A Point-of-Care Diagnostic Prototype for High-throughput, Multiplexed Single-Virus Detection

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Attention to viral infections has increased after multiple recent outbreaks: the global 2009 H1N1 influenza pandemic, the variant-strain Ebola virus epidemic of 2007, and the West Nile virus epidemic of 2003-2004. Despite advances in antiviral drugs, diagnostics have lagged behind [1] [2]. These outbreaks underline the growing need for early virus discovery through rapid and reliable point-of-care (POC) diagnostics. POC diagnostics are limited to symptomatic patients as they rely on antibody-antigen interactions for detection. Plaque titer and real-time PCR are the standard methods for pre-seroconversion detection of an infection. Currently, these methods are laborious and do not lend well to POC applications. The **I**nterferometric **R**eflectance **I**maging **S**ensor (IRIS) is a portable, label-free, and multiplexed method for pre-seroconversion detection of single viral particles.

Rapid POC testing for viral infections will revolutionize the role of primary healthcare physicians. The implementation of a rapid, sensitive, and specific diagnostic instrument will: (1) facilitate immediate containment to minimize the spread of viral outbreaks, (2) accelerate the treatment of viral infections with confirmatory diagnoses, and (3) reduce the inappropriate use of antibiotics. The impact of this work will improve quality of care and aid in stemming the emergence of antibiotic resistant bacteria.

In recent years, several approaches have focused on optical techniques for particle detection [3]. Interferometric detection techniques have shown single nanoparticle sensitivity and the potential for real-time detection [4] [5] [6]. While sensitivity is an important criterion, identification and characterization of the particle will lead to a more specific diagnosis.

To this end, IRIS has been developed to provide high-throughput single particle detection along with size and shape discrimination. IRIS is approaching the sensitivity of PCR with the advantages of improved speed and multi-pathogen discrimination. This is accomplished by imaging the interferometrically enhanced contrast between the particle scattering and the reflection off the buried interface on a SiO2/Si substrate. By illuminating with a partially-coherent source, such as a LED, free-floating particles in the solution are automatically filtered out. This filtering is because the interferometric enhancement is confined to surface-bound particles. Specific virus immobilization is verified by size and shape. First, the virus is captured using specific probes like antibodies or glycans as shown in figure 1a. Next, a predictive model is used to determine the diameter as shown in figure 1b and aspect ratio of the captured

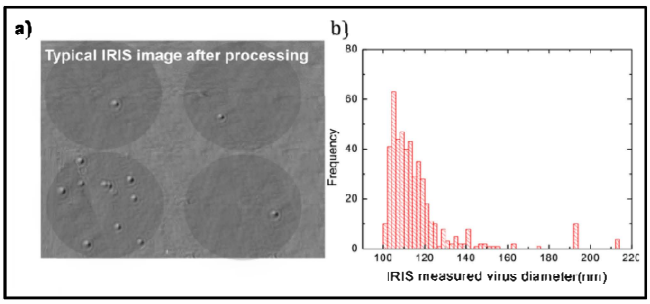

Figure 1- (a) Multiplexed IRIS image. (b) Particle sizing result from a H1N1 virus experiment. Outliers indicate aggregate particles.

nanoparticles [7]. Non-specific binding is reduced by eliminating detected particles not within the expected size or shape for target virus. This added specificity is especially useful when discriminating between viruses with similar symptoms. For example, Dengue and Ebola, two viral hemorrhagic fevers, vary in their shape. Dengue is a round virus with a diameter of 50nm while Ebola is a long and filamentous virus.

To prove this technology can be POC, a self-contained, portable prototype has been designed and fabricated as shown in figure 2. Constructed from off-the-shelf components, the prototype provides automated scanning and processing of the sample while minimizing user-training. Initial characterization of this instrument has shown clinically relevant levels of sensitivity for vesicular stomatitis virus (VSV) at 10^4 plaque forming units per mL (PFU/mL) in undiluted bovine serum. Future iterations of this prototype will optimize power consumption for battery applications and include microfluidics for sample preparation. With these improvements, this system will provide a simple, high-throughput sample-to-answer instrument for POC applications.

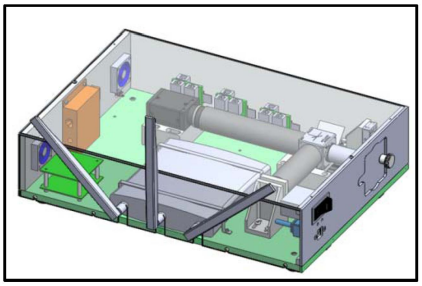

Figure 2 - Self-contained IRIS prototype design. A laptop is interfaced via ethernet or wireless connection. Custom software automates data acquisition and processing

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