

Final Report

Real-Time Monitoring of *E. Coli* Contamination in Wyoming Surface Waters

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Project Duration: 03/01/2005 – 02/29/2008

Abstract

This project shows 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 prototype for *E. coli* enumeration was developed for rapid monitoring of surface waters, including streams, rivers, and lakes. With 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 demonstrated 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 3-year project were 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, and 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.

Final Progress Report, 3 Years of Funding

We completed and tested improvements on a low-cost, portable, highly sensitive, self-contained single-cell detection system for *E. coli* in surface waters, which greatly exceeds the current testing procedures in both speed and reliability. The goal of this project was the development of 1) a low-cost, rapid (\ll 1 hour test), sensitive (< 5 cells/ml), portable, easy to use system for *E. coli* detection in raw surface water. Our objectives were 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. We completed the first objective, and the second is still being pursued, although limited funding precludes us finishing that in a timely way. 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

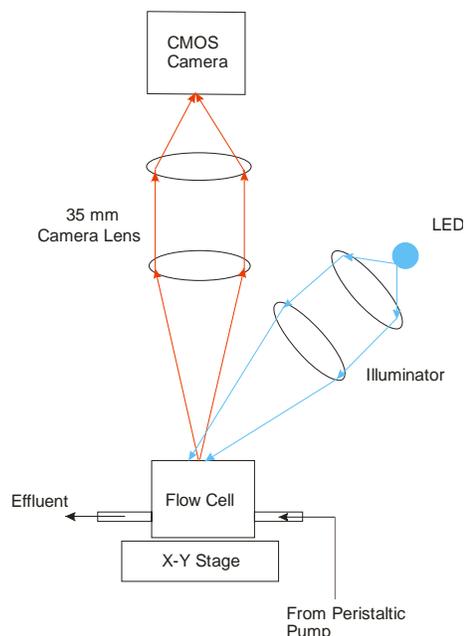


Figure 1. Schematic diagram of an LED-illuminated epifluorescent Fountain Flow Cytometer. A sample of fluorescently tagged cells flows through the flow cell toward the CMOS camera and fore-optics. The cells are illuminated in the focal plane by an LED. When the cell(s) pass through the CMOS camera focal 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.

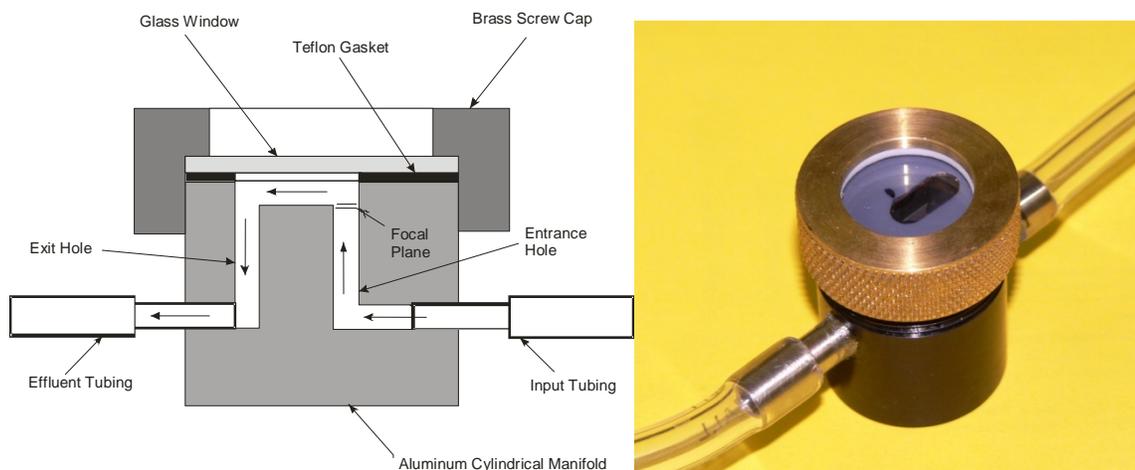


Figure 2. The Fountain Flow™ Cytometer aluminum flow manifold as used in the device shown in Figure 1 (from Johnson et al., 2006). **Upper Panel:** The sample enters the flow block through a Tygon tube connected to a stainless steel tube and exits through a stainless steel tube. Two holes have been drilled into the aluminum flow block: an entrance hole and an exit hole. As the sample flows up the internal entrance hole, it passes through the focal plane of the CCD/CMOS camera. A Teflon tape gasket is sandwiched between the aluminum flow block and a circular window, and tightly held with a screw-on brass cap. The gasket is cut to form a channel through which the fluid is diverted into the exit hole. **Lower Panel:** A photograph of a working flow block with attached tubing.

culturing. These factors combine to impede overall routine monitoring for fecal coliforms in the field and preclude widespread, routine screening of surface waters.

Over the three-years of 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 for a manuscript to be submitted this year,
- 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 patent applications for the software control of Fountain Flow and for cell sorting using Fountain Flow Cytometry. The latter allows for the separation of rare cells from a large volume of water, so that species identification can then be made using other techniques, such as PCR.

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 and bacteria 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 an 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 and fall, will be to improve our detections by using RPE-CY7.

Student Support

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

Publications and Patents

Manuscripts in Preparation

1. **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*, Journal of Applied Microbiology.
2. **Turque, Nathalie, et al.,** *Detection of Metal Contamination in River Water using Fountain Flow™ cytometry of Transgenic Xenopus Larvae*, Biotechnology.

Manuscripts Published

1. ***Johnson, P.E., Deromedi, A.J., Lebaron, P., Catala, P., Havens, C., and Pougard, C.** 2007. *igh throughput, real-time detection of Naegleria lovaniensis in natural river water using LED-illuminated Fountain Flow Cytometry*, **J Appl Microbiol.** **103** (3), 700–710.

2. *Johnson, P.E., Deromedi, A.J., Lebaron, P., Catala, P., and Cash, J. (2006) *Rapid detection and enumeration of Escherichia coli in aqueous samples using Fountain Flow™ Cytometry*. **Cytometry Part A, 69A**, 1212-1221.

Patents Pending

1. Apparatus and Method for Measuring the Fluorescence of Large Multi-Cellular Organisms, *patent pending*, P.E. Johnson.
2. Method and System for Counting Particles in a Laminar Flow with an Imaging Device, *patent pending*, P.E. Johnson.
3. Method for Pre-Sorting Microorganisms in Aqueous Solution prior to Selective Staining and Detection, *patent pending*, P.E. Johnson.
4. Copyrighted software, including XenopeImage, BioImage, and BioCount, P.E. Johnson.

Patent Granted

1. Apparatus and methods for high throughput analysis of samples in a translucent flowing liquid, P.E. Johnson.

Presentations (all invited)

2005 Cytometry Development Workshop, Asilomar, California

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

2006 Cytometry Development Workshop, Asilomar

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*

2007 Cytometry Development Workshop, Asilomar

1. *Fountain Flow cytometry of microorganisms in complex matrices (milk & blood)*
2. *Cytometry of ultra-large multi-cellular organisms*