DNA to Go: Portable, Low-cost PCR-based Diagnostic Assays
PI: Victor M. Ugaz (Chemical Engineering)
Graduate Mentor: Aashish Priye (Chemical Engineering)
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Grand Challenge to be Addressed:
This proposal addresses Grand Challenge #14 for Global Health (Bill and Melinda Gates Foundation): Develop Technologies that Allow Assessment of Multiple Conditions and Pathogens at Point-of-Care

Students involved in this project will design and build simple, low-power instruments to replicate DNA using the polymerase chain reaction (PCR). The simplicity and robustness of this approach will make it feasible to greatly expand availability of a host of nucleic acid-based diagnostic assays, especially in resource limited settings lacking dedicated laboratory facilities and skilled personnel.

Overview of Proposed Research:
The lack of affordable, rapid, and easy to use diagnostic technologies is one of the most critical issues confronting global public health. Expanded availability of such technologies will not only impact health care, but will be a key to enabling rapid identification of infectious agents in environments with little or no laboratory infrastructure. We will address this need by developing an innovative thermocycling system that harnesses natural convection to perform rapid DNA amplification via the polymerase chain reaction (PCR) with smartphone based detection of the reaction products. The design is inherently simple (similar in principle to a lava lamp) and consumes minimal electrical power.

We propose to engage two teams of undergraduate students to address the following project tasks to advance this PCR thermocycling technology beyond the proof of concept stage so that its full potential can be realized as a viable point of care diagnostic tool.

Team 1. Fluorescence Detection. Students will build and test a smartphone-based imaging setup to detect reaction products from the PCR. Team members: Danielle Cope, James Johnson, Alexandra Keller, Richard Lim

Team 2. Thermal Management. Construct a heating platform to actuate PCR-based DNA replication incorporating a single heater powered by a standard USB port. Team members: Jamison Chang, Staley Lu, Alex Millard, Neal Patel, Luke Smith

Overview of Research Tasks for Fall 2013

Both Teams Goal: Become proficient performing DNA replication via the polymerase chain reaction using conventional benchtop-scale instrumentation in order to understand how the instrumentation functions.

Tasks:
- Learn to run PCR using a conventional thermocycling instrument and analyze the reaction products using gel electrophoresis
- Learn to run PCR in our existing convective thermocycling instrument
Detection Team Goal: Demonstrate ability to distinguish between pre- and post-reaction states using a smartphone camera.

Tasks:

- Evaluate new PCR chemistries in the conventional thermocycling instrument to optimize the reaction conditions
- Evaluate ability to detect achievable fluorescence using a smartphone camera

Summary of accomplishments. The detection team focused initial efforts toward evaluating fluorescent dye chemistries for PCR to select a dye formulation and concentration to add into the reagent mixture in order to generate the maximum fluorescence signal upon completion of DNA replication. Two dye formulations were studied: SYBR Green and EvaGreen. The team’s experiments revealed that both dye chemistries can be used without inhibiting the reaction. However in each case it was found that it was difficult to visually detect a significant difference in fluorescence between the post reaction sample (after DNA replication) and the pre-reaction control. Further experiments ruled out the elevated temperatures during PCR as a factor, but instead pointed toward fluorescence quenching with time after completion of the reaction. This hypothesis was verified by observation of significantly higher fluorescence immediately upon addition of dye than after the labeled sample was incubated for 10-20 minutes at room temperature. We anticipate that this will not be an issue in our instrument design because the camera detection will be built in so that it can be automatically performed immediately upon completion of the reaction.

Future work will focus on further enhancing the fluorescence signal by (1) concentrating the replicated DNA on a filter paper surface on one sidewall of the reactor, and (2) performing quantitative analysis of the fluorescence images (i.e., instead of relying on visual inspection alone). A calibration curve will be constructed to directly relate DNA concentration to observed fluorescence.

Thermal Team Goal: Design a single-heater, USB-powerable heating device.

Tasks:

- Determine how to generate sufficient heating (95-100 °C surface temperatures) using USB power
- Apply a combination of experiments and simulations to how to design the reactor geometry to passively attain 55-60 °C on the top surface

Summary of accomplishments. The thermal team focused on selecting resistors capable of heating the bottom surface of the reactor to 95 °C and maintaining this condition for 20 min using a USB phone charger as a power source. Initial experiments showed promise to achieve the desired temperature range, and a potentiometer was chosen to enable finer adjustments. However the team found that the potentiometer setting drifted with time due to internal heating. More stable results were obtained by simply inserting additional resistors into the circuit, allowing the desired temperature conditions to be achieved. The team also designed a device incorporating an exposed surface and clamping tool in which to mount the convective reactor. Preliminary analysis has allowed us to select a reactor design that will achieve the internal temperature gradient needed to drive the reaction.

Future work will involve performing DNA replication using the USB powered heating apparatus. Working with the detection team, we will next attempt to demonstrate successful PCR in the USB powered device, followed by detection of products using the smartphone camera.