A Microfabricated Polymerase Chain Reaction-Based Biosensor

CNF Project # 884-00
Principal Investigator: Dr. Carl A. Batt

Abstract:
A portable, fully-automated, PCR-based detection system has been developed for the rapid detection of bacterial pathogens and forensic DNA analysis. Microfabricated DNA purification and real-time PCR microchips were fabricated and tested for their ability to purify and detect DNA sequences from a variety of bacteria including *Bacillus anthracis* (Anthrax). Chips were also tested for their ability to purify and detect single nucleotide polymorphisms (SNPs) for forensic DNA identification.

To extend the utility of the system, we are currently developing a multiplex bead-based DNA detection module. This module will allow for the rapid identification of up to 20-30 different SNPs, or an equivalent number of target pathogens. DNA purification and PCR amplification would be performed in the previously developed microchip, followed by hybridization of PCR products to multi-colored fluorescent microbeads. Beads will then be analyzed with a miniaturized, microfluidic fluorescent particle counter that we are developing. This particle counter has been constructed in polydimethyl siloxane (PDMS) using photolithographically patterned SU-8 molds. The counter uses five fluid inputs to focus a central fluid stream using three-dimensional sheath flow. We have successfully focused fluids to a 10 µm by 10 µm fluid stream using this system. Furthermore, we have integrated this flow cell with fiber optics and a molded wave guide to excite a 20 µm region of the fluid. This region is also fiber optically coupled to a fluorescence detection system that is capable of counting particles that pass by the excitation region.

Using this system, we have been able to observe and count multiple colors of 10 µm diameter fluorescent beads, demonstrating the potential for bead-based DNA analysis.

Summary:
We have developed a portable DNA purification and PCR-based detection system for bacterial pathogens and human DNA targets. Current work has focused on the development of a fluorescent bead-based detection system for multiplex detection of PCR-amplified DNA.

References:

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- A device capable of detecting a fluorescence microsphere in a microfluidic channel.
- Sample flow is confined in the center of the channel by having the sheath flow in the vertical and horizontal direction.
- Includes an integrated waveguide which confines the excitation region to 20 µm.

Figure 1, left: The fluorescent signal from a 10 µm fluorescent microsphere in a 125 µm wide and 125 µm high microfluidic channel.

Figure 2, below: Detection of green and orange fluorescent microbeads in microfluidic channel. Fluorescence intensity (arbitrary units) are shown on the Y axis with time (seconds) shown on the X axis.