LLNL MBARI ASTEP project update

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Background

LLNL is working for MBARI on a NASA-sponsored project "An Environmental Sample Processor for Deep-Sea Seep and Hydrothermal Vent Applications" from the solicitation "Astrobiology Science and Technology for Exploring Planets" (ASTEP). The goal is to develop a polymerase chain reaction module for oceanographic microbiology in MBARI's ESP by 3/2008. The work areas are:

Task 1: Design the PCR module, 4/2006 – 9/2006.

Task 2: Fabricate and test a prototype, 10/2006 – 3/2007.

Task 3a: Fabricate and integrate production modules into the ESP, 4/2007 – 9/2007.

Task 3b: Support production modules, 10/2007 – 3/2008.

Summary of recent activity (11/18/2006 – 2/2/2007)

We completed the detailed design of the prototype, including electronics and all mechanical aspects (thermal, optical, and packaging). All of the parts are either being fabricated or assembled.

We specified, ordered, and received all of the electronic components for the prototype. The most recent work has been loading the digital board in stages, which started with the power conversion section. We got the voltage conversion, but are tuning the circuit to get the best efficiency. We have also verified that we can program the microprocessor as expected on the manufacturer's development platform.

[Note: The thin-film heater design and the following concept of using the material as an RTD should be considered proprietary for now.]

The first prototype thin-film heater module ran on our Bench 2 fluidics workstation from 9/4/2006 to 1/4/2007 before being taken out so other modules could be tested. This was about 734 PCRs and 31,510 cycles, so the heater durability looks great. We are improving the design to be more thermally efficient and also testing the feasibility of using the resistive film as an RTD for closely-coupled thermal feedback. In our design, the thin film could be very close to measuring the reaction temperature and would give us the best feedback for control.

The dicey aspect of using the thin film as the RTD is that the heater material has a significantly lower resistive temperature coefficient (1.2e-4/°C) than typical RTD materials like platinum and nickel (3.97e-3 and 5.49e-3/°C, respectively). That means that we would have to control with a 0.4 % resistance change from 60 to 95 ºC. We set up a temperature measurement circuit and demonstrated that we can handle that small variation on a thin-film RTD that was going from about 342 Ohms at room temperature to 346 Ohms at 100 ºC. The practical problems, however, are that even small part-to-part variations or variations over time could put the RTD out of

calibration or out of range for the circuit. We are investigating these issues now. The fall-back is to go with the off-the-shelf platinum RTDs that we usually use. The thermal coupling would not be as good, but the temperature measurement would be easier.

The optical components for the four channels (emissions 525, 580, 620, and 680 nm from dyes FAM, TAMRA, Texas Red, and Cy5) are on order or in hand. We have the LEDs and filters for the FAM and TAMRA channels, and we may start by building the first prototype with 2 FAM and 2 TAMRA channels instead of 4 different channels. We have the most experience with these two optical sets, and it would be a good way to test the relative response and variability.

We have four test dyes 5'-coupled to oligonucleotides (ten Ts, or deoxythymidine nucleotides): FAM, TAMRA, Texas Red, and Quasar 670. Quasar 670 is a substitute that we are considering for Cy5. Quasar 670 is less expensive from oligo source Biosearch Technologies because it is better in synthesis and has less license fee. These test dyes will let us check out the optical system sensitivity and dynamic range in relevant chemical conditions, but isolated from the enzymatic reaction.

Several of the structural parts of the heater, optics, and packaging have been fabricated on our rapid prototype machine.

Upcoming activity

The digital board will be loaded and checked out for low-level functionality by 2/16/2007. Further software development is awaiting the digital board. In parallel, the analog board will be loaded and checked out. If there are no major problems, we will have integrated functionality at the end of February. The high-level software will not be complete, but we should have low-level software control of all of the major functions through RS232 at that time.

We will have the heater module assembled by 2/16/2007 and the optics module assembled by the end of February. Optically, this will allow testing with at least FAM and TAMRA dyes to show that we have the required sensitivity. Thermally, this will let us measure heating and cooling rates, energy efficiency, and offsets.

In general, we are going to have a better idea of where we are on 2/16/2007. That will let us know if we can achieve the PCR demonstration in mid-March. After we show 525 and 580 nm (FAM and TAMRA) detection, we can proceed to 620 and 680 detection.

In the technology transfer area, we need to work with MBARI on the interface with the ESP core, particularly the microfluidic interface, the chemical process including nucleic acid cleanup, and the signature and dye chemistry. The software and electrical interfaces are better specified and on firmer ground at this point.

Schedule and budget

As of 1/27/2007, the total spent was 218 k$ and 90 k$ remain, which includes additional funds-in of 69 k$ in January. We understand that MBARI is transferring or has transferred the remaining Year 1 funds, but these have not landed in our account yet. We are under-budget and behind-schedule, as discussed above, and are aiming to make the PCR demonstration in the prototype in mid-March. One mitigating factor in the delay is that the prototype will be closer to the final design fidelity than we initially intended.