

Autonomous Microbial Genosensor

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LONG-TERM GOALS

The overall goal of this work involves the development of a self contained, fieldable microfluidic based biochemical sensor for detecting microbial water quality indicators. The targeted integrated system comprises an opto-analytical segment, integrated detection electronics plus fluidic management system. The long-term goal is to place the autoanalyzer onto field platforms.

OBJECTIVES

The main focus of the effort is to advance the design, fabrication and eventual field testing of a microfluidic analyzer as a biochemical analyzer for detecting microbial water quality indicators. The goal is to develop an autoanalyzer that can be adapted to engineered gene probes for identification of target microorganisms in the field and has applications toward harmful algal blooms, water-borne pathogens and biowarfare agents and also environmental monitoring.

APPROACH

The current period entails several main objectives. Primers specific to the *Synechococcus rbcL* gene with T7 RNA polymerase were to be designed for Nucleic Acid Sequenced Based Amplification (NASBA). After optimizing NASBA amplification, molecular beacons were intended as a specific detection system for *rbcL* transcripts. The Boom extraction technique for cultured *Synechococcus* mRNA was the preferred solid phase extraction method for combination with the NASBA protocol. A linear tape drive format for sample prep was also part of the project. In addition, an integrated heater within an optical block fluorometer comprised a reaction and detection system for the autoanalyzer.

WORK COMPLETED

Molecular Probe Development

We have developed a simplified protocol for RNA extraction from *Synechococcus*. This requires only incubation with a buffer derived from a commercially available kit. No time consuming beadbeating or mechanical disruption is required. We have also performed some solid-state amplification with the solid phase nucleic acid material, Xtrabind, but optimized use of the material has not been achieved.

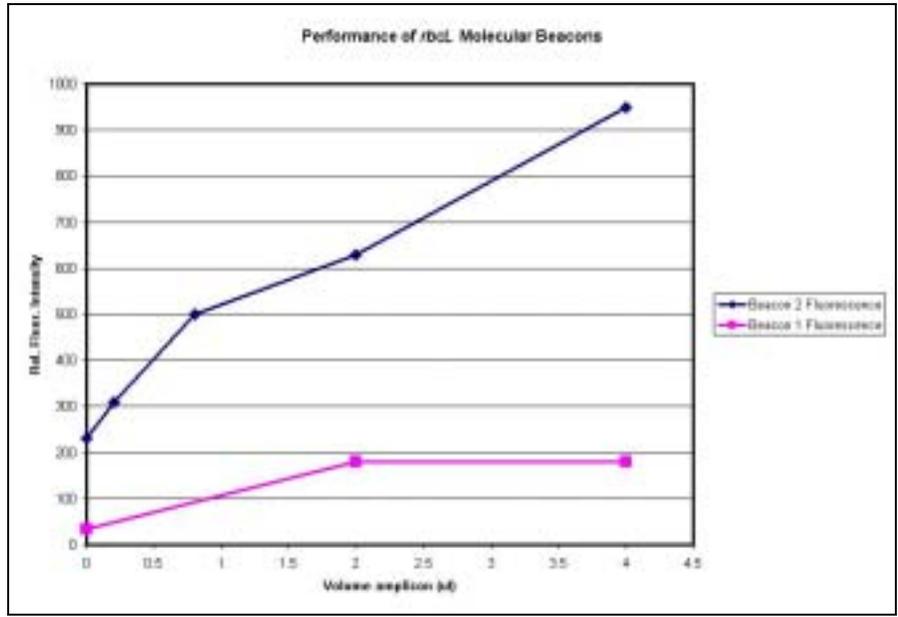


Figure 1. Fluorescence performance of two different molecular beacon engineered probes. The higher fluorescence yielding transducer (beacon 2) was used for subsequent assay development.

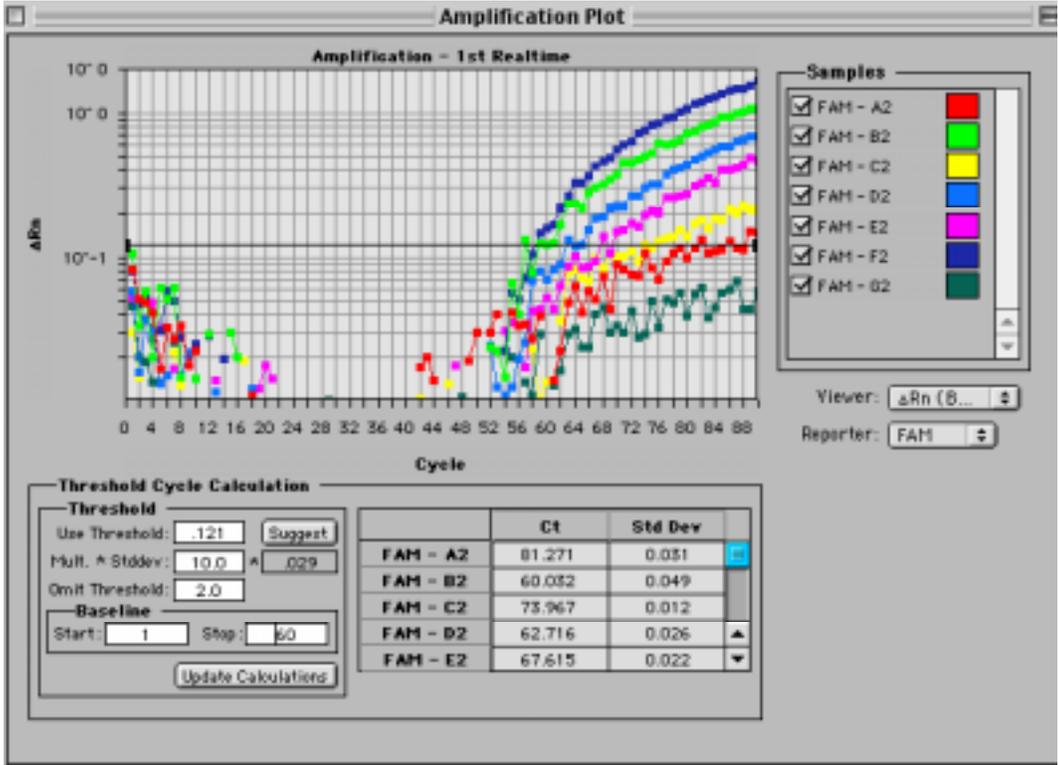


Figure 2. Real-time NASBA using molecular beacon #2. The Orange/red line is a DI blank. The Green is the highest concentration of mRNA. The data shows that NASBA amplification of the targeted organism can be achieved.

We have also designed a new NASBA primer which enables detection of ~ 0.01 fg RNA (~ 95 copies) *Synechococcus rbcL* mRNA. This new primer apparently binds in a loop of the *rbcL* mRNA. This is a significant breakthrough, which will enable smaller sample sizes to be collected.

Instrumentation Development

A heating element, Ion Optics T-05 infrared heater, has been incorporated into the optical detection block along with the needed fluorometric optical components. The heater is controlled through a driver board and is mounted normal to the optical detector's light path (Figure 3). Optimization of the temperature control parameters (current, pulse width, and frequency), are in process.

We have also achieved a functioning fluorometer that is based on 9X9 mm optical filters, an inexpensive LED light source, and photodiode detector. In addition, we have developed an integrating signal detection circuit card assembly to support wide dynamic range detection requirements.

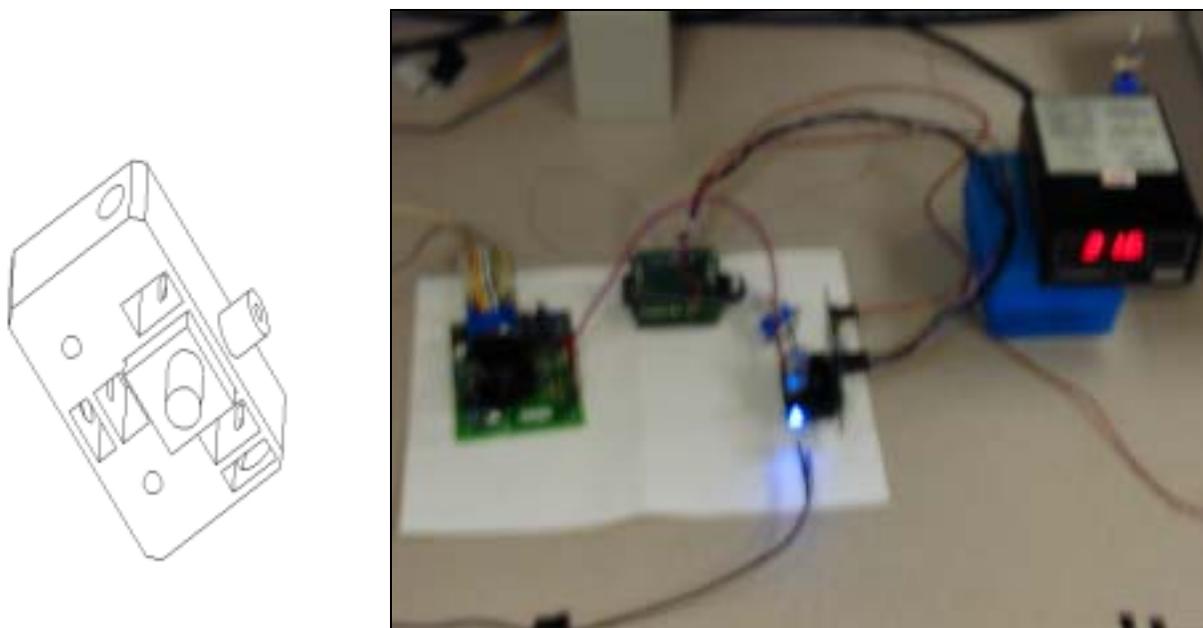


Figure 3. Image showing amplification reactor design (left). Lab system configuration showing fluorometer with microcontroller and heater driver board. The light source is blue LED and the optical detector is photodiode supported by an integrating signal detection board.

Fluidics management is being advanced in a modular development fashion. The first goal is to create an integrated fluidic manifold that emulates the logic of the lab based NASBA assay. The manifold is intended to permit full demonstration of amplification followed by detection in the connected detector block. A fluidic pathway is shown in Figure 4 outlining total fluidic and valve logic for discharge into the reaction chamber. With establishment of the simplified RNA extraction we are in process of developing the online lysis module for sample preparation. Similarly, the linear tape drive awaits the final solid phase material selection.

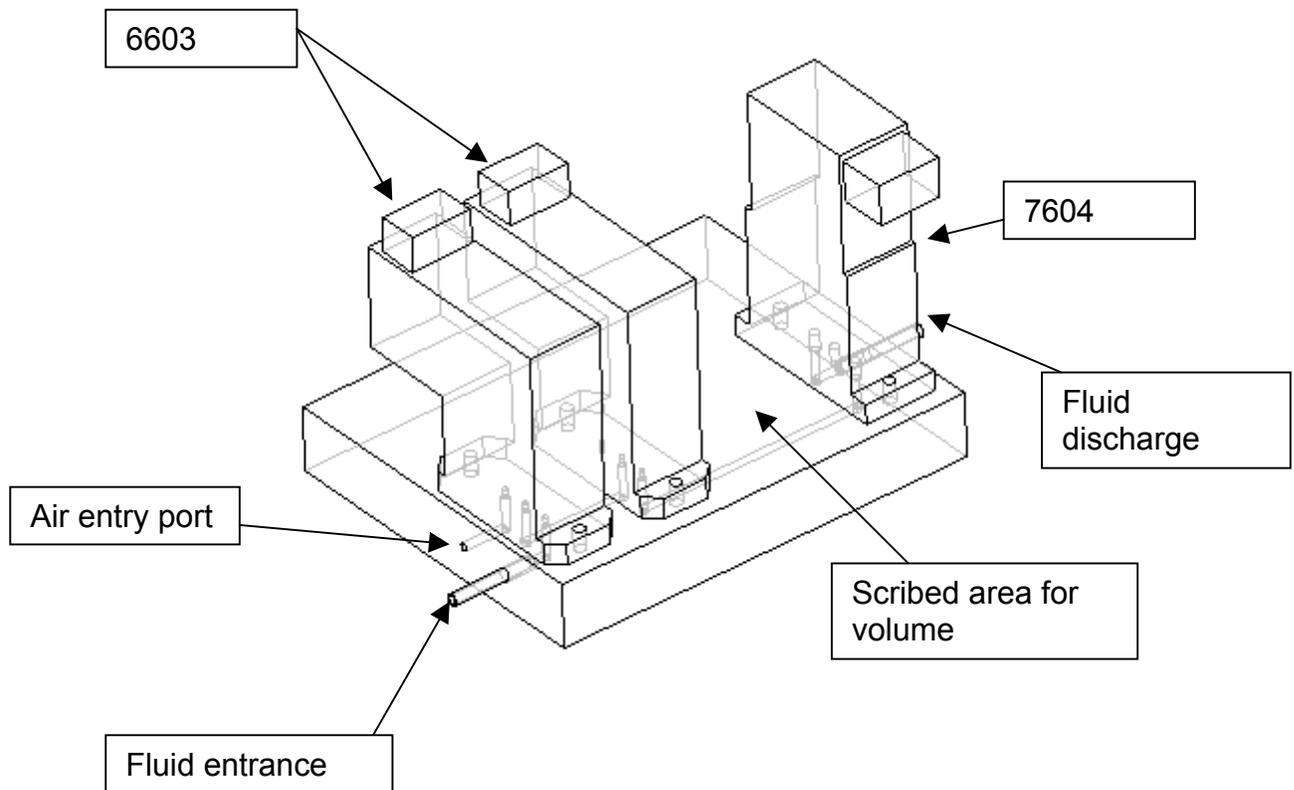


Figure 4. Illustration of the fluidic module to be used in the fluidic management system.

RESULTS

We have advanced both molecular diagnostic technology and instrumentation toward the goal of a remote, deployable microbial system for field genomics. We have specifically demonstrated NASBA amplification of the intended environmental target (*synechecoccus*) and have made several of the key instrumentation modules (fluorometer, heater, controller) needed for the microfluidic-based autonomous analyzer system. This project is encountering difficulty in transferring the solid phase material into a tape format due to initial material properties. We are currently working with a local company who has expertise in creating lateral flow assays and expect resolution of the issue within this quarter.

IMPACT/APPLICATIONS

This proof of technology demonstration has impact for both marine science and ocean applications that seek real-time detection and characterization of microorganisms for water security, modeling the biota, and seeking the detection or discrimination of species that may indicate the presence of man-made or natural changes in a targeted region.

TRANSITIONS

The work described herein on the microbial sensor development is an emerging ocean detection capability. We expect this technology to have application beyond coastal ocean science into freshwater and drinking water security, and as a key detection component in the emerging global ocean observing efforts that seek real-time detection biosensors.

RELATED PROJECTS

We are involved in another (NSF) project aimed at a field sensor for water monitoring applications to detail ecosystems biocomplexity.

PATENTS, PUBLICATIONS, TECHNOLOGY TRANSFER

Development of an Autonomous Microbial Genosensor (AMG), Matthew C. Smith, John H. Paul, David P. Fries & Andrew Farmer, Marine Biotechnology Summit, Ft Pierce. FL. Oct. 2002, accepted

Developing NASBA for Real-Time Detection of Microbial RNA Targets in the Marine Environment Matthew C. Smith, John H. Paul, David P. Fries, ASLO, Salt Lake City, UT. Feb. 2003, oral, submitted