A GENERAL PURPOSE, MULTIWAVELENGTH, MICROFLOW CYTOMETER FOR CLINICAL AND ENVIRONMENTAL APPLICATIONS

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ABSTRACT

A general purpose cytometer design has been developed. It is based on the use of grooves in the channel surface to generate complete sheathing. For clinical applications, the design has been manufactured with integrated fiber waveguides. Reversing the grooves "unsheaths" the solution so that the sheath fluid can be recycled. Assays for up to 13-plex pathogens and toxins have been demonstrated using coded beads with limits of detection that are comparable to commercial instruments.

KEYWORDS: Cytometer, Laminar Flow, Detection

INTRODUCTION

Traditional, bench-top cytometers use an annular design to fully ensheath the sample stream. Unfortunately, that geometry is not easily reproduced by any of the standard microfabrication technologies. Most of the microfluidic cytometers found in the literature only provide sheathing in one dimension. The design is simple to manufacture and operate, but at the cost of measurement precision. Designs that can provide full sheathing are available, but often suffer from increased complexity.[1]

The design presented here finds a compromise between complexity and precision. As with previous microflow cytometer designs, the fluid is hydrodynamically focused in the horizontal direction. Vertical focusing is then provided by a set of chevron shaped grooves cut into the top and bottom of the channel.

Optical detection was accomplished by the incorporation of optical fibers that were inserted in prealigned channels. Illumination was provided by two lasers, and up to four photomultiplier tubes (PMTs) were used for detection, using the appropriate filters to provide discrimination.

THEORY

The sheathing depends on laminar flow conditions. Grooves cut into the channel surfaces rearrange the flow in a controllable and deterministic manor. The behavior is not effected by flow rate as long as the Reynolds number is kept below approximately 250.[2] Above the threshold, inertial effects slowly start to be seen. Below that threshold, the reorganization of the fluid streams is not only deterministic, but reversible. We have taken advantage of this to facilitate recycling of the sheath stream.[3]

Figure 1 presents a flow diagram of the sheath recycling experiments. The sample stream was provided by a syringe pump at a flow rate f_c. The sheath flow rate, f_s , was provided with a peristaltic pump. A second syringe pump recaptured sample fluid from the outlet end at a rate f_o. A reservoir in the sheath loop allowed the flow rate of the recovered fluid, f_r , to differ from f_s. The fluid recycling efficiency was given by f_r / f_s. Obviously, 100% recycling is not possible without contamination of the sheath fluid due to diffusion from the core into the sheath as the fluids move through the device, but considerable savings of the sheath fluid could be achieved.

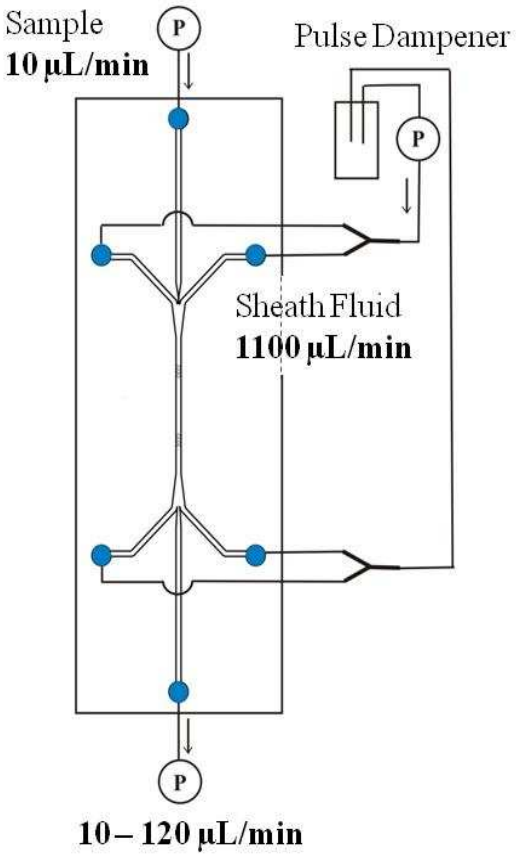


Figure 1: Flow diagram for recycling experiments

EXPERIMENTAL

Figure 2 shows a schematic of the layout used for bioassays using the Luminex beads. The typical protocol was for a sandwich assay with phycoerythrin as the tracer.

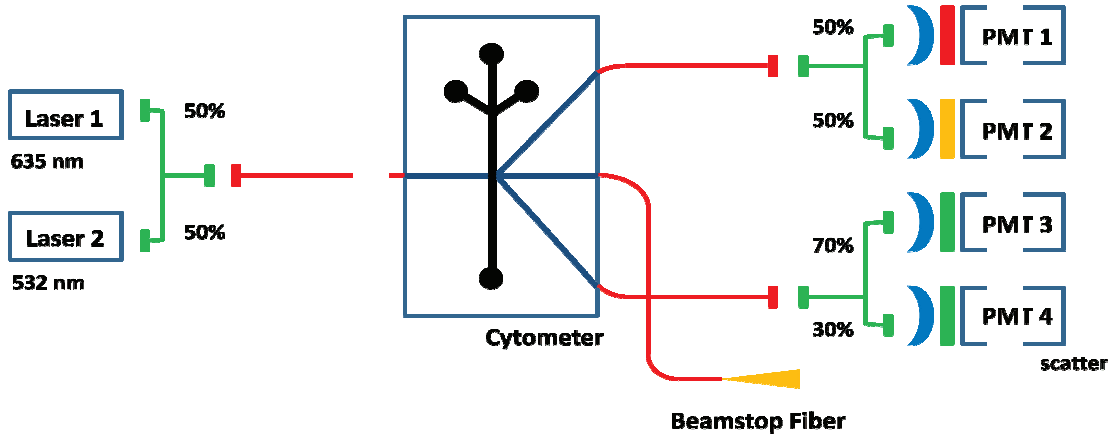


Figure 2: Layout of the microcytometer for use with Luminex beads. PMT 1: >700 nm (bead ID 1); PMT 2: 670+/-20 nm (bead ID 2); PMT 3: 565+/-10 nm (Phycoerythrin); PMT 4: 635 +/- 5 nm (light scatter)

RESULTS AND DISCUSSION

Figure 3 is a composite image of the chip during a recycling test. The sample stream has been labeled with a dye for visibility. The broadening of the sample stream seen at the outlet end of the channel is due to the removal of sheath fluid out the side channels.

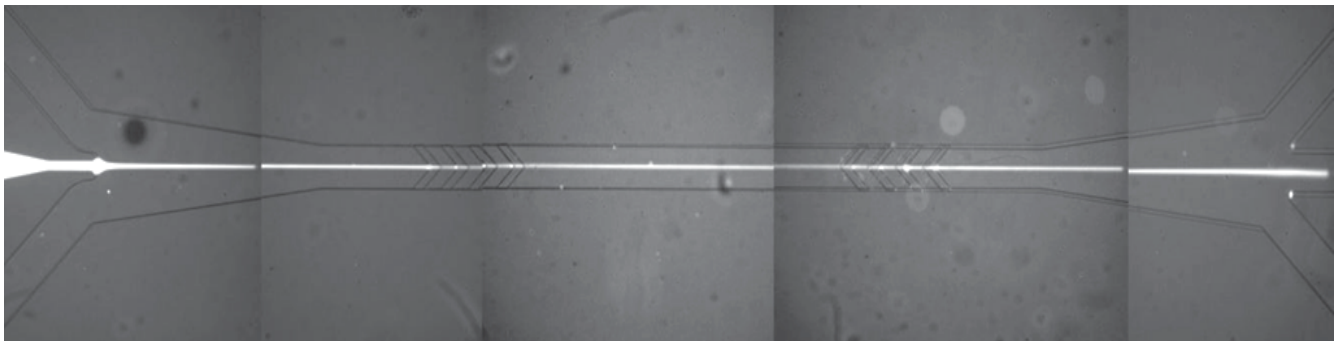


Figure 3: Photocomposite of sheath recycling. Sample stream contains fluorescent dye.

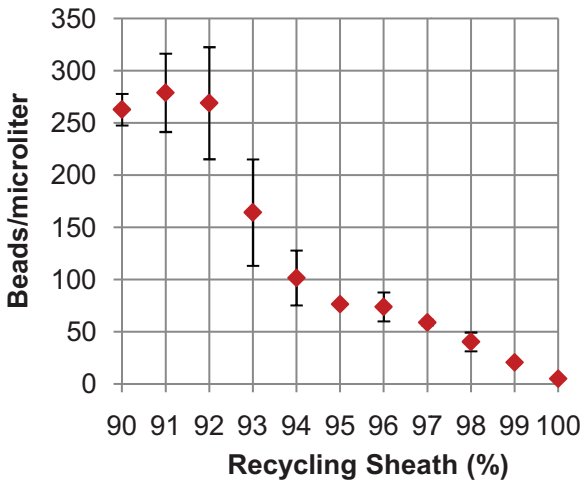
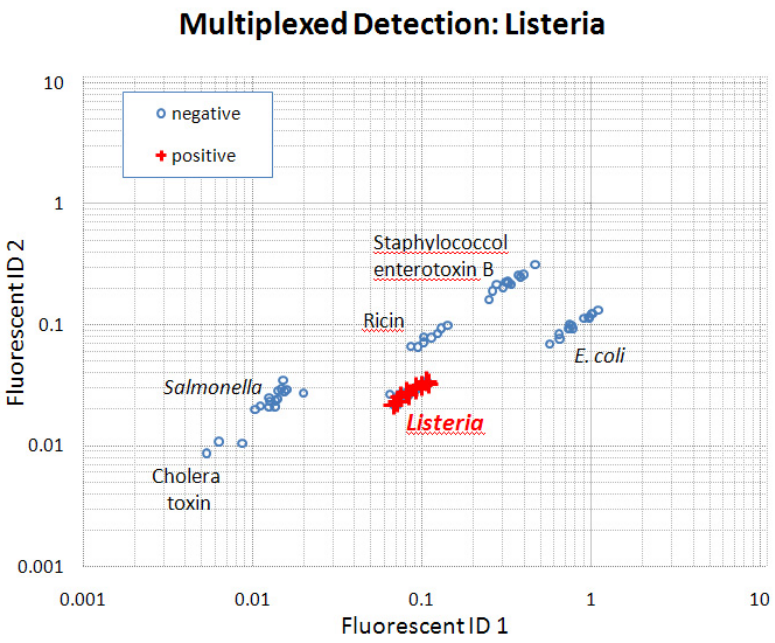


Figure 4: Recovery of beads

When dye was used as the tracer, the maximum recycling efficiency without contamination of the sheath was 90%. While diffusion undoubted played a role in limiting the maximum efficiency, small errors in pump and manufacture were most likely also contributors. Figure 4 presents the data for recovery of 5µm polystyrene beads during sheath recycling. The bead concentration in the recaptured sample stream remains flat up to a fluid recovery of 92%. Under these conditions, no beads were observed in the recovered sheath fluid. Beyond 92%, however, progressively more beads are lost to the sheath fluid.

RESULTS AND DISCUSSION

The microflow cytometer was used with the Luminex beads for the 12-plex assay for category B biothreat agents. Figure 5 presents the results for a test that proved positive for Listeria. The two axes are the bead ID channels for the Luminex beads. Blue circle indicate negative for the tag (phycoerythrin), and red crosses are positive.



CONCLUSION

We have developed a microflow cytometer capable of at least 12-plex assays using both sandwich and displacement assays. It can be adapted to recycle the sheath fluid to extend the operation time of stand-alone monitoring systems by as much as 12-fold. Future work will focus on automated sample processing and downstream sorting systems.

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