Published in final edited form as:

Lab Chip. 2012 April 24; 12(10): 1793–1799. doi:10.1039/c2lc21189k.

Spinning magnetic trap for automated microfluidic assay systems†

Jasenka Verburg, Kian Kamgar-Parsi, Adam R. Shields, Peter B. Howell Jr., and Frances S. Ligler*

Naval Research Laboratory, 4555 Overlook Avenue, SW, Washington, DC, 20375, USA.

Abstract

While sophisticated analyses have been performed using lab-on-chip devices, in most cases the sample preparation is still performed off chip. The global need for easy-to-use, disposable testing devices necessitates that sample processing is automated and that transport complexity between the processing and analytical components is minimal. We describe a complete sample manipulation unit for performing automated target capture, efficient mixing with reagents, and controlled target release in a microfluidic channel, using an array of spinning magnets. The “MagTrap” device consists of 6 pairs of magnets in a rotating wheel, situated immediately beneath the microchannel. Rotation of the wheel in the direction opposite to the continuous flow entraps and concentrates the bead-target complexes and separates them from the original sample matrix. As the wheel rotates and the active pair of magnets moves away from the microchannel, the beads are released and briefly flow downstream before being trapped and pulled upstream by the next pair of magnets. This dynamic and continuous movement of the beads ensures that the full surface area of each bead is exposed to reagents and prevents aggregation. The release of the target-bead complexes for further analysis is facilitated by reversing the rotational direction of the wheel to sweep the beads downstream. Sample processing with the MagTrap was demonstrated for the detection of *E. coli* in a range of concentrations (1×10^3 , 1×10^4 and 1×10^6 cells ml^{-1}). Results show that sample processing with the MagTrap outperformed the standard manual protocols, improving the detection capability while simultaneously reducing the processing time.

Introduction

There are two main reasons that “lab-on-a-chip” devices have remained “chip-in-a-lab” components. One is that the equipment used to operate or interrogate the chips is large and often expensive. The second is that sample processing prior to introduction into the chip remains to be automated. We have developed a technology that can address this latter problem for many lab-on-a-chip analytical systems. Critical to this approach is the ability to perform the following operations on chip: (1) preconcentrate the target out of variable volumes of complex sample matrices, (2) expose the target to sequentially-added reagents under conditions that maximize the binding kinetics, and (3) deliver the processed target in a concentrated form into the interrogation region of the microfluidic chip.

The ultimate goal of most lab-on-a-chip (LOC) sensors is the detection of targets at ultra-low concentrations and/or in minimal sample quantities, using a portable and disposable

†Electronic supplementary information (ESI) available: Video of capture and release of magnetic beads in a microfluidic channel *via* rotating magnets. Video of simulated magnets, in parallel and antiparallel orientations, rotating over a line, with the corresponding graphs of the magnetic flux density gradients. See DOI: 10.1039/c2lc21189k

© The Royal Society of Chemistry 2012

*frances.ligler@nrl.navy.mil; Fax: +1 202 404 8897; Tel: +1 202 404 6002 .

device. Reductions in incubation times, reagent quantities, and skill required for operation are widely recognized advantages of analytical methods based on microfluidics.

One example of a microfluidic analyzer is the microflow cytometer (MFC), which is capable of detecting bacteria in levels as low as 10^3 cfu ml⁻¹ and toxins at levels of nanograms to picograms per millilitre.¹ The MFC utilizes fluorescently coded beads for sample processing and multiplexing. Multiplexed assays on beads have been used for detection of a wide variety of targets in conventional flow cytometers.^{2,3} In the MFC, a stream containing the beads is focused by a sheath stream to a diameter sufficiently small that each bead passes individually through the laser beam used for interrogation. However, while the on-chip detection technology has proven effective, to date all samples have been processed manually prior to introduction into the cytometer.³⁻⁵

In order to achieve on-chip sample processing, the targets must be captured on the coded beads and retained during exposure to the reagents required to produce the signal. Multiple groups have worked with on-chip magnetic particle trapping; several of these have employed magnets to control movement of magnetic beads with respect to flow.⁶⁻¹⁰ In most cases, an external, stationary magnet is brought near the chip to entrap the beads against the wall of the channel while the reagents are being added. Similarly, in suspension-based assays, the relatively dense magnetic beads rapidly settle to the bottom of the container where they may aggregate or contact the container walls. Beads treated in either fashion may not be uniformly exposed to the reagents and suffer from a reduced molecular binding.

The use of beads for sample processing builds on the well established technique of immunomagnetic separation, which dates back to the 1980s^{11,12} and is usually conducted with target binding to the magnetic beads in suspension. The utility of magnetic beads has been reported for immunoseparations in microfluidic channels as well.¹³⁻¹⁹ One method is the movement of target from one region of the microfluidic chip to another, which can be accomplished with a rotary magnet that moves target between different wells on the chip.²⁰ For example, Yager's group reported on a device where two fluids (preincubated magnetic bead sample and buffer) flow side-by-side in the same microchannel. As the beads traverse the channel, a stationary block magnet pulls aggregated magnetic beads (complexed to multivalent targets) from the sample stream to the buffer, separating them from non-aggregated beads.¹⁴

Similarly, Pamme's group developed a free-flow magnetophoresis device with multiple inlet and outlet channels and a common central chamber. A single channel was used to flow in the sample, while an orthogonally placed magnet was used to drive the lateral movement of magnetic particles as they flow down the chamber. The result was a size- and/or magnetic susceptibility-dependent separation of beads into different outlet channels. The free-flow layout has been used for the separation of both magnetic beads and magnetic nanoparticle-labeled cells, as well as the separation of nonmagnetic particles *via* diamagnetic repulsion.²¹⁻²³

Magnetic beads concentrated in a microchannel using a fixed magnet exhibit several problems: (1) They aggregate, which may confound subsequent analysis; (2) Bead surfaces are not uniformly exposed to reagents if the beads are concentrated on the channel wall;²⁴ (3) Bead clusters may alter or even occlude flow in the microchannel. Ramadan and Gijs alternately create regions along the side of the microchannel that sequentially trap and release beads. While this solution is quite creative, it requires a very long channel and complex magnet assemblies.²⁵ A solution lies in the ability to trap the beads while maintaining dynamic motion in a continuously flowing stream

The spinning magnetic trap, or “MagTrap”, reported in this work combines the advantages of immunomagnetic target capture with the ability to dynamically manipulate magnetic beads inside a microfluidic channel. Multiple permanent magnets are arranged on a rotating wheel placed directly beneath the channel. The passage of each magnet concentrates the immunomagnetic beads from a sample stream, without aggregation, and moves them both against the flow and from one side of the channel to the other (Fig. 1). When the leading magnet rotates away from the channel, the trapped beads are briefly released and then trapped by the next magnet that rotates into close proximity of the beads. Reversal of the wheel’s rotation sweeps the beads downstream by the magnets. When the magnets move away from the channel at the downstream end, the particles are free to exit the device. The reversible rotation technique allows particles to be captured, mixed, and released using permanent magnets that are always in contact with the channel.

Experimental

Materials

Reagents, including bovine serum albumin (BSA), phosphate buffered saline with 0.1% Tween-20 (PBST) with 0.1% sodium azide, sodium phosphate buffer, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (Sulfo-NHS), dimethylsulfoxide (DMSO) and methanol (MeOH) were obtained from Sigma Aldrich (St. Louis, MO, USA). Fluorescent, superparamagnetic, carboxylated beads, approximately 6.5 μm in diameter, were purchased from Luminex Corporation (MagPlex™-C microspheres, Austin, TX, USA). *E. coli* 0157.H7 and α -*E. coli* IgG were obtained from KPL (Gaithersburg, MD, USA), chicken IgY and biotinylated α -chicken IgY were from Jackson ImmunoResearch Labs, (West Grove, PA, USA). Streptavidin phycoerythrin (SAPE) was purchased from Prozyme (San Leandro, CA).

The magnets used in the trapping device were neodymium iron boron (NeFeB, K&J Magnetics, Jamison, PA) in three geometrical shapes: axially magnetized cylinders, 19 mm in length and 6.35 mm in diameter with a surface field of 6510 gauss; equilateral triangles with 19 mm sides, magnetized through their 3.18 mm thickness with a surface field of 2400 gauss; and strips, 19 mm long, 1.6 mm wide, and magnetized through their 6.35 mm thickness with a surface field of 2060 gauss.

Microchannel embossing and bonding

The microchannel was hot-embossed into poly-(methylmethacrylate) (PMMA, Goodfellow Cambridge Limited, Huntingdon, England) using an aluminum mold. The mold consisted of a straight 6 cm channel with a trapezoidal cross section. The microchannel structure was hot embossed in a 7.5 cm $\times$ 2.5 cm piece of 250 μm -thick PMMA, sandwiched between the aluminum mold and a 10 cm $\times$ 10 cm 0.3 cm piece of glass. The press was heated to 160 $^{\circ}\text{C}$ and, once the temperature was reached, 2.6 kN was applied on the top platen using a DAP19 pneumatic press (Air-Mite Devices, Inc., Chicago, IL). The temperature was held for 10 min and then lowered to 50 $^{\circ}\text{C}$ while maintaining the pressure on the platen. Finally, the PMMA/mold assembly was removed from the press and the resulting embossed channel shape was visually evaluated for imperfections. The height of the embossed channel was 125 μm , with widths of 500 μm at the top and 355 μm at the bottom. The thin channel bottom minimizes the distance between the magnets and microfluidic channel.

In order to provide strength for ease of handling and maintaining device integrity, the embossed PMMA chip was bonded to a 3.2 mm-thick PMMA chip, having holes for the inlet and outlet ports. The bonding procedure began with a 15-minute sonication of the two pieces in an ethanol bath, followed by drying with pressurized air. Then, 200 μl of the

solvent (47.5% DMSO, 47.5% ddH₂O and 5% MeOH) was dispensed onto the channel substrate and the thick PMMA chip was positioned against it, with the inlet and outlet holes aligned over the channel. The assembly was placed into a manual press, consisting of three aluminium platens, stacked on top of each other and held in place by four screw posts. The two interfaced chips were between the bottom and middle platen, while two springs (2 cm, relaxed) were inserted between the middle and top platens. For the first 30 min, the press was used with only the gravitational force. Then, the top platen was lowered by 12 mm, compressing the springs and applying a force of 165 N. The assembly was placed in a heated chamber at 90 °C for one hour, after which the heat was turned off and the system was allowed to cool for one hour. Finally, the bonding was visually evaluated, and the remainder of bonding solution was removed from the microchannel.

Magnetic trap design analysis and selection

Three magnet designs were evaluated for the MagTrap. As shown in Fig. 2, all three designs consisted of 6 discrete magnetic regions arranged around a common center in a PMMA wheel, 5 cm in diameter. The three designs differed in the size and shape of the magnets, as well as the distance between neighboring magnets. The NeFeB magnets were rod-, strip-, and triangleshaped. The selection of the three magnet geometries was based on small, commercially available magnets, which could be integrated into a rotor placed outside the microchannel and project the field into the channel. Size, field strength, and future miniaturization were major considerations. A custom holder for each set of magnets was machined and mounted on a rotator with translational stages that controlled the distance of the magnets from the microchannel. The poles of the magnets were perpendicular to the plane of the holder.

In addition to magnet shapes, two pole orientations were evaluated for each design: 1) parallel (where all the magnetic poles were oriented in the same direction), and 2) anti-parallel (where each magnet's pole orientation was opposite from that of its neighbors). As discussed later, the final design selected for the optimal capture and release of the beads included the strip magnets having parallel polarities.

Capture and release of beads

In the presence of a magnetizing field, superparamagnetic beads are subjected to a magnetic force, which is proportional to the gradient of the square of the magnetic field strength. In order to trap magnetic beads in the presence of a fluid flow, the magnetic force must be sufficiently strong to balance the drag force exerted by the fluid. The effectiveness of each magnetic trap design was evaluated by quantifying capture and subsequent release of the magnetic beads with a continuous fluid flow through the channel. The only degree of freedom available to the magnets was rotation. Bead solutions were pumped through the microchannel using a P625 peristaltic pump with a 300 μm ID silicone tubing (Instech Laboratories, Plymouth Meeting, PA, USA) at a concentration of 50 beads μl^{-1} and at a flow rate of 10 $\mu\text{l min}^{-1}$.

Magnets were mounted on a house-built stage. Rotation was provided by a stepper motor *via* a set of gears with a 12 : 1 ratio. The gear ratio coupled with the use of microstepping of the motor prevented significant pulsing in the rotation. The motor controller was built using an Arduino board and motor shield (Adafruit Industries, NY, NY), and provided a dial to set rotation rate and a switch to set direction. The controller software was configured such that turning the dial did not have an effect on the rotation rate until the motor power was cycled or the direction was changed. This prevented accidental changes in rotation rate during an experimental run. In future multiplexed assays, the bead concentration could easily be raised by a factor of 10.

The flow rate, magnetic strength, rotation rate, and magnetic susceptibility of the beads are important interactive values, critical for the function of the MagTrap. As the MagTrap is adapted for different applications, the values of these parameters will be changed. We established the values for flow rate, bead characteristics and concentration, magnetic strength, and rotation rate based on our intent to integrate the MagTrap with our microflow cytometer. The bead concentration and flow rate are those currently used by the microflow cytometer. The rotation rate was selected so as to minimize additional drag on the beads during the capture phase. This was important in order to use the smallest, weakest magnets possible.

As previously described in a proof of principle experiment by Memisevic *et al.*,²⁶ capture was achieved by rotating the magnets in the direction opposite to the flow (Fig. 1). In order to release the beads, the direction of rotation was reversed so that the magnets pulled the beads in the same direction as the flow, sweeping the beads toward the outlet.²⁷

During each stage of the experiments, effluents from the channel were collected in 3- or 5-minute time intervals. Capture efficiency (CE) was calculated as a fraction of the number of beads captured, compared to the number of beads passing through the microchannel in the allotted time in the absence of magnetic trapping,

$$CE = \left(1 - \frac{N_E}{N_0}\right) \times 100\%$$

where N_E was the number of beads that escaped the MagTrap during capture and N_0 was the number of beads that would have been collected with effluent in the same length of time and in the absence of magnets.

Release efficiency (RE) was the ratio of beads released after the reversal of the magnets' rotation compared to the number of beads captured.

$$RE = \left(\frac{N_R}{N_C}\right) \times 100\%$$

where N_R was the number of beads collected during magnetic release and N_C was the number of beads captured by MagTrap.

Beads were counted after collection from the channel using an Accuri C6 flow cytometer (Accuri Cytometers Inc., Ann Arbor, MI, USA), at a $66 \mu\text{l min}^{-1}$ flow rate and having a $22 \mu\text{m}$ core diameter.

COMSOL modeling

A simulation of the strip magnet trap design was created using the electromagnetism module in the finite element modeling software COMSOL Multiphysics (COMSOL, Inc., Burlington, MA, USA). The magnets were assigned characteristics according to the manufacturer's specifications: a remnant magnetization of 2060 gauss and relative permeability of 1.05. The model was solved for the magnetic flux density B , in gauss, for both the parallel and anti-parallel orientations of the magnetic poles, using an adaptive solver to optimize the mesh.

Post-processing of solved models was performed with MATLAB (Math Works, Inc., Natick, MA, USA). Using flux density data exported from COMSOL, a simple MATLAB function

was written to calculate field gradients in the space around the magnetic trap. Additionally, the magnetic gradient magnitude along the channel was evaluated as a function of position of incrementally rotating magnets over a line, which represented a microchannel. The magnetic field gradient attenuation at different distances from the trap was also calculated from the simulation data.

Bead preparation for assay utilization

Antibodies were attached to the magnetic beads using the protocol described by Taitt *et al.*²⁸ Briefly, the beads were suspended in 0.1 M sodium phosphate buffer (pH 6.2) to activate the surface carboxyl groups. Then, the EDC linker was used in presence of sulfo-NHS to achieve an amine-rich surface, ready for attachment of α -*E. coli*, α -chicken IgY and BSA.

The beads are manufactured with an internal dye code and grouped into sets for multiplexing. Triplex assays were designed to use three bead sets to detect *E. coli*, with chicken IgY acting as the positive control and BSA as the negative control. Luminex 100 and MagPix cytometers (Luminex Corporation, Austin, TX, USA) provided the fluorescence and numerical analysis of magnetic beads used in assays.²⁹

Sequential microfluidic assay processing

Following a 20-minute incubation with *E. coli* at 1×10^3 , 1×10^4 , or 1×10^6 cells ml^{-1} , solutions of 50 beads μl^{-1} were pumped into the microchannel at a rate of $10 \mu\text{l min}^{-1}$ for bead concentration, separation from the original sample, and reagent addition. Once the beads were captured by the magnets, the sequential addition of assay reagents ensued at the same $10 \mu\text{l min}^{-1}$ flow rate. The biotinylated tracer reagents, including $10 \mu\text{g ml}^{-1}$ α -*E. coli* and 750 ng ml^{-1} chicken IgY, were added for five minutes. Then, a 30-second wash with PBST containing 1 mg ml^{-1} BSA (PBSTB) was followed by a 3-minute addition of the fluorescent reporter, SAPE at $7.5 \mu\text{g ml}^{-1}$. A final 5-minute wash with PBSTB removed any unbound SAPE.

Upon completion of the microfluidic assay on the beads that were captured in the microchannel, the rotation of magnets was reversed to release the beads through the outlet of the microchannel. The strip magnets with parallel polarities were used in the MagTrap sample processing experiments.

Static control assays

Performance of the microfluidic assay was compared to two controls: short and long “static assays.” While all three methods followed the same sequence of steps and had the same reagent concentrations, the static assays were carried out in microcentrifuge tubes in contrast to a microchannel. Stationary magnets were used to move the beads to one side of the tube for removal and replacement of solutions.

The long static assay was similar to the protocol provided by Luminex³⁰ and involved 30-minute incubations of functionalized beads, having a concentration of 50 beads μl^{-1} , with the analyte, the tracer antibodies, and finally with SAPE, in $100 \mu\text{l}$ volumes. On the other hand, the short, static assay had the same incubation times as the microfluidic assay. A 20-minute incubation of capture beads with the analyte suspension was followed by a 5-minute incubation with $50 \mu\text{l}$ of the tracer antibodies and a 3-minute incubation with $30 \mu\text{l}$ of SAPE. In both static assays, suspensions of beads in the newly added reagents were moved away from the magnets, mixed once after each reagent addition, and washed with PBSTB after each incubation step.

Results and discussion

Design of the MagTrap: Magnetic capture and release of beads

Straight, trapezoidal microchannels were utilized to evaluate the capture and release efficiencies of three magnetic trap devices. The positioning of the narrow side of the trapezoid with the obtuse angles closest to the magnets helped to prevent trapping of beads in the corners of the channel. The three MagTrap devices differed in the shape and size of the magnets. The shapes of magnets were rods, triangles, and strips, as depicted in Fig. 2, and each was evaluated with pairs of magnets having parallel and anti-parallel magnetic polarities.

The capture and release efficiencies of each magnet configuration were calculated based on their effect on superparamagnetic beads that flowed through the microchannel. The capture and release efficiencies, as defined above, are presented in Table 1 for each magnet geometry and pole orientation.

For the rod and triangle magnets, there was not a clear distinction in capture and release efficiencies between the parallel and anti-parallel magnets. Both sets of magnets captured the beads with reasonable efficiencies. However, for these designs the large majority of beads remained trapped in the microchannel when the direction of rotation was reversed. In order to release beads with these designs, it was necessary to completely remove the magnets from any proximity to the microchannel.

In the case of strip magnets, differences were observed between the two pole orientations. While both parallel and anti-parallel orientations of strip magnets were successful at capturing beads, the parallel magnets released the beads more readily upon reversal of rotation. Therefore, subsequent studies were performed using the strip magnets with the parallel pole orientation. A video of the capture and release of magnetic beads with the strip magnets can be viewed in ESI Video 1, where the movement and position of the beads is shown. During release, the magnets move the beads to the edge of the microchannel, along which they flow due to the drag force until the next magnet arrives.

It is clear that the drastic difference in the ability to release the trapped beads between the rod and triangle designs and the strip magnet designs is attributed to the separation distance between the magnets in a given design. For the relatively large rod and triangle magnets, there is very little space between adjacent magnets, such that the trapped beads do not have sufficient time to escape the trap when the device is operated in release mode.

As shown in ESI Video 2 (MATLAB simulation), for the strip magnets the magnetic field gradient is present only near the magnets and decreases to zero between the magnets. Therefore when the rotation of the magnets is reversed, they move the beads towards the outlet of the channel, where they can escape due to the large separation between the magnet pairs in that region.

COMSOL simulations

Further analysis of the differences between the magnetic fields generated by the parallel and anti-parallel strip magnets was done with COMSOL. Post-processing and visualization data, shown in Fig. 3, was performed at a distance of 125 μm (z-distance) from the top of the magnets' surfaces, corresponding to the bottom surface of the microchannel.

At this distance from the magnets, the magnitude of both the flux density and the magnetic gradient are larger for the antiparallel orientation than the parallel orientation. Thus, for the parallel orientation of the strip magnets the correspondingly smaller magnetic force allows

easier release of the beads upon reversal of trap rotation, with the weaker gradients still being strong enough to effectively trap beads during capture. While the magnets continually pull beads upstream during the capture phase, there is a period during the release phase where the flux density gradient at the end of the channel rapidly approaches zero (Fig. 3). It is at this time when the beads are released from the outlet of the channel.

Assay performance

The efficiency of processing bead-based immunoassays for detection of *E. coli* using the MagTrap was examined utilizing the strip magnet design with parallel polarities. The assay processing consisted of binding the *E. coli* analyte to antibodycoated, superparamagnetic, fluorescently-coded beads, and subsequently introducing the beads into the microchannel. The rotating magnetic trap captured the beads and dynamically retained them in the microchannel. While beads were trapped, the sample matrix was removed and assay reagents were added to the channel in succession.

In addition to the processing of beads for the *E. coli* assay, two bead sets were included as positive and negative controls: beads coated with α -chicken IgY or BSA, respectively. The results using the MagTrap were compared to those from beads processed manually in microcentrifuge tubes using incubation times specified for the conventional bead-based assays by Luminex (long static control) and times equivalent to those used in the spinning magnetic trap (short static control).

The signal generated for the detection of *E. coli* was obtained for each of the three processing procedures at a range of concentrations. Fig. 4 shows normalized median fluorescence (NMF) as the concentration of *E. coli* increased from 1×10^3 to 1×10^6 cells ml^{-1} . NMF was calculated using the positive control (chicken IgY) and negative control (BSA) as the 1 and 0 values, respectively. While results show that all three processes were capable of detecting a high concentration of *E. coli*, the MagTrap processing method produced a higher signal-to-background ratio at concentrations near the limit of detection reported previously.¹

Importantly, these results demonstrate that a dynamic, automated sample processing device can provide increased sensitivity, while simultaneously decreasing the time required to process the sample. The superior performance of the MagTrap over control assays is attributed to the unique feature of the dynamic and continuous bead movement during processing. As seen in ESI Video 1, the magnets' rotation not only entraps the magnetic beads as they flow through the microchannel, but also moves them in both the transverse and longitudinal directions within the channel, thus increasing the mixing during the incubation stages. Mixing of beads maximally exposes their surfaces to solution that has not been depleted of reagent, thus providing a higher binding efficiency. In contrast, the static assay allows the beads to fall to the bottom of the tube and stack on top of each other, which greatly reduces exposed surface area available for binding. Furthermore, depletion zones along the surface of a channel or tube can reduce the interaction of the reagents with the bead surface if the beads settle or are pulled against a wall.³¹

The two static control assays were designed to emphasize the importance of dynamic and continuous mixing in sample processing. Namely, the long static assay is the standard in conventional performance, with the long incubation times (30 min each) ensuring time for the reactions between the beads and reagents to reach completion. On the other hand, the short static assay utilized the reduced incubation times (20 min for initial analyte capture, 5 min for tracer antibodies, and 3 min for SAPE incubation). Ultimately, neither method was capable of achieving the signal levels demonstrated by the MagTrap. In the MagTrap experiments, the initial analyte capture was performed outside the microfluidic channel on

the assumption that in a real assay, variable amounts of sample would be collected in a tube containing beads. Additionally, the incubation time could be adjusted to optimize the initial binding for the particular affinity of the capture agent and viscosity of the sample.

It is also worth noting that the ability of the MagTrap to concentrate samples means that this processing method is easily transferrable to detection of samples in highly diluted matrices. The initial introduction of the beads with captured analyte can concentrate the beads from any volume and remove the sample matrix. From that point on, most reagents and steps to be used in the MagTrap assays should be approximately equivalent, independent of the actual targets and samples of interest.

Conclusions

Efforts to achieve lab-on-chip analytical systems are hindered by the need for off-chip sample preparation and processing. The MagTrap introduces a method of on-chip processing of the sample for analysis. The concentration of target was achieved by utilizing specifically modified magnetic beads for target capture and manipulating the movement of those beads in a microfluidic channel. An external, rotating magnetic field was strategically designed for capture, concentration, and separation of the beads from the sample, mixing of the beads with successively added reagents, and release of the concentrated beads from the channel. Results demonstrated increased efficiency of reagent binding and decreased processing time. In addition, performing the reagent processing in a microfluidic channel reduced the quantity of reagents required, thus reducing cost. Future work will focus on further miniaturization of the spinning trap and integration with sensors such as the microflow cytometer. The MagTrap is appropriate for any analytical device that involves the capture of target, exposure to multiple reagents, and the release of processed target into an analytical instrument. The integration with the microflow cytometer is particularly appropriate because not only can the analysis be performed with target captured and processed on magnetic beads, but also the effluent from the MagTrap can be directly introduced into the microflow cytometer as the core stream.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

J. Verbarg is a National Research Council postdoctoral associate. A. R. Shields is a National Research Council postdoctoral associate supported by the Defense Threat Reduction Agency. This work was supported by ONR 6.2 work unit 6336 and NIH grant UO1 A1075489. The views are those of the authors and do not represent opinion or policy of the US Navy, Department of Defense, National Institutes of Health or Department of Health and Human Services. Authors would also like to thank colleagues Nastaran Hashemi, Jeff Erickson, Kirsten Jackson, Joel Golden and Lisa Shriver-Lake and University of Maryland collaborators Ben Shapiro and Alek Nacev for their assistance and support with this project.

Notes and references

1. Kim JS, Anderson GP, Erickson JS, Golden JP, Nasir M, Ligler FS. Anal. Chem. 2009; 81:5426–5432. [PubMed: 19496600]
2. Bjerre M, Hansen TK, Flyvbjerg A, Tonnesen E. Vet. Immunol. Immunopathol. 2009; 130:53–58.
3. Kim J, Taitt C, Ligler F, Anderson G. Sens. Instrum. Food Qual. Saf. 2010; 4:73–81. [PubMed: 20953301]
4. Thangawng AL, Kim JS, Golden JP, Anderson GP, Robertson KL, Low V, Ligler FS. Anal. Bioanal. Chem. 2010; 398:1871–1881. [PubMed: 20658281]

5. Golden JP, Kim JS, Erickson JS, Hilliard LR, Howell PB, Anderson GP, Nasir M, Ligler FS. *Lab Chip*. 2009; 9:1942–1950. [PubMed: 19532970]
6. Pamme N. *Lab Chip*. 2007; 7:1644–1659. [PubMed: 18030382]
7. Chandler DP, Brown J, Call DR, Wunschel S, Grate JW, Holman DA, Olson L, Stottlemire MS, Bruckner-Lea CJ. *Int. J. Food Microbiol.* 2001; 70:143–154. [PubMed: 11759752]
8. Straub TM, Dockendorff BP, Quinonez-Diaz MD, Valdez CO, Shutthanandan JI, Tarasevich BJ, Grate JW, Bruckner-Lea CJ. *J. Microbiol. Methods*. 2005; 62:303–316. [PubMed: 15979746]
9. Gassner A-L, Abonnenc M, Chen H-X, Morandini J, Josserand J, Rossier JS, Busnel J-M, Girault HH. *Lab Chip*. 2009; 9:2356–2363. [PubMed: 19636467]
10. Teste B, Malloggi F, Gassner A-L, Georgelin T, Siaugue J-M, Varenne A, Girault H, Descroix S. *Lab Chip*. 2011; 11:833–840. [PubMed: 21253647]
11. Lund A, Hellemann AL, Vartdal F. *J Clin Microbiol.* 1988; 26:2572–2575. [PubMed: 3068250]
12. Jacobsen CN, Fremming C, Jakobsen M. *J. Microbiol. Methods*. 1997; 31:75–81.
13. Guan XA, Bi YN, Zhang L, Hao DL, Zhang HJ. *Biomed. Microdevices*. 2010; 12:683–691. [PubMed: 20300854]
14. Lai JJ, Nelson KE, Nash MA, Hoffman AS, Yager P, Stayton PS. *Lab Chip*. 2009; 9:1997–2002. [PubMed: 19568666]
15. Peyman SA, Iles A, Pamme N. *Lab Chip*. 2009; 9:3110–3117. [PubMed: 19823727]
16. Anderson NL, Jackson A, Smith D, Hardie D, Borchers C, Pearson TW. *Mol. Cell. Proteomics*. 2009; 8:995–1005. [PubMed: 19196707]
17. Corchero J, Villaverde A. *Trends Biotechnol.* 2009; 27:468–476. [PubMed: 19564057]
18. Kim KS, Park JK. *Lab Chip*. 2005; 5:657–664. [PubMed: 15915258]
19. Teste B, Malloggi F, Siaugue J-M, Varenne A, Kanoufi F, Descroix S. *Lab Chip*. 2011; 11:4207–4213. [PubMed: 22033539]
20. Shikida M, et al. *J. Micromech. Microeng.* 2008; 18:035034.
21. Pamme N, Wilhelm C. *Lab Chip*. 2006; 6:974–980. [PubMed: 16874365]
22. Tarn MD, Hirota N, Iles A, Pamme N. *Sci Technol Adv Mat.* 2009;10.
23. Manz A, Pamme N. *Anal. Chem.* 2004; 76:7250–7256. [PubMed: 15595866]
24. Lin C-C, Hsu J-L, Lee G-B. *Microfluid. Nanofluid.* 2011; 10:481–511.
25. Ramadan Q, Gijs MAM. *Analyst*. 2011; 136:1157–1166. [PubMed: 21270982]
26. Memisevic, J.; Shriver-Lake, LC.; Howell, PB.; Golden, JP.; Hashemi, N.; Jackson, KB.; Ligler, FS. *BioPhotonics*. 2011 International Workshop on, IEEE; 2011. p. 1-3.
27. United States Pat US 2011/0188339. 2011. p. A1
28. Taitt, CR.; Shriver-Lake, LC.; Anderson, GP.; Ligler, FS. *Biomedical Nanotechnology*. Hurst, SJJ., editor. Vol. vol. 726. Humana Press; 2011. p. 77-94.
29. Corporation, L. *xMAP Technology Technical Note*. 2010.
30. Corporation, L. *Technical Notes*. Luminex Corporation; 2010.
31. Lund-Olesen T, Dufva M, Hansen MF. *J. Magn. Magn. Mater.* 2007; 311:396–400.

Fig. 1. Schematic of rotating MagTrap as it is capturing magnetic beads in a microchannel. Curved arrows show rotational direction of magnets during capture and release, and the straight arrow depicts the direction of the flow.

Fig. 2.
Three MagTrap designs: rods (left), triangles (center), and strips (right).

Fig. 3. Simulation of magnetic flux densities and gradient magnitudes for anti-parallel (top) and parallel (bottom) orientations of the strip magnets. Z-distance was 125 μm , which corresponds to the bottom of the microchannel. The flux density magnitude images [(a) and (d)], as well as the magnetic gradient magnitude images [(b) and (e)] are shown. Graphs (c) and (f) show the gradient magnitudes along the white line (which represents the microchannel) in (b) and (e).

Fig. 4. Normalized median fluorescence (NMF) of *E. coli* detection using three processing methods: black – MagTrap, gray – short control (static), and white – long control (static).

Table 1

Magnetic bead capture efficiency (CE) and release efficiency (RE) of three MagTrap designs: rods, triangles and strips, with parallel and anti-parallel orientations

	(%)	RODS	TRIANGLES	STRIPS
Parallel	CE $\pm$ SD	94 $\pm$ 3	97 $\pm$ 1	98 $\pm$ 1
	RE $\pm$ SD	2 $\pm$ 1	1.5 $\pm$ 1	80 $\pm$ 11
Anti-parallel	CE $\pm$ SD	93 $\pm$ 1	99 $\pm$ 0.5	99 $\pm$ 0.5
	RE $\pm$ SD	5 $\pm$ 4	1 $\pm$ 0.5	23 $\pm$ 13