PAPER MICROFLUIDIC DETECTION OF SALMONELLA USING A SMART PHONE

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ABSTRACT

We describe a novel demonstration of latex immunoagglutination assay in a paper microfluidics format and subsequent Mie scatter detection using the smart phone's flash and digital camera as optical sensing platform. The background noise was eliminated through careful optimization of Mie scatter parameters: the diameter of latex beads, surfactant concentration, the angle of scatter detection, etc. The detection limit was 10 CFU/mL for *Salmonella typhimurium* using the paper microfluidics and smart phone. This method can be used for any field deployable assays (demonstrated for foodborne pathogens here; can be used for medical, veterinary, and environmental diagnostics) and is low-cost, easy-to-use, near-real-time and handheld.

KEYWORDS

Paper microfluidics, Salmonella typhimurium, latex immunoagglutination, Mie scatter.

INTRODUCTION

Paper microfluidics has gained great popularity in recent years, potentially as a low-cost, field deployable assay device [1]. The paper itself, however, is not a homogeneous medium and generates substantial background noise (optically and electrochemically). To use this paper microfluidics for field assays, the sensitivity must be improved significantly and the corresponding detection system must be made handheld and easy-to-use. To this end, we demonstrated, for the first time, the immunoagglutination assay in a paper microfluidics and its Mie scatter detection using the smart phone's flash and digital camera as an optical sensing platform. The immunoagglutination assay provides the improved sensitivity (very low detection limit), the angle-specific optimized Mie scatter detection eliminates the effect of background reflection/scatter, and the smart phone provides a handheld and easy-to-use optical detection platform.

EXPERIMENT

The paper microfluidics was fabricated using cellulose chromatography paper (Whatman) and SU-8 negative photoresist [2]. The pattern was printed on a transparency film using a laser printer, which was used as a mask. The paper was immersed within photoresist solution, dried, and UV-exposed with the aforementioned mask. Acetone and isopropyl alcohol (IPA) were used for rinsing. The resulting channel is SU-8-free and hydrophilic, allowing the sample to spontaneously flow through it by capillary action. Anti-Salmonella were conjugated to 920 nm, highly carboxylated polystyrene beads by covalent bonding as described previously [3,4]. This bead suspension was applied to the detection area of paper microfluidics and fully dried. The final paper microfluidics would have multiple channels, each with different antibody-conjugated beads pre-loaded in it (Fig. 1), detecting multiple pathogens simultaneously. (The result with a single channel is shown in this work.) The paper microfluidics was dipped into the target Salmonella solutions (together with a blank = phosphate buffered saline), which traveled through the channel by capillary action. The target molecule (Salmonella) in the solution caused immunoagglutination of antibody-conjugated beads within paper fibers, thus increasing the effective diameter and morphology of the beads, which was detected by measuring light scatter intensities (Fig. 2).

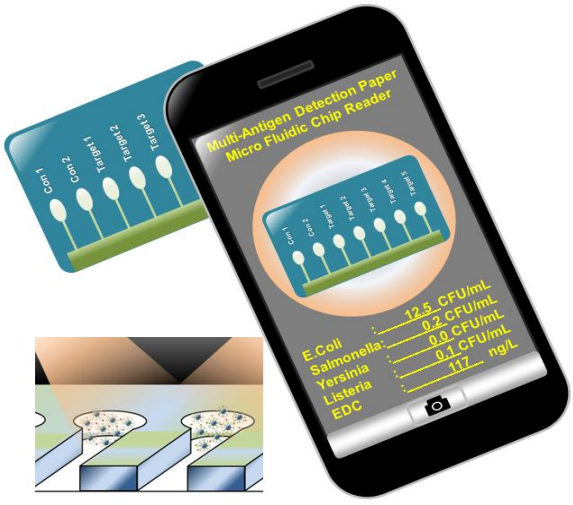

Figure 1. Illustration of smart phone detection from multi-channel paper microfluidics.

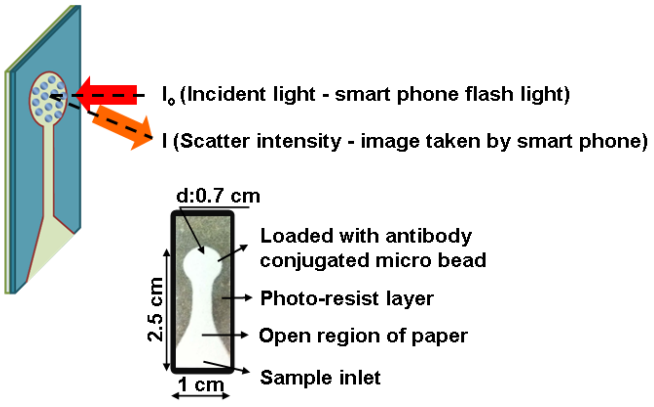

Figure 2. Schematic of the paper microfluidics and the subsequent optical detection.

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Latex immunoagglutination assay and subsequent Mie scatter detection is a label-free, non-spectrophotometric detection, which may improves the detection limit down to 10 CFU/mL bacteria or 10 pg/mL antigens [3,4]. There is a difficulty in demonstrating latex immunoagglutination assay and subsequent Mie scatter detection in a paper platform: difficulty in differentiating the true light scatter caused by the bead immnoagglutination from the reflection or non-specific scatter by the paper fiber. Therefore, a benchtop apparatus was fabricated using fiber optic cables, blue (λ=475 nm) LED light source (LS-450, Ocean Optics) and a miniature spectrometer (USB4000, Ocean Optics), and the angles of light irradiation and detection were varied using rotational positioning stages (Fig. 3). Forward scatter (i.e. light passes through the paper) from 0° to 80° and back scatter (i.e. light reflects from the paper) from 100°-160° were tested (Fig. 4).

RESULTS AND DISCUSSION

The benchtop experiments revealed that back scatter showed stronger signal due to the non-transparent nature of the paper, and the optimum angle was obtained at 150°.

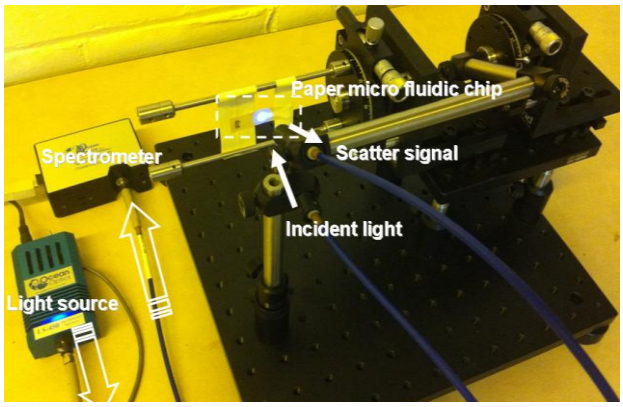

Figure 3. Benchtop system for optimizing the angles for incident and detection light.

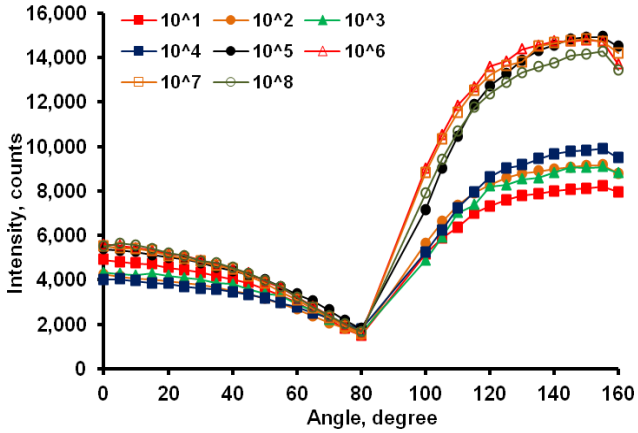

Figure 4. Light scatter intensities over scatter angle, varying Salmonella concentration.

Using this setup and the optimized angle, the light scatter intensities were collected varying the target (Salmonella) concentrations. The paper microfluidic strips were pre-loaded with anti-Salmonella-conjugated polystyrene beads and dipped into a series of Samonella solutions varying their concentrations. The presence of target (Salmonella antigens) in the solution caused the antibody-conjugated beads to immunoagglutinate in paper fibers, thus increasing the effective diameter (and morphology) of the beads, resulting in the change in scatter intensities at the optimized angle. All data were normalized to that of a blank (PBS), and a standard curve was constructed (Fig. 5). The curve shows initial increase, followed by a dip, which is purely an optical phenomenon of Mie scatter as demonstrated previously by Mie scatter simulations (Fig. 6) [3], then by a continued increase until the antigen saturation occurs at 10^4 CFU/mL. This dip will become less pronounced through reducing the size of channels and the use of Tween 80 (thus limiting the growth of beads only up to doublets or triplets) [4].

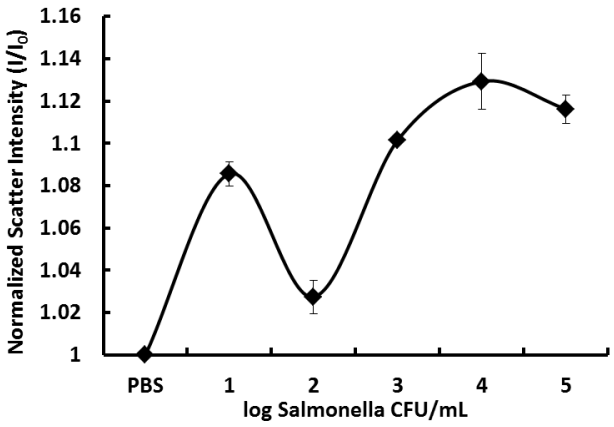

Figure 5. Normalized light scatter intensity at 150° over target concentration using the benchtop system. Average of three different experiments. Error bars are standard errors.

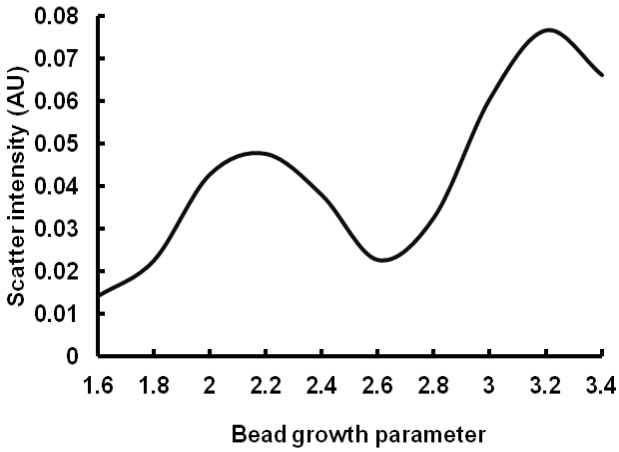

Figure 6. Simulated light scatter characteristics as the effective bead size grows upon immunoagglutination.

The whole experiment was repeated, this time using two smart phones (iPhone 4), one as a light source (white flash) perpendicular to the paper and the other as a detector (digital camera) at 150° (Fig. 7). The acquired images were adequately cropped to show only the area of interest (antibody-conjugated beads zone) and the pixel intensity was averaged using the ImageJ software. In these experiments, Tween 80 surfactant was also pre-loaded in the

channel, in between the sample loading zone and the antibody-conjugated beads zone. In addition, the channel width was substantially narrower (1 mm).

The resulting standard curve (Fig. 8; average of three different experiments, each time with different paper strips and different reagents/samples) does not show dip at 10^2 CFU/mL and the detection limit is again 10 CFU/mL. However, the data at 10^3 CFU/mL became erratic. In fact, only one data set showed continued increase in normalized scatter intensity while the other two showed the normalized scatter intensities the same as that of PBS. This indicates that the highly concentrated Salmonella solution could not travel to the antibody-conjugated beads zone for those two cases, i.e., no immunoagglutination occurred. Further investigation is necessary to address this issue. The overall normalized scatter intensities in Fig. 8 were smaller than those in Fig. 7, primarily due to the presence of Tween 80 and the narrower channel width, which limited the growth of beads, as well as the lower resolution of the camera (8-bit for each color) compared to 16-bit of a spectrometer.

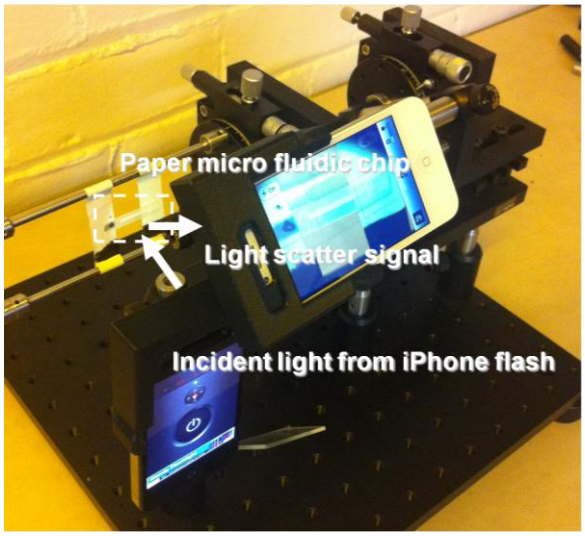

Figure 7. Two smart phones replaced the Ocean Optics light source, Ocean Optics miniature spectrometer, and a pair of optical fibers to measure light scatter intensity.

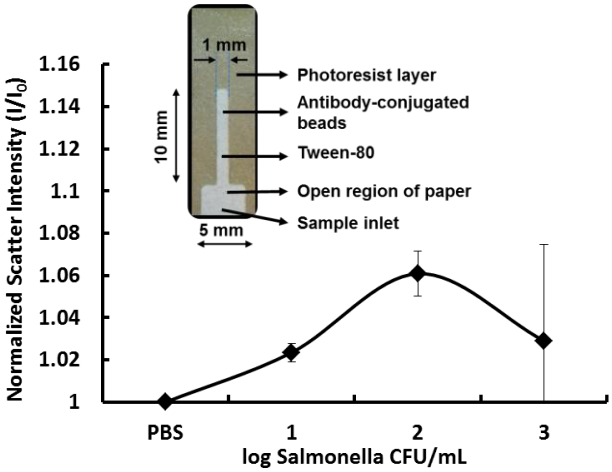

Figure 8. Top inset: the layout of a "new" paper microfluidic strip with narrower channel and pre-loaded Tween 80. Bottom: normalized light scatter intensity at 150° over target Salmonella concentration using the smart phone system. Average of three different experiments. Error bars are standard errors.

Future work should include designing and fabrication of a small reader device [5] that can accommodate the paper microfluidic strips and a single smart phone (for both light irradiation and image detection), to detect at a specific optimized angle (150°). Alternatively, a multi-channel paper microfluidic strip can be designed and fabricated with multiple negative control channels, which can be used to normalize the scatter reading at any (acceptable) angle of detection. A smart phone software application should also be developed that will quantify the extent of Mie scatter from the acquired digital images.

The proposed system demonstrates a strong potential to be used in field situations at extremely low cost.

REFERENCES

[1] A. W. Martinez, S. T. Philips, E. Carrilho, S. W. Thomas, H. Sindi and G. M. Whitesides, *Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis*, Anal. Chem. 80, 3699-3707 (2008).

[2] A. W. Martinez, S. T. Philips, B. J. Wiley, M. Gupta and G. M. Whitesides, *FLASH: A rapid method for prototyping paper-based microfluidic devices*, Lab Chip, 8, 2146-2150 (2008).

[3] B. C. Heinze and J.-Y. Yoon, *Nanoparticle immunoagglutination Rayleigh scatter assay to complement microparticle immunoagglutnation Mie scatter assay in a microfluidic device*, Colloids Surf. B, 85, 168-173 (2011).

[4] D. J. You, K. J. Geshell and J.-Y. Yoon, *Direct and sensitive detection of foodborne pathogens within fresh produce samples using a field-deployable handheld device*, Biosens. Bioelectron., 28, 399-406 (2011).

[5] D. J. You, T. S. Park and J.-Y. Yoon, *Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays*, Biosens. Bioelectron. doi:10.1016/j.bios.2012.07.014 (2012).

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