Lehigh University

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Formula Grant Overview

Lehigh University received $119,007 in formula funds for the grant award period January 1, 2010 through December 31, 2012. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Development of a Point-of-Care Opto-Fluidic HIV Viral Load Detector - The intended result of this work is the design and construction of a hybrid platform that uses a combination of filtration and concentration methods in order to quantify the number of circulating HIV virons directly from patient blood samples. The purpose of this project is to perform fundamental studies to gauge the feasibility and performance of the device. After primary feasibility studies, we expect that the device will address the challenges associated with a point of care diagnostic platform that is both portable and easy to use in both resource-limited and general settings.

Anticipated Duration of Project

1/1/2010 - 12/31/2012

Project Overview

The purpose of this project is to create a novel, low-cost, globally deployable diagnostic device capable of detecting viral loads in HIV patients. HIV infection affects more than 33 million people worldwide, most of whom live in areas without even the most basic laboratory facilities. Although state-of-the-art technologies based on nucleic acid amplification are sensitive and quantitative for viral load analysis, they are widely accepted to be technically too demanding and too costly, thus severely limiting their applicability at the point of care (POC). The goal of this project is to furnish the device in a usably portable high-performance platform. Since the device will quantify whole viral particles from blood using a combination of filtration and detection, we must first verify the individual components of the technique. There are two main factors in the operation of the device; one being the straining/concentration element and the other being the labeling and detection of the filtered viral particles.

In order to detect the viruses, they must first be isolated from the other blood-borne debris and concentrated significantly in order to be optically detected. The clinically relevant level of circulating viruses can be as low as nine orders of magnitude below even the most sensitive

optical detection techniques. In order to accomplish the necessary concentration enhancement, we will combine nanoporous filtration membranes with an optical concentrator. The filtration membranes will strain and concentrate the viruses by six orders of magnitude. After this step, an optical concentrator will be used to further enhance the concentration. Additionally, a systematic fluorescent labeling of the virions will occur in tandem with the membrane element to allow optical detection.

Thus the isolation of the viruses requires a combination of mechanical and optical techniques, both of which require fundamental research to move forward. In addition to system development, we will also evaluate the system performance using clinical samples obtained from Lehigh Valley Hospital. The funding of this project will support both the fundamental research and clinical evaluation.

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Timothy Friel, MD – employed by Lehigh Valley Hospital

Expected Research Outcomes and Benefits

The end result of this project will be a compact device capable of quantifying HIV viral loads directly from whole blood. We expect that at the completion of this study, we will have explored the fundamental questions that surround the device; including the performance of the filtration membranes, the effectiveness of the fluorescent labeling and the concentrating power of the optical elements. Once the device has been tested with model systems and clinical trials, we expect that in the future a compact version of the platform will improve the public health by providing a cheaper, faster and more accessible diagnostic method for viral infections.

Although the focus in this project is HIV viral load quantification, the concentrating and detection platform could easily be applied to the detection of other pathogens for clinical diagnosis, homeland security and epidemiological monitoring purposes.

Summary of Research Completed

Research effort of the current funding period has been focused on 3 aspects: (1) Establish the concentration range in which the optical sensor can be applied to count nanoparticles of different sizes; (2) produce model viral particles with intrinsic fluorescence and (3) construct a

miniaturized set up towards real-time viral counting. The details of the research outcome in this period are summarized in the following text.

1. Experimental Methods and Materials

Instrumentation: Fig.1 shows the experimental setup for this study. An IR laser (1064 nm) was used to trap fluorescent nanoparticles, while a blue Argon laser (473 nm) was used to excite the fluorophores. The IR laser power is adjustable up to 90 mW and the blue laser power can be adjusted up to 3 mW. The trapping and excitation beams were combined through a Dichoric mirror and aligned confocally. A microscope objective lens (PlanFluor 100 X N.A.=1.3, Olympus) was used to focus the laser beams and collect the emitted fluorescence. The fluorescence emission was passed through a band-pass filter and directed into a photoncounting avalanche photo diodes (APD) (SPCM-AQRH-13-FC Perkin Elmer). The auto-correlation functions of the APD outputs were analyzed by a correlator (Corcle.com).

Production of pseudo-HIV virus: HEK 293T cells were transfected with JRFL, pcRev, R8 deltaEnv and p55 GAG/GFP plasmids by calcium phosphate update. After incubation for two days, the supernatant containing pseudo virus was filtered using 0.2 micron filter and centrifuged at 20,000 rpm on top a 20% sucrose cushion. The pellet was then collected and re-suspended in cell culture medium. The resulting particles have the same capsid structure and membrane proteins as the wild-type HIV virion, but lack some of the viral genome to minimize its pathogenicity. Moreover, these particles are labeled intrinsically by the green fluorescent protein genetically fused to a capsid protein.

2. Experimental results and data analysis

Validation of the optical technique using fluorescent nanoparticles

We used aqueous suspensions of 110 nm, 160 nm, 190 nm, 210 nm and 300 nm fluorescently labeled polystyrene particles prepared at 0.02% volume fraction to validate the optical technique combining FCS with optical trapping. We first acquired FCS autocorrelation function curves at different trapping laser powers ranging from 0 mW up to 30 mW. The resulting correlation curves for 110 nm particles are summarized in Figure 2(a), where each curve represents an average of 10 independent measurements. The typical autocorrelation function (ACF) $G(\tau)=(1/\langle N\rangle)(1+4D\tau/w_0^2)^{-1}(1+4D\tau/z_0^2)^{-1/2}$ was used to fit the curves at zero trapping laser power, where $\langle N\rangle$ is the average number of particles in the observation volume illuminated by the excitation laser beam, D is the diffusion coefficient of the particle, w_0 and z_0 are the beam waist and axial dimension of the excitation volume. The values of w_0 and z_0 for our setup were determined to be 0.22 µm and 2 µm, respectively. The fitting yielded $\langle N_0\rangle = 0.165$ and $D = 4.5\ \mu m^2/s$, where $\langle N_0\rangle$ is the particle number density within the detection volume $\langle N\rangle$ at zero laser power. The ACF equation above was then used to fit the curves under trapping laser for $G(0)$. Independently, the average brightness of each particle ε was then determined through photon counting histogram measured by the same APD. The average number of particles in the trap $\langle N_F\rangle$ was calculated from the average photon counts $\langle F\rangle$ divided by ε. When the inverse of $G(0)$ was compared with $\langle N_F\rangle$, a linear relationship was observed with a slope of 1 and intercept of 0 (Figure 2(b)). The linearity in Figure 2(b) validates $G(0)=1/\langle N\rangle$ for dilute

nanoparticles in an optical potential. Hence, the average number of nanoparticles in the center of optical trap $<N_{trap}>$ can be determined through the inverse of the ACF prefactor.

In order to quantify the ambient concentration from the number of particles in the center of the trap, the optical trapping energy on each particle needs to be determined. In Figure 2(c), we plot the normalized $<N_{trap}>$ versus the trapping laser power for particles with different diameters. At low particle concentration, when particles are far away from each other so that the interaction can be ignored, the number density of the trapped particles increases with the trapping laser power, following a Boltzmann distribution $G(0)^{-1}_{trap}/G(0)^{-1}_{0}=N_{trap}/N_0=exp(U_{trap}/k_BT)$. The trapping energy U_{trap} can be determined through an exponential fitting of the curves.

We tested the above analysis on nanoparticles with diameter ranging from 110 nm to 300 nm, and the trapping energies follow a linear relationship with the volume of the particles as expected for Rayleigh particles (Figure 2(d)). Since the concentration enhancement increases exponentially with the trapping energy, it is possible to enrich nanoparticles optically by orders of magnitudes. Thus, this combination of optical trapping with FCS allows us to detect the concentration of nanoparticles several orders lower than the lowest detection limit of FCS.

Miniaturized set up for pseudo-HIV counting

After validation of the method using nanoparticles, we tested our instrument using pseudo-HIV viral suspensions. The autocorrelation curve of the viral suspension (shown as the black curve in Figure 3) indicates that the viral concentration is about 10^9 particles/mL, which was independently confirmed by using a commercial p24 ELISA kit. When applying an optical trapping power of 40 mW to the suspension, the number of HIV viral particles in an excitation volume was enhanced by an order of magnitude. This result suggests that the optical property of viral particles permits their trapping.

The results so far demonstrate that we are able to lower the detection limit of FCS significantly by combining FCS with an optical trap. Analysis of the number density enhancement permits measurement of individual particle trapping energy and determination of nanoparticle ambient concentration. Moreover, the trapping effect of biological particles was observed and the concentration of pseudo-HIV viral particles can be determined by this method in a preliminary test.

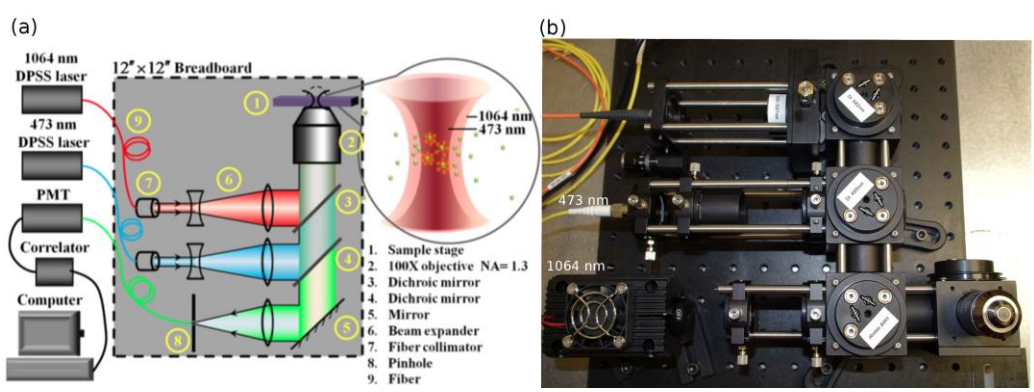

Fig. 1 A sketch for the experimental setup (a) and a photo of the miniaturized system (b).

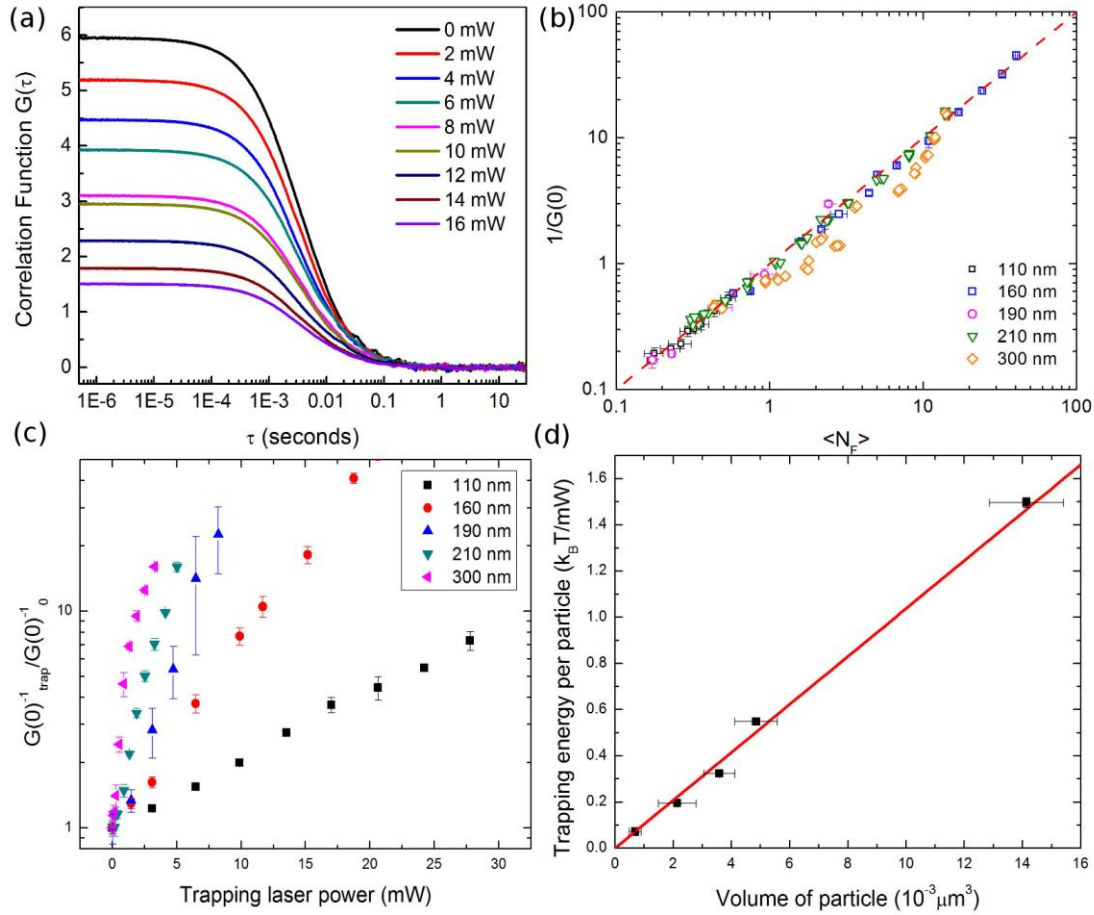

Fig. 2 (a) Correlation curves at different trapping laser power. (b) The inverse of the prefactor of autocorrelation function equals the average number of particles in the excitation volume. (c) The average number of particles in the excitation volume increases exponentially with trapping laser power. (d) The trapping energies of particles with different sizes can be determined from (c) and follows a linear relationship with the volume of the particle.

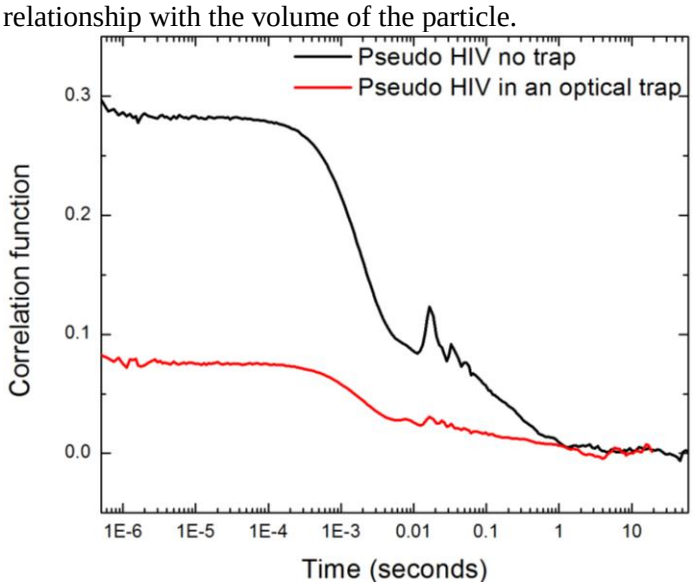

Fig. 3 Autocorrelation function of Pseudo-HIV viral particles in cell culture medium without an external optical trap (black line) and in an optical potential (red line).