tested using paramagnetic and latex beads. First, fluorescent paramagnetic beads were suspended above the wires. Beads were attracted by the wires in a striking fashion, accumulating at the edges where the field gradients were the highest. As a control, nonmagnetic polymer beads were then suspended. They disseminated uniformly, showing no preference for the magnetic wires.

4 Magnetic field gradients

Magnetic fields are produced by Co-Cr-Ta wires positioned at a 45° angle to the input stream of cells. Each wire is 10 μm wide and 0.2 μm thick, and neighboring wires are separated by 25 μm. The following calculations presume uniform magnetization of the wires, with a remanent field of 6000 Gauss. Wires may be magnetized in either of two directions: in the plane of the wafer or perpendicular to the wafer. The two orientations are shown in Fig. 4, with a consistent coordinate system. For neither method is there a force directed along the length of the wire; ∂B/∂z = 0 in each case. However, for both orientations, there are very large gradients in the magnetic fields near their edges. These gradients attract paramagnetic objects to the wires and redirect their flow through the chamber. The orientation that can provide higher fields gradients should be chosen for optimal results. Field gradients were also calculated analytically. Since cells travel at a fixed height y, above the wires, only ∂B/∂x is significant. Although gradients for both in-plane and perpendicularly magnetized wires are comparable at large y, gradients at lower heights are significantly greater for the case of perpendicular magnetization. Thus, in order to maximize the magnetic force for separation, wires were magnetized perpendicular to the plane of the wafer.

A bead that is not attached to a cell will roll along the floor of the chamber. The bead is separated from the wires by only a 0.2 μm layer of SiO2. Since its magnetic moment aligns with the field and Fm(μ·V)B, the bead will experience horizontal forces of up to 3 × 10⁻¹¹ N, or 3 × 10⁻⁶ dynes. A bead attached to a cell will feel a smaller force because it is elevated above the floor of the chamber. Lymphocytes are approximately 8 μm in diameter. Beads will therefore be, on average, 4 μm above the wires in the chamber. At this height, fields are 0.0045 Tesla and gradients are 1300 T/m. Consequently, beads will experience forces up to 1.5 × 10⁻¹³ N, or 1.5 × 10⁻⁶ dynes. For comparison, a constant force of 1.5 × 10⁻⁶ dynes will cause a leukocyte to reach a terminal velocity in water of 1 μm/s, a modest speed for a cell in a microchip. In reality, though, the forces on the cells will be considerably higher. Because the beads are so small, several thousand may bind to each cell. Also, many of these beads will be closer to the wires than 4 μm, where forces are significantly greater. Cells will experience the net effect of the magnetic forces on all beads bound to them. It is important to note that magnetic forces point in the x-direction, perpendicular to the wires. The magnetic force is greatest at the edges, and it is always attractive. Since the cells flow at a 45° angle to the wires, there is an x-component to the hydrodynamic force which should exactly cancel the magnetic force. All that is left is the z-component to the
hydrodynamic force (and viscous drag). As a result, the cells flow in the z-direction, along the lengths of the wires. Although they continue to be propelled by the input jet stream, they are constrained to follow the wires by the high field gradients at their edges.

5 Hydrodynamic considerations

In order for the deflected cells to be resolved, the input jet stream must be confined to a narrow width through the entire chamber. If the jet stream broadens out over time, magnetic separation becomes impossible. However, for a single jet that stands alone, this is exactly what happens. As soon as the jet enters the chamber, it experiences an abrupt change in environment. It slows down almost at once to the velocity of the surrounding fluid and, by conservation of mass, broadens immediately. Designed with this in mind, our device contains an alternative to the single input jet: the N-port injector [23]. The foundation for this idea can be derived from elementary fluid dynamics. In our device, fluid essentially flows in a sheet, where two dimensions are much larger than the third. This is a departure from normal fluid flow, as in pipes, where one dimension is dominant. Darnton [23] refers to this kind of flow as “2½-D hydrodynamics” because it is effectively two-dimensional but the third dimension cannot be ignored. Due to the large viscous force in our chip, boundary conditions become very important and can greatly affect the flow through the chamber. To solve for the fluid flow in our chip, one must consider the effects of the height $h$ of the chamber and, more specifically, the effects of the boundary conditions at both surfaces. It is here that Darnton’s “2½-D” approximation enters. By separating the variables of the velocity function, $v(x, y, z) = u(x, y) f(z)$, and choosing $f(z) = 1 - (2z/h)^2$, the Navier-Stokes equation becomes

$$\nabla^2 u(x, y) f(z) + \frac{2}{h^2} u(x, y) = \nabla P(x, y)$$

A scalar stream function $\Psi(x, y)$ is defined such that $u \equiv \nabla \times (\Psi \hat{z})$ [23]. As a result, we have

$$(f(z) \nabla^2 + \frac{2}{h^2}) \nabla^2 \Psi = 0$$

In the limit of small $h$, this is just the Laplace equation:

$$\nabla^2 \Psi = 0$$

From here, fluid flow is easy to solve for, as long as one sets the appropriate boundary conditions. In this derivation, the flow of cells appears to be passively subject to whatever boundary conditions happen to exist. This is the case for a single jet stream, where the much slower flow rate of fluid in the chamber causes the jet to broaden. Since boundary values explicitly determine the flow in an area, it would be beneficial to be able to control these conditions. Our design does just this. A wide array of channels feeds into the chamber on both sides of the input jet stream. The flux of fluid through these channels becomes the new boundary condition and can easily be adjusted. As a result, fluid flow remains uniform as cells enter the chamber, and the shearing forces on the jet are not as great. With this “N-port injector,” undeflected cells may be confined to a narrow jet, allowing deflected cells to be effectively separated.

6 Device interface

The apparatus consists of a series of channels and chambers etched into a silicon wafer (Fig. 5). Channels are sealed by a glass coverslip. Large hydrodynamic forces produced by a pressure gradient drive cells through the device, where they are collected in nine outlet portals. All structures in the apparatus are etched 16 μm deep, allowing ample room for cells to flow. Once cells are labeled, the sample is fed into the main input channel at a high hydrodynamic pressure. The input channel is 40 μm wide and feeds into a central rectangular chamber. Buffer flows through multiple channels on both sides of the input at an even higher pressure. This confines the sample to a narrow stream as it flows through the chamber. The central chamber (5 mm x 18 mm) contains the magnetic structures and is where magnetic separation occurs. Wires of a ferromagnetic material are countersunk into the floor of
the chamber at a 45° angle to the flow of cells. The wires are 10 μm wide and spaced 25 μm apart. The stripes were magnetized by placement in a 10 kG external magnetic field from an electromagnet. The high magnetic field gradient at the edge of each wire imposes a force on the superparamagnetic beads at an angle to the hydrodynamic force. The component of the magnetic force perpendicular to the flow causes the lateral deflection of all labeled cells. Cells are constrained to move along the magnetic wires by the large field gradients that exist at their edges. Figure 2 shows fluorescent images of magnetic beads. However, the undeflected cells must stay confined to a narrow stream across the length of the chamber in order for perpendicular deflections to be resolved. Channels are positioned at the sides and end of the chamber to capture cells as they exit. More than 350 channels, 24 μm wide, feed into nine outlets where cells are collected. All unlabeled cells will flow into the central outlet. In the outlets to the right, the fraction of labeled cells will be enriched. No cells should migrate to the left.

If cells can indeed be sorted by the high gradients in our device, rare cell types expressing known surface antigens can be isolated. Though its implications are the same as for fluorescence-activated cell separation (FACS) and MACS, our method comes with several unique advantages. First, it allows for the continuous input of cells. It therefore holds the promise of tolerating a large volume of cells with high efficiency. Second, its two-dimensional design allows simultaneous isolation of different cell types. By labeling different populations of cells with particles of differing magnetic susceptibilities, one could expect different degrees of lateral separation. Third, its small size would make it a cheap and convenient mechanism for separating cells. The central chamber (5 mm x 18 mm) contains the magnetic structures and is where magnetic separation occurs. Wires of a ferromagnetic material are countersunk into the floor of the chamber at a 45° angle to the flow of cells. The wires are 10 μm wide and spaced 25 μm apart. The high magnetic field gradient at the edge of each wire imposes a force on the superparamagnetic beads at an angle to the hydrodynamic force. The component of the magnetic force perpendicular to the flow causes the lateral deflection of all labeled cells. Cells are constrained to move along the magnetic wires by the large field gradients that exist at their edges. However, the undeflected cells must stay confined to a narrow stream across the length of the chamber in order for perpendicular deflections to be resolved. Channels are positioned at the sides and end of the chamber to capture cells as they exit. More than 350 channels, 24 μm wide, feed into nine outlets where cells are collected. All unlabeled cells will flow into the central outlet. In the outlets to the right, the fraction of labeled cells will be enriched. No cells should migrate to the left.

Once a chip is prepared with a coverslip, it is loaded onto a lucite chuck which mediated the flow of fluid (Fig. 6). The chuck was machined in the Princeton Physics Department machine shop. It is designed so that a high pressure could be applied to the fluid in all three inlets to drive it through the chip. Twelve tubes are drilled in the chuck so as to exactly line up with the holes in the chip. Fluid can enter the chip from the bottom, through the holes drilled at the inlets. It can then exit through one of the nine outlet holes in the chip, eventually filling up the tubes in the chuck that were exposed to atmospheric pressure. From there, the cells can be collected. The sealed chip is placed on top of the o-rings and held down by a rectangular steel frame. It is recommended that spring washers be used with the screws that hold down the frame so that the chip does not crack under stresses. However, controlling the flow through the input channels is not easy. Bubbles are a big obstacle to establishing hydrodynamic flow. Small bubbles that became trapped in the apparatus blocked off channels to flow. To work around this problem, the empty chuck was submerged in buffer before it was even assembled. The chip was then loaded onto the chuck, completely underwater.

Figure 6. Chuck assembly.
Additional problems came from loading the inlets with the syringe. The right angles in the input tubes turned out to be an awkward design for filling them. When bubbles are encountered, vacuum pumping of the chip under buffer can often liberate them. Otherwise, the chuck can be soaked overnight to let the bubbles gradually disappear.

7 A preliminary blood cell run

Since this is really a progress report on the development of a technology as we develop this idea it is unfortunate that we cannot present at this time pictures of cells labeled with magnetic beads separating in the device. However, we have been able to show at this point that whole blood samples can be run in the device and that ultra narrow jets of cells can be run across the entire 2 cm length of the chip with precision, and that white blood cell adhesion to the device walls can be controlled. White blood cell adhesion is a major problem because of the high concentration of proteins presented at the cell surface, particularly white blood cells. Our chip was fabricated out of silicon and has approximataly a 0.2 µm thick silicon dioxide overcoat. However, silicon dioxide surfaces are highly charged and strongly bind cells to the surface. We have been exploring ways to avoid cell adhesion, and one of the most promising materials is the tri-block copolymer polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO), also known as Pluronics under the manufacture of BASF. A good reference on the use of Pluronic surfactants to prevent the adhesion of cells to surfaces can be found in the papers of Karin Caldwell [27]. The great advantage of the Pluronic system is that the hydrophobic center bloc (PPO) can bind to hydrophobic surfaces of silicone elastomers.
(polydimethylsiloxane, PDMS) while the end group polyethylene oxide (also commonly known as polyethylene glycol, PEG) is a very unusual polymer that is neutral but very hydrophilic and strongly hydrogen bonding. If the PPO end groups and the center PPO group are chosen to be of the appropriate molecular weights, a very robust surface on a virgin PDMS surface can be created which does not bind proteins due to a combination of the shielding of the hydrophobic surface of PDMS by the Pluronics and an entropic repulsion of the surface PPO groups of the protein.

In our case, we used a 0.1% solution of Pluronic F108 from BASF (BASF Corporation, Mount Olive, NJ, USA) in a saline buffer to wet our magnetic array wafer that was sealed with a cover slip upon which a thin layer of PDMS had been spun and polymerized. Although PDMS is very hydrophobic and normally water will not penetrate a sealed PDMS structure, a saline buffer solution which contains 0.5% F108 will over a period of 24 h penetrate and wet a sealed PDMS structure. This then provides a very interesting surface which is hydrophilic, uncharged and not “biofouling”. Once the array was successfully wet and the surface passivated a 20 μL sample of blood from a finger prick was put into Becton Dickinson Microtainer tube treated with lithium heparin to prevent clotting of the blood (Becton Dickinson, Franklin Lakes, NJ, USA). The white blood cells in the blood sample were then stained with the vital nuclear stain Hoechst 33342 (Molecular Probes, Eugene, OR, USA) by incubation at 37°C for 30 min. The sample of stained blood was then loaded in the center chamber and positive air pressure of approximately 0.2 psi where applied to the center jet and the side fluid curtain flow.

Figure 11 shows in epi bright field illumination the blood sample at roughly ×40 magnification leading up to the injection jet. Figure 12 shows in epifluorescence the same blood sample only now the labeled white blood cells are visible. Figure 13 shows the stream of blood cells

![Figure 11. Brightfield image of blood at entrance to the array.](image1)

![Figure 12. Epifluorescence image of labeled white blood cells at entrance to the array.](image2)

![Figure 13. Brightfield image of blood cells flowing (a) from injection port and (b) in the center of the array about 1 cm in from the injection port.](image3)

![Figure 14. Epifluorescence image of two white blood cells in Fig. 13b.](image4)
flowing from right to left into the magnetic line array, illustrating how the hydrodynamics ensures a smooth non-expanding flow into a large open area, both at the entrance (a) and well within the array (b). No evidence of red blood cell adhesion to the device was seen. Figure 14 shows an image of two labeled white blood cells constrained within the red blood cell stream seen in Fig. 13b, demonstrating how the white cells move smoothly with the stream of red cells and show very little adhesion to the surface. Clearly, the next step now is to label these cells with paramagnetic antibody beads and deflect them from the main stream, but alas due to the deadline constraints of this paper that project remains to be done in the next several months.

8 Looking ahead

Progress has been made in constructing a magnetic cell separation device. However, its potential to fractionate cells remains unfulfilled. Several procedures still need to be optimized, and several experiments still need to be run. In this section, a few main areas are highlighted. It is important to determine how to optimize the hydrodynamic forces in scale with the magnetic forces produced by the wires. Magnetic separation of beads must be demonstrated. If the hydrodynamic forces are so strong that they overshadow magnetic deflections, the pressure gradient must be diminished in a way that does not compromise the jet width. If instead the beads adhere to the wires, pressures may need to be increased. The electrostatic adhesion of beads to wires is a concern, in which case the SiO$_2$ layer on top of the wires should be made thicker. At all points along the way, careful control experiments need to be conducted. Paramagnetic beads can be run alongside latex beads, preferably of two different colors. Also, it will be important to have more confidence in handling cells. Labeling cells is crucial for achieving magnetic fractionation. Experimentation with different stains and different markers will likely determine a good test sample for the apparatus. Further, multiple markers and fluorescent stains will be necessary to conduct control experiments. Surface passivation measures must also be optimized in order to prevent the adhesion of cells to silicon structures.

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9 References

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