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NSF GFRP 2015 Research Proposal

Autonomous, real-time detection of Dengue virus

Dengue virus, once contained to a few countries, has become the leading cause of illness in the tropics and subtropics, infecting more than 100 million people per year[1]. With no vaccine or specific course of treatment, continuous surveillance of disease incidence is key preventing epidemics, which occur with alarming and increasing regularity. *Aedes* mosquitos are the virus's primary method of transmission, so **I propose to design a mosquito trap that autonomously catches, processes, and transmits incidences of Dengue virus detection.** A network of autonomous kits will strengthen the efficacy of public health measures – saving lives – as well as link data on viral spread with local environmental data to better understand the effects of climate change on disease. Moreover, this work will advance the field of microfluidics, improving the scalability of point-of-care diagnostics.

Problems with the state of the art technology

By and large, Dengue is monitored manually. This method is costly, time consuming, and puts public health workers at risk. For example, in coastal Ecuador where we plan to conduct our first field trials, researchers embark on weekly excursions to 60 randomly selected locations with backpack aspirators to catch mosquitos. These are processed back in a central laboratory. **My device will automate collection and laboratory work and do it in the field, for a safer, cheaper, scalable option for virus detection.**

Current trapping methods include nets, fragrances, and CO_2 emissions that mimic mammalian respiration; commercial traps exist, but are intended solely for vector control, not sampling. My system needs to attract and capture a small number of uncontaminated mosquitos.

In order to detect Dengue, genetic material needs to be extracted from the mosquito. Commercial, automated processing and purification machines also exist, but have been developed almost exclusively for laboratory environments that require high-throughput extraction methods that are energy intensive and exceed the needs of field monitoring. Nucleic acid sequence based amplification (NASBA) is an easy, reliable method of RNA amplification that detects the presence of a virus or bacteria by attaching a fluorescing protein to specific nucleic acid sequence. Promisingly, NASBA is isothermal, and has been shown to effectively detect serotype-specific occurrences of Dengue[2].

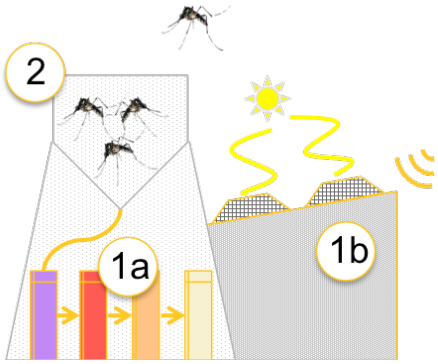

Figure 1: 1a and 1b will be an integrated microfluidic chip that extracts, purifies and detects Dengue RNA

An integrated microfluidic chip is the most contained, cheapest option for the latter two steps. Dimov, et. al[3] has published papers on partially-automated purification and NASBA integration, but their device lacked the ability to process whole samples, e.g. could not lyse the viruses, and used bacterial tmRNA, which is more stable than viral mRNA. **As yet, no one has developed a fully integrated RNA extraction, purification and amplification microfluidic device.**

The heating and necessary pressure gradients can be produced by solar panels. As proof of concept, the

[1] Center for Disease Control. "Dengue." http://www.cdc.gov/dengue/epidemiology/index.html
[2] Jittmittraphap A+ Southeast Asian J Trop Med Public Health. 2006 Nov;37(6):1117-24.
[3] Dimov+ ***Lab Chip***, 2008,**8**, 2071-2078

Erickson Lab published work on solar-powered polymerase chain reaction (PCR)[4]. Fluorescence detection and wireless transmission are easily achievable with cheap smartphone technology.

Project Aims

This project has two aims (Figure 1): develop an integrated, autonomous, solar-powered microfluidic chip (1a, b) and trap mosquitos while avoiding environmental contamination (2).

Primary Aim – microfluidic extraction and purification

Design PDMS chip with two chambers, for extraction and amplification in coordination with the Cornell NanoFabrication Lab using standard soft lithography techniques. Extraction involves: lysing the viruses, binding nucleic acids to silica, washing to remove impurities, elution to unbind the nucleic acids from the silica. This can be simplified, as the final two steps are not always necessary. Determine whether a third chamber is necessary to lyse the viruses.

The NASBA process is well defined, but has never been done using solar power, which will require thermal modeling to determine if a lens can stably reach and maintain the required temperature of 41°C, as shown by Jiang, et. al. for higher PCR-related temperatures[4].

Secondary Aim – Trapping

With input from our Ecuadorian partners, I will determine which method (nets, fragrances, CO_2 "respiration") effectively captures mosquitos with minimum contamination from the environment and without affecting the efficacy of the proceeding assays.

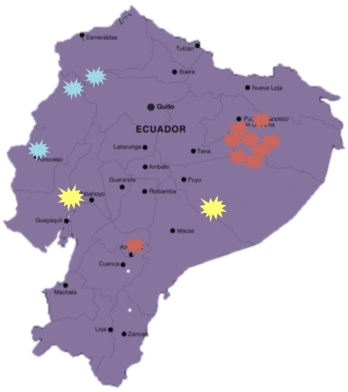

Figure 2 – Sample map: public health officials can pinpoint real time Dengue outbreaks before the epidemic reaches humans

Resources and experience

As a first-year PhD student at Cornell, I recently joined the Erickson Lab, which has significant experience working on global health applications of microfluidics-based diagnostics. The lab has established partnerships in Ecuador. Additionally, I worked as an environmental journalist and covered vector-borne disease and mosquito control in in California. I have done research with solar cells, at the UC Davis NanoFast lab, and developed the Sol Cycle, a miniature, solar-powered bicycle.

Intellectual Merit and Impact on Basic Science

Currently, autonomous PCR processes are bulky, costly, and power-intensive – unsuitable for field sites. A integrated microfluidic chip for extraction and amplification of RNA has never been developed and could be adapted to numerous diseases, tracking disease outbreak on time scales much faster than previously possible. Moreover, data on exactly how the disease spreads can indicate the way mosquitos are adapting to climate change.

Broader Impact

At its core, this device will reduce epidemics, saving lives while decreasing the financial burden on and of the millions of people who require hospital care from vector-borne diseases.

As I develop the mosquito trap, I will design lesson plans around portable diagnostic devices for Cycle for Science, an education-focused initiative I co-founded in April 2015. Once the first aim is complete, I will host a two-part science-focused hackathon to provide a way for local high school students to interact with state-of-the-art science. Over one weekend, students will develop hypothesis and preliminary report about the diseases or insect life around Ithaca, New York, and then provide research support for the best ideas throughout the semester.

[4] Jiang+. *Scientific Reports* **4**, Article number 4137 (2014)