AUTOMATING SAMPLE PREPARATION IN MICROFLOW CYTOMETRY

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ABSTRACT

This report describes a microflow cytometer capable of five-plex bacteria detection with an automated sample-to-answer processing time of 30 minutes. The limit of detection of a magnetic bead-based immunoassay run on the sample preparation portion of the microdevice closely matched that of the current, manual assay. Fluidic driving and optical interrogation was performed within a compact instrumentation footprint, offering clear advantages over the laboratory-based flow cytometers currently in use for medical diagnostics. Moreover, an individually addressable array of micromagnets was developed as a method to overcome challenges involved with coupling magnetic bead-based sample preparation with microflow cytometry.

KEYWORDS: Microflow Cytometer, Microfluidics, Immunoassay, Magnetic Beads, Sample Preparation

INTRODUCTION

Flow cytometry remains a key technology in a diverse range of applications, including medical diagnostics. Traditionally, flow cytometers used to perform diagnostics have been housed in centralized laboratory settings and run by trained technicians. In contrast, recent interest has centered on smaller and less expensive instruments that provide a greater level of automation. Microfluidics has played an important role in this transition, providing simpler methods of sample injection, cell sorting, and optical interrogation. However, sample preparation remains an unmet challenge in bringing these cytometers into point-of-care and on-site environments.

This report describes a complete microflow cytometry system capable of five-plex bacteria detection with an automated sample-to-answer processing time of 30 minutes. The limit of detection of a magnetic bead-based immunoassay run on the sample preparation module closely matched that of the current, manual assay. Automated fluidic manipulations were performed using an elastomeric microvalve assembly adapted from GE Healthcare's Biacore ® System[1], and used to perform the labeling and reaction steps necessary for the sandwich-immunoassay. Moreover, fluidic driving and optical interrogation was performed within a compact instrumentation footprint, offering clear advantages over the large and complex laboratory-based flow cytometers currently in use for medical diagnostics.

In addition, we report the initial development of an addressable micromagnet array designed to overcome the limitations of magnetic bead-based sample preparation in microfluidic cytometers. The utilization of active magnetic elements in the array yielded greater control during bead loading, resulting in an even distribution of beads throughout the fluidic channel. Moreover, active control of each magnet enabled separate release of the 100's – 1000's of beads trapped at each magnet and direct injection into the downstream microflow cytometer. Similar, methods of optimizing surface-based immunoaffinity capture in microfluidics have been investigated [2]; our aim in future experiments will be to approach the efficiency of surface-based immunoaffinity capture, while retaining the flexibility of magnetic beads for downstream analysis.

EXPERIMENTAL

Figure 1A shows a schematic of the integrated system, consisting of two microfluidic modules. The sample preparation module (left) contained a fluidic manifold with five, 100 μL capacity reservoirs, each addressable by a valve. The cytometer portion of the device was based on a simple microfluidic sheath flow technique developed at the Naval Research Laboratory and was key to assay miniaturization.[3] Figure 1B depicts the hydrodynamic focusing portion of the device, which uses transverse flow across chevron-based grooves to wrap a single sheath stream around a sample stream. This scheme results in sample isolation from the microchannel surfaces within a common T-junction. Volume requirements for this scheme remain low; thus, compared to the multiple fluid inlets required for most 3D focusing schemes, the chevrons remain amenable to self-contained devices.

Prototyping for active fluidic components in figure 1 was accomplished using sterolithography (Fineline Prototyping). Figure 1C shows the fluidic control box which contained both positive and negative pressure sources operated using four separate diaphragm pumps, and an array of 16 solenoid valves for control of the pneumatic valve lines. The sandwich-immunoassay was performed in continuous flow by washing the pre-functionalized and trapped magnetic beads with each reagent. Briefly, carboxy-functionalized luminex ® microspheres (sets 56, 59, 77, 81, 98) were coupled to Goat-IgG, specific for Shigella, Listeria, Salmonella, E. coli, and Campylobacter. After the bacteria capture step, immunolabeling was completed by flowing biotinylated antibody and streptavidin-conjugated phycoerythrin through the device. Excitation light from diode lasers at 532 nm and 635 nm enabled both bead identification (emission: 705 nm and 660 nm) and quantification of bacteria (emission 565; figure 1C).

In separate experiments, 200 or 500 μm magnets were housed in an array of cylindrical holes fabricated directly above the microfluidic channels (Figure 2). A sliding mask above the holes kept the micromagnets from being attracted to a larger magnet placed directly above the array. Each magnet was individually actuated for bead release by simply moving the slide and exposing the cylindrical hole to the larger magnet. The micromagnets were reset by simply re-inserting

the slide and breaking attraction to the larger magnet. Experiments were performed to determine the optimal conditions to load the array, and capture and release from individual micromagnets was demonstrated.

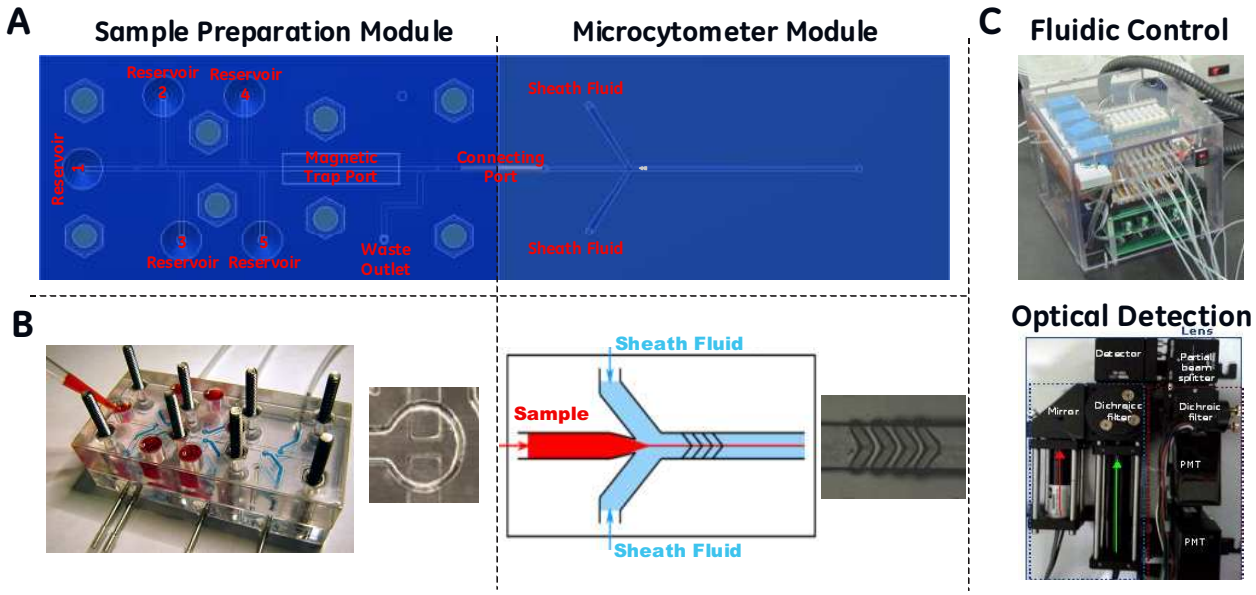

Figure 1. A. Schematic of the integrated device with both the sample preparation and microflow cytometer modules. **B.** Separate images of the two modules with zoom-in's of the key components (left: microfluidic valve seat, right: chevron structures). **C.** An image of equipment used for both fluidic control and optical monitoring.

RESULTS AND DISCUSSION

E. coli labeled within the sample preparation device resulted in a limit of detection of 6 x 10^3 CFU/mL, compared to 2 x 10^3 CFU/mL using manual processing. However, unlike magnetic traps used for sample preparation in the larger cytometers, placement of the magnet directly upstream of the microflow cytometer resulted in high detection efficiencies of the trapped beads. In addition, while bead loss during loading remained an issue due to surface adsorption (typically ~40% retention, data not shown), the number of beads trapped and then counted from each magnet position remained consistent (689.33 +/- 80.21 for 3 separate trials). Thus, unlike the large-scale magnetic traps that are used for qualitative pre-concentration of cells in cytometry applications, further optimization of microfluidic bead capture and release may result in quantitative detection of rare biological targets.

In addition, use of the chevrons for flow focusing enables multiplexed detection of the luminex® beads, as shown in figure 3. The complete assay was performed in under 30 minutes, and remained hands-free except for the initial loading of the reservoirs. An alignment procedure could be performed at the on-set of the experiment by sending a small portion of the beads past the magnetic trap and into the cytometer portion of the device. Further processing was completed using only the pressure source and valve control available in the fluidic control box described above. For these initial, single magnet assay runs, speed was limited by the holding force of the magnetic trap, as flow rates exceeding 5 uL/min resulted in bead loss during continuous flow processing.

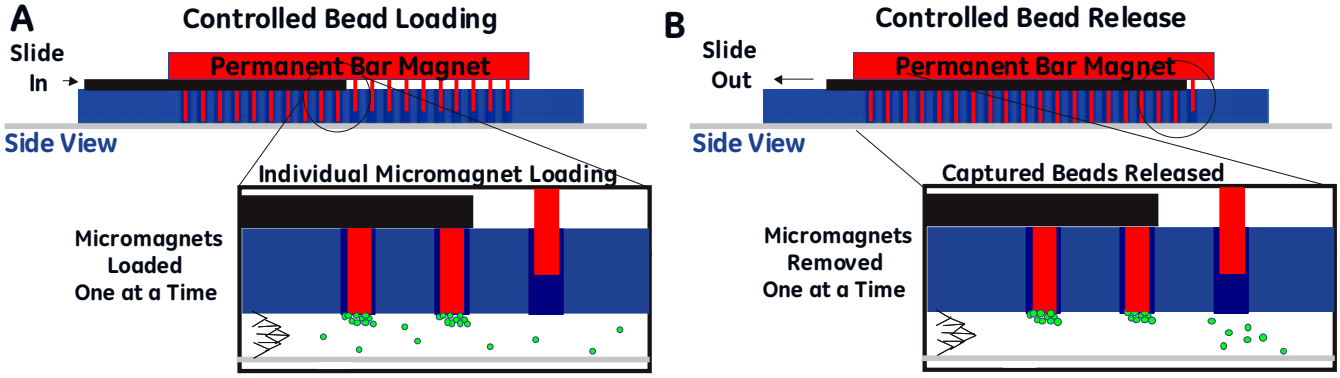

Figure 2. A. A schematic of the active array of micromagnets during bead loading. **B.** A schematic of the magnet array during individual release of beads from each separate magnet.

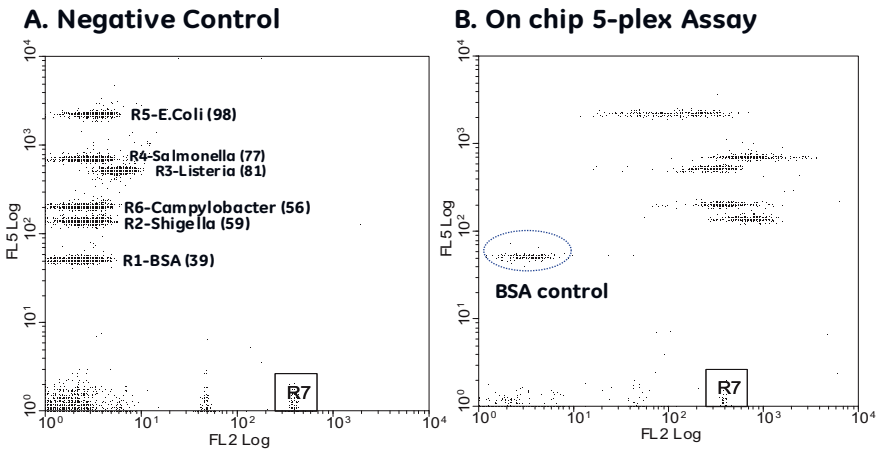

Figure 3. A. Negative controls for each of the antibody-functionalized bead sets run on the sample preparation portion of the device and a commercial cytometer. **B.** Results from the 5-plex assay performed on-chip, showing signal from each of the bacteria contained within the sample. The limit of detection for e. Coli reached 6 x 10^3 CFU/mL using a single magnet trap, compared to 2 x 10^3 CFU/mL for the bulk assay.

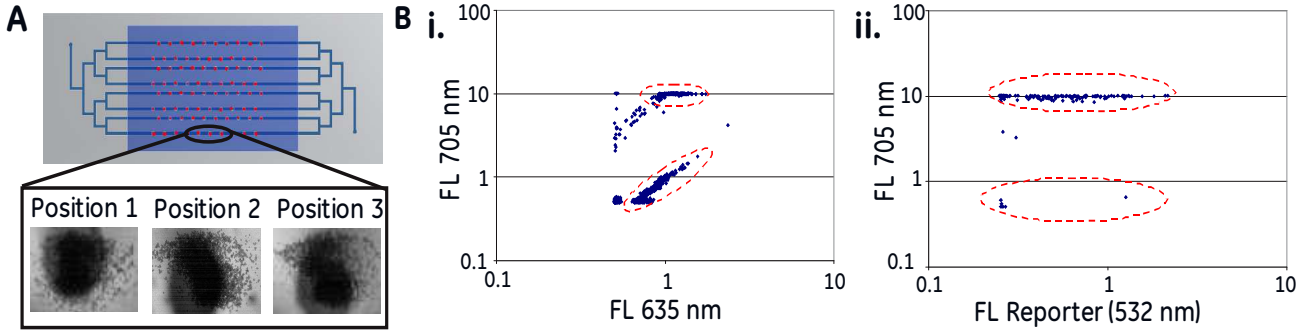

Figure 4. A. A schematic of the micromagnet array with images of beads captured at three separate magnet positions. **B.** The number of beads trapped at each magnet in the array was controlled by loading one magnet at a time, and then flowing a known bead concentration across that magnet. At higher bead loading concentrations the number of beads at each point in the array reached 100's. **i.** The fluorescence peak data of these 100's of beads in the 705 and 635 nm channels shows the distinct signature of two separate luminex bead populations (#39, lower oval, BSA Control) and (#98, upper oval, E. coli). **ii.** Fluorescence data from the reporter 532 nm channel shows specific staining of the beads functionalized with the E. coli antibody (upper circle, #98 E. Coli).

Figure 4 shows preliminary results in using the addressable magnet array to overcome challenges associated with microfluidic sample preparation that inhibit either sample/target interaction with capture beads or transfer for downstream analysis. These challenges include bead crowding in larger magnet field lines, bead clumping at high densities, and variation in bead retention during loading or transfer. The results show that the capture beads could be spread out over a microfluidic channel array, thus increasing the number of target-bead interactions for capture. Bead numbers of each individual magnet could be controlled, and release of only a few 100 beads from each magnetic trap could be detected in the downstream microflow cytometer.

CONCLUSION

A fully integrated microflow cytometry system has been demonstrated for a sandwich immunoassay using microfluidic valve technology adapted from GE's Biacore ® system [1] and a magnetic trap. Combination of the sample preparation and cytometry modules enabled automated bacteria detection in a short period of time at detection limits that are competitive with commercial cytometers. However, the compact fluidic control and optical interrogation equipment used herein demonstrates that the necessary instrumentation associated with device control is amenable to point-of-care or on-site analysis.

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