Lehigh University

Annual Progress Report: 2009 Formula Grant

Reporting Period

January 1, 2010 – June 30, 2010

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 during the current funding period has been focused on developing the optical sensor for nanoparticle counting. This optical approach uses an optical trap to enrich fluorescently labeled viral particles at the focal volume of the beam. The concentration of particles is then detected by fluorescence correlation spectroscopy (FCS) aligned co-focally with

the optical tweezers. We constructed the optical setup and evaluated its performance using synthetic nanobeads of comparable size to HV virions.

Experimental Methods and Materials

Our experimental setup is shown schematically in Figure 1. The optical trap and FCS measurements are performed on an Olympus IX81 inverted confocal microscope. An IR (1064nm) laser is used as the trapping source, while a 488 nm argon laser is used to excite the fluorescent nanoparticles for detection. The intensity of the IR laser beam is controlled through a combination of a half-wave plate and polarizing beam splitter (Thorlabs). After attenuation, the IR beam is coupled into the right side port of the microscope. The power of the beam is modulated between 0 and 20mW by changing the angle of the half-wave plate. The trapping and excitation beams are aligned to be collinear upon coupling into the microscope, thus the objective focuses both beams into a common region. A 100X oil immersion objective with a numerical aperture of 1.3 is used both to focus the two laser beams and to collect the fluorescence emission from the nanoparticles. The collected fluorescence then pass through an optical filter and a 50:50 beam splitter (Thorlabs) before feeding into two avalanche photo diodes (APD) (SPCM-AQRH-13-FC Perkin Elmer). The photocurrent outputs of the two APDs are analyzed by a correlator (Flex02-01D, Correlator.com). The correlation function of the emitted fluorescence signal determined by the correlator is further analyzed and fitted with a PC.

All glassware is soaked in ethanol, thoroughly rinsed with de-ionized (DI) water (Barnsted, Easy Pure RF) and dried under nitrogen purge (99.999% purity) prior to use. We use 100nm polystyrene particles labeled with Texas-Red (Fisher Scientific, excitation maximum: 543nm and emission maximum: 612nm) as our test sample. The stock solution was serially diluted using pre-filtered de-ionized water to a final concentration of 0.01% (v/v) to minimize particle interaction. Ten microliters of particle suspension was then sandwiched between a cover glass and a standard microscope slide. Afterwards, nail polish was brushed around the edge of the coverslide to seal the liquid and prevent evaporation. The average thickness of the liquid layer sealed in was estimated to be 0.13μm.

The analyses of FCS spectra are conducted using the intensity *autocorrelation function* (ACF), $G(\tau)$, whose mathematical form can be predicted from the inherent fluctuation of fluorescence in the observation volume.

$$G(t) = \frac{\langle \delta F(t_0)\delta F(t+t_0)\rangle}{\langle F(t_0)\rangle^2} \tag{1}$$

Where $\delta F(t) = F(t) - \langle F(t)\rangle$, and $F(t)$ is the fluorescence, t_0 is the time lag. When single fluorescent, dimensionless molecule freely diffuses in and out of an ellipsoidal 3D-Gaussian observation volume at a low concentration, the standard autocorrelation function can be written as:

$$G(t) = \frac{1}{\langle N\rangle}\cdot\left[1+\frac{t}{\tau}\right]^{-1}\cdot\left[1+\frac{w_0^{\,2}t}{z_0^{\,2}\tau}\right]^{-1/2} \tag{2}$$

Where τ is the diffusion time a molecule spends on average in the observation volume ($\tau = {w_0^2}/{4D}$), w_0 is the beam waist of the excitation beam, z_0 is the axial dimensions of the observation volume and $\langle N \rangle$ is the average number of molecules in the observation volume. When time approaches 0, the autocorrelation function and the average number of molecules are related by the following relationship:

$$G(t \to 0) = {1}/{\langle N \rangle} \tag{3}$$

<u>Experimental results and data analysis</u>

We obtained the auto-correlation curves from FCS measurements within the optical trap, with the trapping laser power ranging from 0mW to 18mW. At least three correlation curves under each laser power were acquired and the averaged curves under different powers are shown in Figure 2.

While the nanoparticles have a finite size, the correction factor to the original dimensionless equation (Equations 2 and 3 above) for the average number in the FCS observation volume is < 5%. Thus the size effect is not taken into account in our data analysis. The particle interaction is also ignored in this study since we use diluted samples in a low conductive media: even under the highest trapping power in our study, there are only a few particles on average in the detection volume of 0.8 femtoliter. As a result, the distance between particles is much greater than that required for a strong particle interaction.

Free diffusive particles follow the 0W correlation curve (top black line) in Figure 2 and the average number of particles can be described by $1/G(0) = N_{Avg}$ (Equation 3). However, when particles experience an optical trapping force, their diffusion is hindered. In order to find out the physical meaning of *1/G(0)* for optically trapped nanoparticles, we compare the enhancement of 1/G(0) with the enhancement of average fluorescence intensity in the FCS measurement volume as shown in Figure 3. The enhancement values are calculated by taking the ratio of measurements under different trapping powers to those with zero trapping. The fluorescence intensity is the average of directly measured fluorescence fluctuation profile.

Assuming that all nanoparticles have the same fluorescent intensity, the fluorescence enhancement (F_{trap}/F_0) represents the particle number enhancement under optical trapping. In Figure 3, it is clearly observed that the experimental data follow the reference line with a slope of 1 and an intercept of 0. Mathematically, the relationship in Figure 3 can be described by the following equation:

$$\frac{\left({1}/{G(0)}\right)_{Trap}}{\left({1}/{G(0)}\right)_{0}} = \frac{F_{Trap}}{F_0} = \frac{\langle N \rangle_{Trap}}{\langle N \rangle_0} \tag{4}$$

In other words, not only is $1/G(0) = \langle N \rangle$ valid for free diffusive particles (Equation 3), 1/G(0) from the auto-correlation analysis can also be used to describe the average number of particles within an optical trap.

Next, we compared the enhancement of particle number (N_{trap}/N_0) in the observation volume as a function of the trapping laser power. Figure 4 demonstrates that the number of particles in the trap increases exponentially with the trapping power. Since the particle concentration in these experiments is very low, the inter-particle distance is much greater than the Debye length and the particle interaction is ignored in our experiments. Thus, we treat this highly diluted suspension as a semi-ideal gas and the particle number enhancement follows the Boltzmann distribution. As a result, the slope of a linear fit of the data in Figure 4 is the trapping energy per particle per milli-watt laser power, which is $0.10 \pm 0.04 k_B T$ in this study. This value well matches a previous reported trapping energy of 0.12 $k_B T$ per milli-watt of trapping power from our group, measured separately using very high concentration of fluorescent particles.

Future work

In the coming funding period, we plan to miniaturize the above microscope-based setup into a smaller platform that fits into a biosafety cabinet. Function of the miniaturized cage setup will be optimized with synthetic nanoparticles, pseudo-HIV viral particles carrying GFP and wild HIV viral particles.

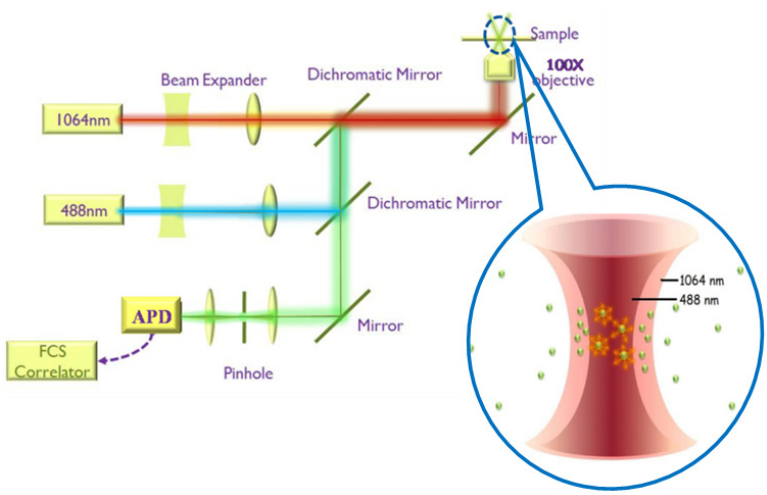

Figure 1. *A schematic showing the experimental setup of the optical components used in the experiment. (Inset) a cartoon depicting the FCS excitation laser (488nm) inside the trapping volume created by an IR laser (1064nm). Due to the their different wavelengths, the focal volume of the 488nm laser is roughly 10 times smaller than that of the 1064 nm laser.*

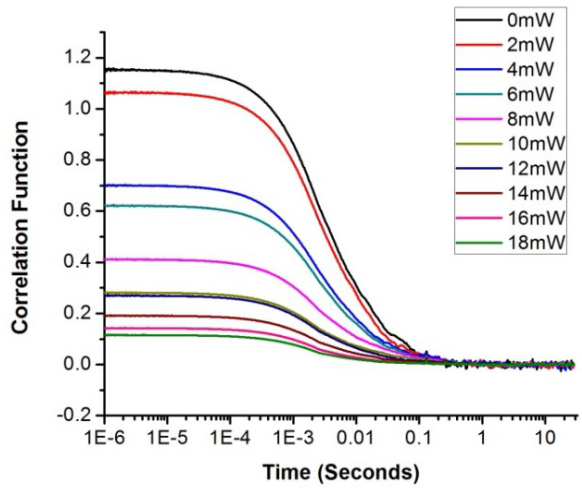

Figure 2. *FCS autocorrelation functions of 0.01% nanoparticle suspension under different trapping laser powers. Each curve represents an average of >3 measurements.*

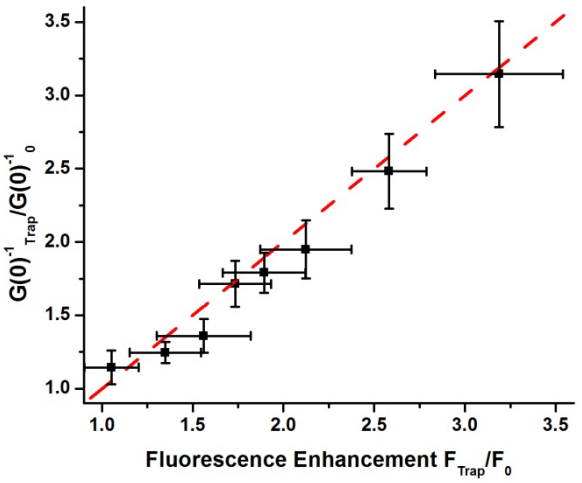

Figure 3. *The relationship between normalized 1/G(0) and fluorescence intensity in the FCS measurement volume under various trapping power from 0-18 mW. Black squares with error bars are the measured results (n>3) and the red line is a reference with a slope of 1 and an intercept of 0. This graph indicates that 1/G(0) in the auto-correlation analysis represents the average number of particles in the FCS observation even in the presence of an optical trapping power.*

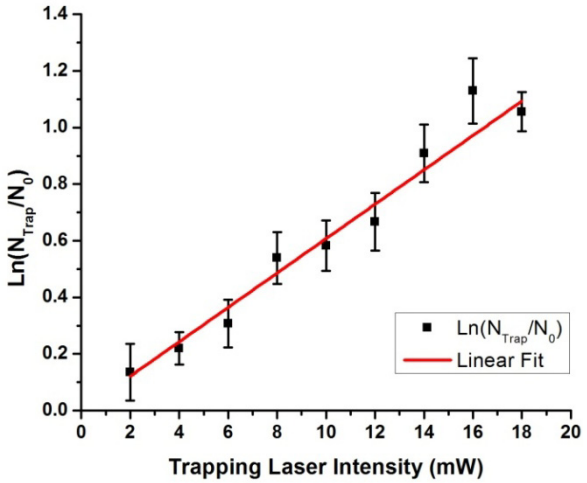

Figure 4. *The enhancement of particle numbers in the observation volume in a logarithmic scale versus the laser intensity. The number of particles in the trap increases exponentially with laser intensity, indicating that the particle number follows a Boltzmann distribution. Moreover, the slope of the graph can be used to calculate the trapping energy per particle.*