Improved detection techniques for foodborne pathogens (multi-plex detection platforms component)

Project Rationale
Microbial contamination of meat and, more importantly, fresh fruits and vegetables has become a mounting concern during the past decade due to an increased emphasis of these products in a healthy diet and the recognition of new foodborne pathogens such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. The availability of rapid methods for detecting pathogens in food production, processing, and distribution systems could enable the real-time assessment of risks. However, the speed of the method is usually indirectly correlated with the cost (less time, more money). The prime example is nucleic acid amplification technologies, which can provide rapid quantitative results, but instrumentation costs can exceed one hundred thousand dollars. Therefore, this effort is focused on the development of a low-cost nucleic acid amplification detection platform that can quickly screen for multiple pathogenic microorganisms and can be operated in the field by non-technical personnel.

Project Objectives
- Integrate a peltier heating/cooling device into the previously designed spatial beacon format.
- Define the parameters needed for utilizing mRNA from previously determined amplicons to differentiate between live and dead cells.
- Develop an improved low-cost optical detector system for analyzing the dedicated DNA microarray FRET-probe platform.
- Develop optimized protocols for the developed platform.

Project Highlights
We developed a prototype integrating a peltier heating/cooling device into the previously designed spatial beacon format. The basic functions of the prototype device are to amplify the gene region of interest using common thermocycling methods (i.e. PCR), and to detect a color change in the fluorescence of the capture probe. The system is capable of quickly heating/cooling the sealed reaction chamber, which we prototyped using a microscope slide ‘sandwich’ with a small gasket between them.

We achieved the heating/cooling functions using peltier thermoelectric modules adhered to a thin aluminum plate placed against the microscope slide sandwich. The heating/cooling system was controlled by using an integrated thermocouple at the microscope slide surface, which provides real-time temperature readings from the reaction chamber. This feedback loop (heating/cooling signals to the peltier modules and reaction chamber temperatures from the thermocouple) provided the basic input/output for the development of a control software system and supporting circuit board. Initial PCR reactions analyzed by gel electrophoresis validated the prototype’s performance. Currently, we are interfacing the prototype thermocycler with a CCD-based optical detection system, as well as a software-controlled laser-based excitation system, and are conducting preliminary tests.