Report as of FY2007 for 2005WY24B: "Real-Time Monitoring of E. Coli Contamination in Wyoming"

Publications

- Articles in Refereed Scientific Journals:
  - Johnson, P.E., A.J. Deromedi, P. Lebaron, P. Catala, and J. Cash, 2006. Rapid detection and enumeration of Escherichia coli in aqueous samples using Fountain Flow Cytometry, Cytometry, 69A, 1212-1221.

Report Follows

Abstract

This project will demonstrate the feasibility of economical, simultaneous, real-time detection of individual Escherichia coli and their viability in surface waters. The Clean Water Act requires states to monitor surface waters for fecal coliforms or specifically for *E. coli*. Fecal coliform monitoring is an indicator of the sanitary quality of the water and can determine the extent of fecal contamination in the water from warm-blooded animals. A low-cost, portable, highly sensitive, self-contained single cell detection system for *E. coli* enumeration is being developed for rapid monitoring of surface waters, including streams, rivers, and lakes. With first-year USGS/WWDC funding, the P-I and his team have demonstrated and significantly improved an innovative technique for detection of pathogenic microorganisms in surface water, economically and in real time. This technology is based on LED-induced fluorescence of antibody- and DNA-labeled cells. *The project will demonstrate the detection of individual E. coli simultaneously in two wavebands in order to detect and determine viability of individual microorganisms.* The suspended bacteria are stained using both an immunofluorescent antibody and a fluorescent cell viability label. The resulting aqueous sample is passed as a stream in front of an LED, which excites the fluorescent labels (Figures 1 and 2). The resulting fluorescence is measured with a CCD or CMOS imager using an innovative integration scheme (called *Fountain Flow*), giving a dramatically higher signal-to-noise ratio than conventional techniques. In addition, we are investigating the extension of the fountain flow technology to imaging, to provide increased discrimination capability among *E. coli*, other biological particles, and small geological particles.

Objectives

The major tasks of this project are to: 1.) fabricate and test a two-color, LED-illuminated detection system in order to simultaneously detect and determine the viability of *E. coli*, 2.) perform laboratory measurements on quantified *E. coli* samples to determine the detection efficiency and sensitivity of the two-color monitoring system, 3.) enumerate *E. coli* in stream and lake water samples using both our proposed method and the standard method currently recommended by the US Environmental Protection Agency, 4.) determine the feasibility of a rare-cell, fountain flow *imaging* system based on an extension of our current technology, and 4.) fabricate and test a prototype fountain flow imaging system for proof of concept.

Progress Report, Second 12 Months of Funding

We are testing and engineering improvements on a low-cost, portable, highly sensitive, self-contained single cell detection system for *E. coli* in surface waters, which will greatly exceed the current testing procedures in both speed and reliability. The goal of this project is the development of 1) a low-cost, rapid (<< 1 hour test), sensitive (< 5 cells/ml), portable, easy to use system for *E. coli* detection in raw surface water. Our objectives are to: 1) develop and test a system for simultaneous detection and viability testing of *E. coli* and 2) develop and test a proof-of-concept prototype for multi-spectral high resolution FF imaging. This proof of concept will allow for the design and fabrication of a remote monitoring system that will automatically screen water in real time. Alternative methods necessitate the shipping of bulk water samples or concentrates to laboratories and labor-intensive screening technologies, which may include bulk water concentration, incubation, and culturing. These factors combine to impede overall routine monitoring for fecal coliforms in the field and preclude widespread, routine screening of surface waters.

In the 12-months of year 2 funding, we have:

- successfully fabricated a two-color detection system for detection of microorganisms,
- continued successful proof of concept experiments for a fountain flow (FF) imaging system, using a syringe pump to consistently stop fluorescent beads in the focal plane of the FFC,

- collected data on the two-color detection of amoebae in natural river water,
- drafted a paper on the previously mentioned detection of amoebae in natural river water, using LED illumination, to be submitted to the Journal of Applied Microbiology (JAM),
- published a paper on the detection of *E. coli* in water to the journal Cytometry,
- published a paper on the detection of amoebae in natural river water using LED-illumination, against a background of organic detritus, in the Journal of Applied Microbiology, and
- have pending a patent application for the software control of FF.

The paper that we have written and are about to submit to JAM concerns the use of Fountain Flow Cytometry (FFC) for detection of protozoa in raw water with a two-color LED-illuminated FFC system. The system was tested with a flow throughput of 10 ml/minute and amoebae concentrations of 0.06 to 3 amoebae/ml. Two dyes were used, Chemchrome V6, a viability dye, and and R Phycoerytherin immunolabel. Detections were made in two colors, simultaneously using two cameras and two LED illuminators. Water samples for the Tech River (France) were sampled and tested for background autofluorescence from organic and non-organic material. These experiments show that two-color simultaneous measurements allow us to successfully separate living amoebae at 0.5 to 4 amoebae/ml from background detritus and that we will be able to separate E. coli detections from background detritus. Our final experiment in this series, this summer, will be to detect E. coli at low concentrations in natural river water.

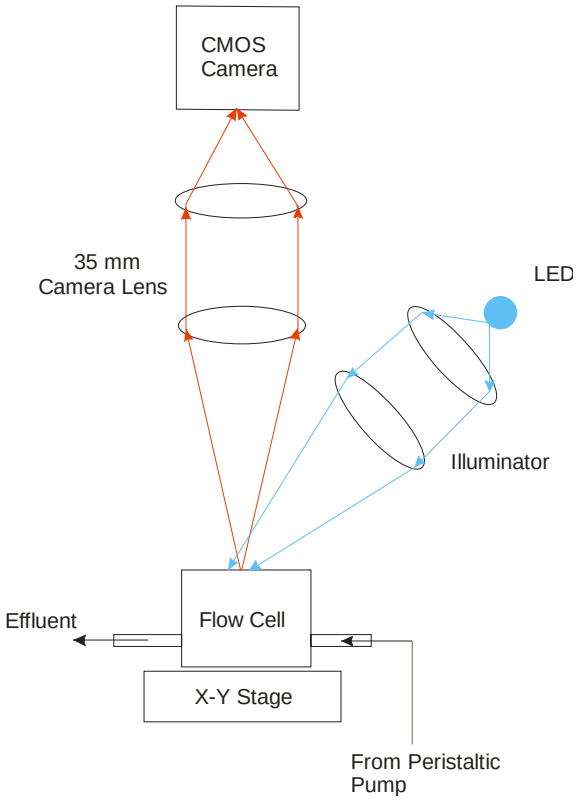

Figure 1. *Schematic diagram of an LED-illuminated epifluorescent Fountain Flow Cytometer. A sample of fluorescently tagged cells flows through the flow cell toward the CMOS camera and fore-optics. The cells are illuminated in the focal plane by an LED. When the cell(s) pass through the CMOS camera focal plane they are imaged by the camera and lens assembly through the transparent flow cell window, and a filter that isolates the wavelength of fluorescence emission. The fluid in which the cells are suspended then passes by the window and out the flow cell drain tube.*

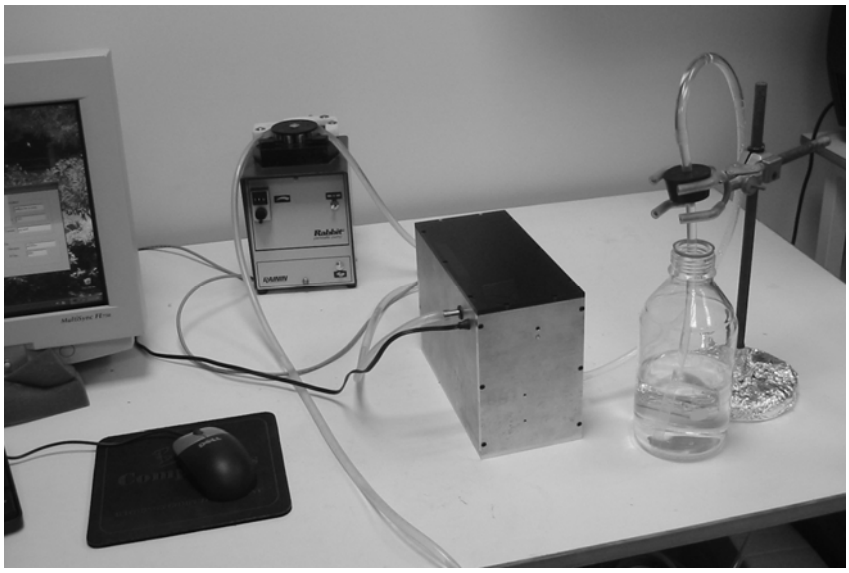

Figure 2. *The Wyoming Biodetection System Fountain Flow Cytometer, shown with peristaltic pump, and sampling reservoir*

Student Support

During Year 2, the P-I employed one former undergraduate Pre-Med student, Chris Havens (BS graduate 2006), and one geology student, Joseph Johnson (provisional graduate student), in this research. The interaction among personnel of varying backgrounds has provided a highly educational experience for everyone in research biodetection technology.

Publications in Preparation (and other project products) from Year 2

Manuscripts (in preparation or submitted)

Johnson, P.E., Deromedi, A.J., Lebaron, P., Catala, P., Havens, C., and Pougnard, C. 2007. *High throughput, real-time detection of Naegleria lovaniensis in natural river water using LED-illuminated Fountain Flow™ Cytometry*, in press, J Applied Microbiology.

Johnson, P.E., Havens, C., Lebaron, P., and Catala, P. *High Throughput, Real-Time Detection of Naegleria lovaniensis in Aqueous Samples using Two-Color Fountain Flow™ Cytometry,* to be submitted to Journal of Applied Microbiology.

Invited Presentations

2006 Asilomar Cytometry Development Workshop

1. *High-Throughput-Axial Imaging Flow Cytometry with LED illumination*
2. *Imaging Flow Cytometry*

2006 Select Water Committee Meeting, Wyoming State Senate

1. *Detection of Pathogenic Organisms in Wyoming Surface Water*