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Particle Diffusometry: PIV based method for Pathogen Detection on Smartphone-Based Platform

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Abstract

Finding clean drinking water in developing countries is a critical problem. Non-governmental organizations are devoting considerable effort toward testing the water sources in these countries. Typically testing such water sources consists of detecting the level of various pathogens endemic to the area in a sample of the water source. However, many extant testing techniques require collecting samples from the source and shipping them back to university labs or research centers that possess the expensive equipment necessary to conduct the test. In this study, a novel method for pathogen testing is introduced. Loop-mediated isothermal amplification (LAMP) is used to selectively amplify the DNA of the pathogen in a sample leading to a viscosity change in the solution. Particle diffusometry (PD), an extension of particle image velocimetry (PIV), has been shown in previous studies to be an effective yet simple technique to study the change in viscosity of solution (Figure 1.A) (Sie and Chuang, 2014), both spatially and temporally resolved. In our previous work in the lab setting, PD was used with a simple microfluidic chip and fluorescent microscope setup to calculate the Brownian motion of particles in a quiescent solution (Clayton, et al. 2017). To widen the scope of this lab technique making it fully field deployable, a robust, low cost, and portable hardware platform is developed based on smartphone technologies (Figure 1.B). The smartphone is inserted in a 3D printed case. The case provides a dark environment for the fluorescent microscopy. It also houses a low-power CW laser, optical system, and wavelength filter. In effect it turns the smartphone into a low-cost microscope. The smartphone camera is used as the recording device. It is proven to be capable of replacing CMOS camera commonly used in lab settings (Lee, 2018). By creating such a mobile application, lab-based sample processing is eliminated. Results of the water sample testing are obtained locally and quickly. In this study, we targeted the detection of vibrio cholerae in environmental water samples as a proof of concept. After the LAMP DNA amplification, the sample is mixed with polystyrene particles and 2 μL of the mixture is transported to the microfluidic chip and inserted to the device for recording and analysis. The diffusion coefficients are calculated directly on the smartphone. A statistical one-way ANOVA with Dunnett's Post-hoc test was used for confirmation of significance. There was statistically significant difference among the measured diffusion coefficients down to 10 cells/reaction when compared to the negative sample (Figure 1.C).

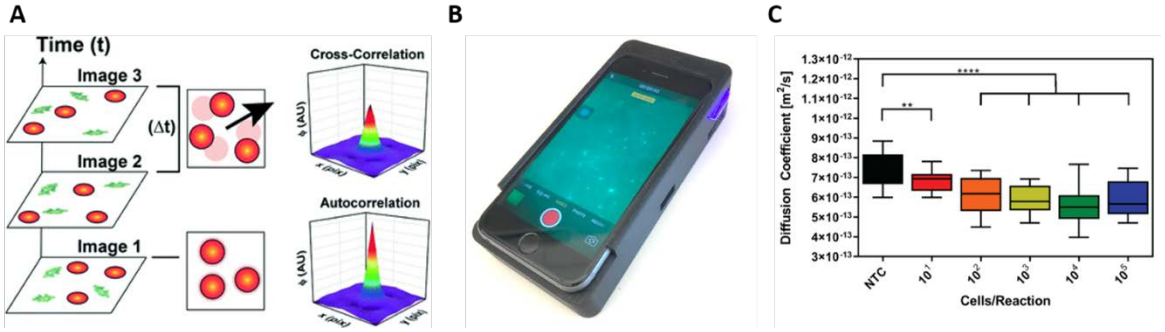

Figure 1. (A) Particle diffusion and image correlation. A stack of images is split into smaller interrogation regions, as pictured. Images which are correlated with themselves produce an autocorrelation peak (image 1). The correlation of sequential images (image 2 with image 3) provide cross-correlation peaks. Note that the cross-correlation peak is both wider and shorter as compared to the autocorrelation peak (Clayton, et al., 2017). (B) Fluorescent microscopy using iPhone as the recording/analyzing device. 30 seconds recording is obtained and another 60 seconds for the analysis. (C) Limit of Detection of V. cholerae cells in pond water. The diffusion coefficient measurements show a decreasing trend as a function of starting cell concentration with significance at 10^1 (** $p < 0.01$), 10^2, 10^3, 10^4, and 10^5 (**** $p < 0.0001$) cells/reaction. (n = 5) (Clayton, et al., 2019).

1 Introduction

Access to clean water should be a basic right to any given population in the world. Filtering pollutants out of water takes time, effort, and enormous financial support to be accomplished. Even in well developed countries, clean water supply does not fully extend to all parts of the country. In certain parts of the world, the quality of the water is not dependent on the taste, or color, but rather on its safety to drink at all. Still, thousands of people suffer due to lack of access to clean water and must choose between dying from dehydration or a pathogen present within their water supply. In order to minimize such problem, there are non-profit organizations that regularly test local water sources in an effort to maintain an updated map of pathogens.

To test, large quantities of water are collected throughout the designated testing sites and are tested locally if there are enough lab supplies; if not, they are sent to larger lab facilities in different cities or even different countries. Then the results are sent back to the sampling sites for the updates on their water map. These processes can take a few days to several weeks since most of the testing sites lack resources to complete the test process.

Our developed method allows for *in situ* testing of water samples, cutting down the processing time from days or weeks to hours. This method, using a smartphone and a 3D-printed portable device, involves particle image velocimetry (PIV) called particle diffusometry (PD). This new solution statistically quantifies the movement of seed particles thus detecting changes of viscosity of pathogens within water samples.

2 Theoretical Background

Particle diffusometry (PD) is a diffusion coefficient calculation using correlation analysis between two subsequent frames, statistical method quantifying the average motion of particles within the region of interest. Series of images of particles under Brownian motion is taken using a microscope setup. These images are then cut into a smaller window size containing 8 to 10 particles in each window (Clayton, et al., 2017). Subsequent frames are then correlated showing the time resolved particle movements. Movements of particles in time are directly proportional to the peak within the correlation peak, which is defined by width of e^{-1} height of the maximum correlation peak height (Sie and Chuang, 2014; Adrian and Yao, 1985). The relationship between peak width of the auto and cross-correlations to the diffusion coefficient was found by Olsen and Adrian (2000) to be

$$D = \frac{s_c^2 - s_a^2}{16M^2\Delta t} \tag{1}$$

In the numerator, representing the widening of the correlation peak width due to Brownian motion, note subscript *c* and *a* stands for cross- and auto-correlation respectively. In the denominator, *M* is for the total magnification used in the measurement, and Δt is the time between two subsequent images. Correlation analysis in calculating the peak width is performed using Fast Fourier Transform (FFT) for its known computational efficiency compared to double or quadruple looping structure for individual pixels in the region of interest (Raffel, et al., 2007).

3 Platform Development

Technological advancements have made possible the migration of various lab-based techniques to a portable platform. In order for any traditional lab-based techniques to become fully functional out in the field, the development of this smartphone application and miniaturization or combination of existing machines are necessary.

3.1 Software Development

In this study, an iPhone 6 and Xcode, Apple's software development platform, is used in the translation of previously developed MATLAB code. A built-in camera control system (AVFoundation), digital signal processing package (vDSP), and vast image processing libraries (vImage) have made this process seamless.

The iPhone's native camera was used to obtain a video of particle movements, and then frames were extracted at an appropriate frame rate. A total of 300 frames were extracted in every experiment video taken. Then, these raw frames went through various image processing algorithms to eliminate uneven background illumination, and to boost the signal to noise ratio as these processes are seen in Figure 2.

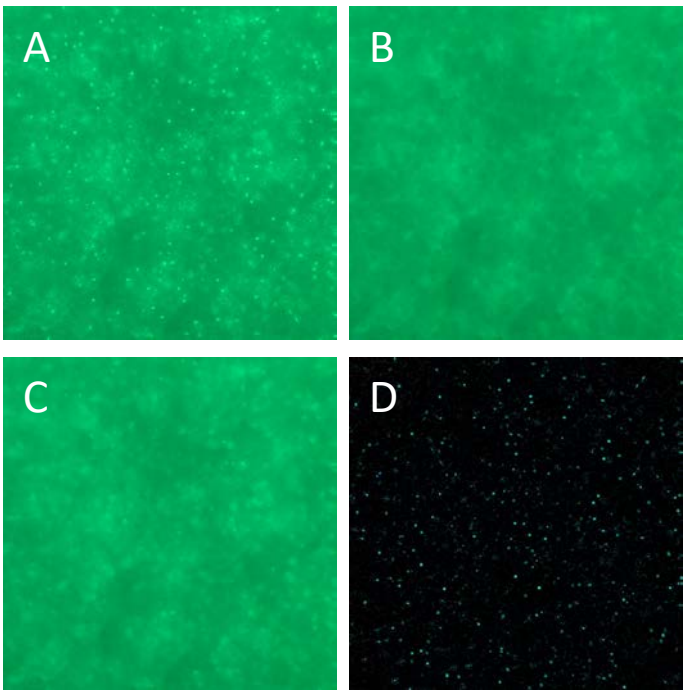

Figure 2. As image processing takes its steps from A to D, noise removal on background and boost of particle signal is noticeable. (A) Original extracted frame, (D) Final result of image processing algorithm.

With these processed images, a correlation analysis is completed to obtain 3 diffusion coefficients results. Averages of 100 correlation pairs were used to minimize statistical error within measurements. Each of these frame sets were obtained from the beginning, middle and towards the end of the video to observe outside factors within the time frame of experimentation.

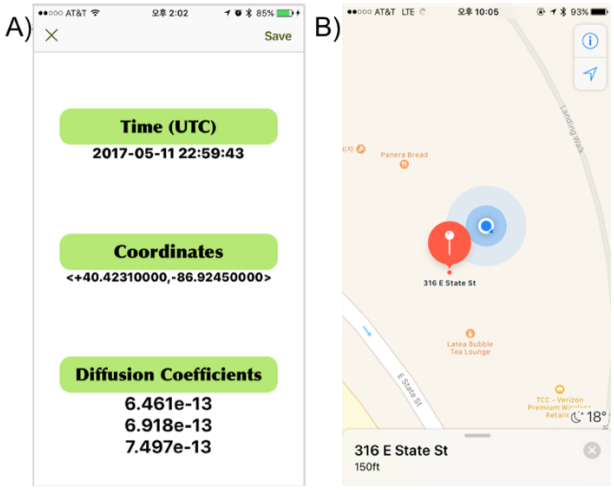

Figure 3. Result page. (A) Result page displaying diffusion coefficient results along the time and coordinate the measurement is taken, (B) Coordinate from the app (red pin) and the actual location of measurement (blue) (Lee, 2018).

As seen from Figure 3: Result page, the results contain both numeric values generated from the correlation analysis and the metadata extracted from the experiment video. The usage of a smartphone gives greater benefits from its inherent technology than simple miniaturization of processing power. The built-in GPS allows the extracted metadata location information to be used in the geolocation study to display the further spread of pathogen: i.e. the distribution of cholera. This accuracy is dependent on the percentage of CPU power designated for the task. If set for its maximum accuracy with moderate cell signal throughout the region, the distance between detected location to real-time location is within 1-200 feet.

3.2 Experimental Verification

Before starting the miniaturized hardware development, verification on the accuracy of the app was required. To provide the validation of PD on mobile device, experiments were conducted and results were compared to a CCD camera measurements. This experiment was held to test the accuracy of the iOS application built from MATLAB code and to compare the recording quality of the traditional CCD camera versus the CMOS camera on the iPhone 6.

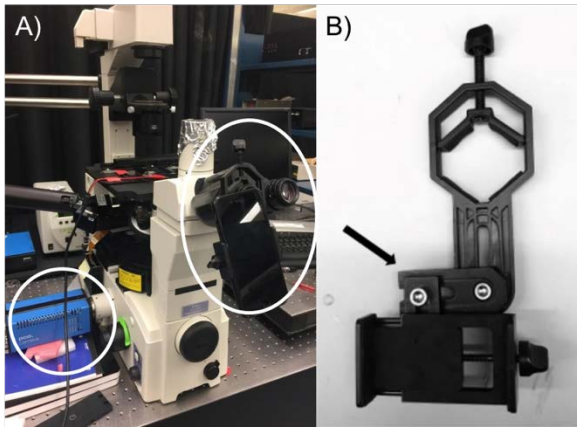

Figure 4. (A) iPhone attached to the microscope eyepiece, left circle is CCD camera, right circle is iPhone attached to an eyepiece, (B) Adaptor used to attach iPhone to microscope, black arrow indicates the aluminum spacer block (Lee, 2018).

To record using an iPhone, Gosky Universal Cell Phone Adapter Mount (Gosky Optics, Zhejiang Province, China) was used to attach the phone to the microscope eyepiece (as seen in Figure 4.A). For the sample, %V/V concentrations of glycerol were used with varying concentration from 0 to 80%. Videos were taken from the identical region, using PCO 1600 CCD camera and iPhone CMOS camera. Then the CCD videos were processed by both MATLAB and iOS code, and CMOS videos were processed by iOS code for comparison. A total of 15 data points were obtained during the experiment.

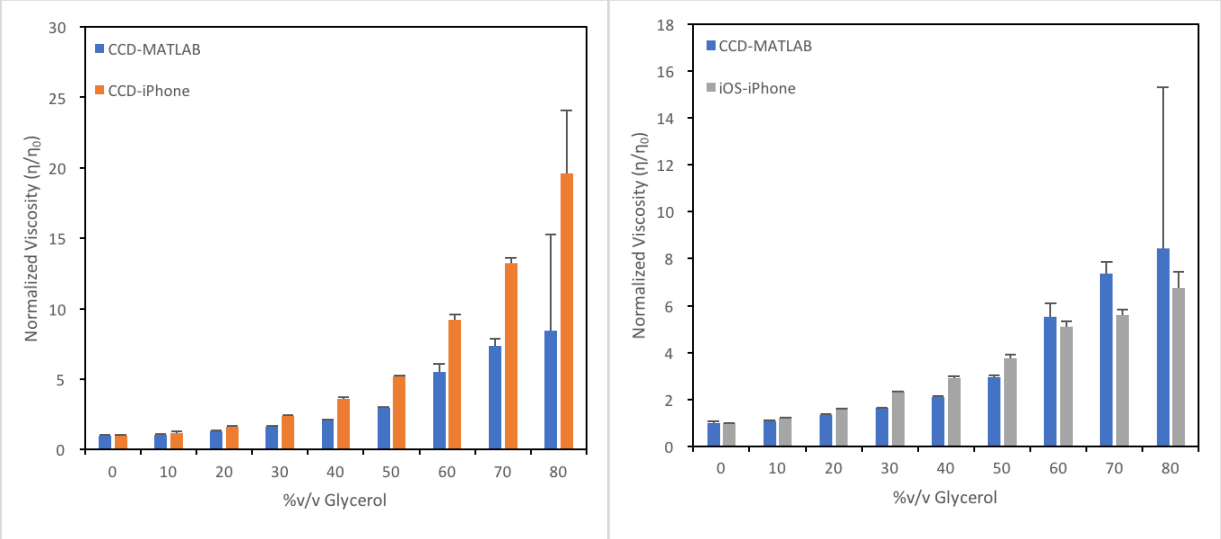

Figure 5. CCD measurement comparison (Left). iPhone and lab comparison (Right). Notice the trend is identical (Lee, 2018).

With controlled temperature value and obtained diffusion coefficients from the experiment, the viscosity of each experiment's results were calculated using Stokes-Einstein equation, (Equation 2). The viscosity values were then normalized with results from the water experiment.

$$D = \frac{k_B T}{3\pi\mu d} \tag{2}$$

Taking CCD obtained video processed by MATLAB code as the base standard of this experiment, both the CCD and iPhone obtained videos, processed by iOS code, shows an increasing trend of viscosity. CCD-iPhone from Figure 5 shows higher values of normalized viscosity due to a higher level of noise that is present during the conversion process when CCD images were imported to the iPhone for analysis.

3.3 Hardware Development

To miniaturize the lab equipment, several key components are identified. Post processing computing power with a MATLAB program is converted into iOS application. Recording capability of PCO 1600 CCD camera was exchanged with CMOS camera of iPhone 6. A full size fluorescent inverted microscope was the next step. Most smartphone cameras use a CMOS sensor, which consists of one red, two green, and one blue sensors per pixel called a Bayer filter to capture incoming light. To utilize this filter, green polystyrene 400 nm microspheres (Bangs Laboratories) were selected. To excite this particle, a blue laser with wavelength of 450 nm at 80 mW power

was used. The elimination of excessive blue light was required to better image the excited green particles. Yellow straw filter was selected to work as an emission filter. For the replacement of original 40X objective, a 0.5 mm diameter ball lens made of N-BK7 from Edmund optics was used.

Figure 6. Components of the hardware platform developed

A containment for the lens was built using aluminum for durability purposes; therefore, it contains a simple drilled hole to hold the lens which then can be inserted into the rest of the device for camera alignment. A microscope stage was made with a simple bolt and nut mechanism with a 3D printed structure working as the knob handle for rotation and as a stage for the microfluidic chip to be placed on. A combination of the blue laser, the ball lens, and the yellow filter worked as a replacement of the microscope's fluorescent excitation and emission filter, and the magnification objective. Fluorescent microscopy is done in the dark room setting for a better capture of excited particles under the laser. To encase all the components of the hardware, a black 3D printed case was designed. Assembled hardware with iPhone placed is shown in Figure 1.B.

4 Experiment

The purpose of this experiment was to determine the limit of detection of the smartphone-based platform. Varying concentrations from 10^0 to 10^5 copies of DNA or cells per reaction of V. Cholerae were prepared for this experiment (Clayton, et al., 2019). At the end, 5 different reactions were prepared, which yielded 3 data points in each reaction per concentration. A total of 15 data points per reactions were used for the post-analysis.

4.1 Method

Loop-mediated isothermal amplification (LAMP) was performed on these reaction tubes. LAMP primers were to target the cholera toxin A, the gene within the toxigenic strain (Okada, et al., 2010). For particle preparation, 400 nm green streptavidin coated polystyrene fluorescent particles were used (Bangs Labs, Fishers, IN, USA) to maximize the benefit of Bayer filter provided by

CMOS camera. Streptavidin coated particles were necessary as the biotinylated biological sample was prepped. By mixing the prepared sample before inserting into the chip, binding process and hindrance of the Brownian is achieved.

Figure 7. Recording snip of the video taken using iPhone 6. Center square area was cropped for further image processing and PD analysis. Notice the circular aberration occurred at the outer edges due to the usage of ball lens as optical system.

The microfluidics chip was prepared by using cyclo olefin polymer (COP) of thickness 376 um (Zeon, Japan) as the base plate while double-sided tape (Therm-O-Web, Weeling, IL) with a hole punched through was used to form the fluid chamber (Lee, 2018), and 60 um COP was used for the imaging side. Thickness of the imaging side was tuned specifically to compensate focal length of the optical system used in the device.

4.2 Results

For the post processing, one-way ANOVA with Dunnett's post-hoc against the negative sample was performed. As seen from Figure 1.C, a decreasing trend of diffusion coefficients were observed as the concentration of the biological sample increased in accordance with the theoretical trend calculated using Equation 2. From the analysis, there were statistically significant differences for all the samples (p-value < 0.001 for 10^1 and p-value < 0.0001 for 10^2 – 10^5 DNA copies/reaction).

5 Conclusion

The development of a smartphone-based portable platform to perform PD measurements, previously developed as an in-house MATLAB code, was translated into an iOS application to be utilized as a full lab-scale, miniaturized, fluorescent microscope using an iPhone 6 camera with the aid of an optical and laser system. Validity of the app was confirmed by performing a separate experiment by affixing the iPhone to microscope eyepiece. Varying the concentration of V. Cholerae was used to perform the detection of a pathogen within an environmental water sample which showed a decreasing trend of diffusion coefficient as the biological sample concentration was increased. The post-analysis shows statistically significant results down to 10^1 DNA copies/reaction for the limit of detection. This proof of concept of quick detection of a pathogen

within the water sample can provide a gateway to simple water testing throughout regions where clean water supply is challenging due to pollutants.

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