# RAPID BACTERIOPHAGE DETECTION VIA HOST CELL AMPLICATION IN A DROPLET-BASED OPTOFLUIDIC SYSTEM

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## ABSTRACT

This paper presents a real-time and label-free bacteriophage detection technique with high sensitivity and low cost by using a droplet-based optofluidic system. The bacteriophages and host cells (*E. coli*) mixture are encapsulated in microdroplets during their formation in mineral oil. The scattering patterns of the bacteriophages and the host cells containing droplets are captured by an optical sensor and analyzed by a signal processing system. The scattering patterns of microdroplets with different host cell concentrations are measured. The optofluidic system is developed to effectively detect and quantify bacteriophages in real-time with a detection resolution of $10^3$. A higher resolution can be realized by using a data processing system to analyze the scattering pattern, which will be done in our future work.

## KEYWORDS

Optofluidics, bacteriophage, scattering pattern

## INTRODUCTION

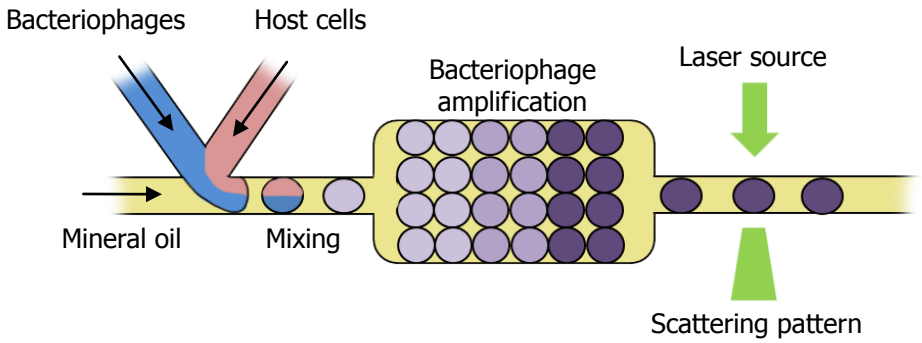



Figure 1: Schematics of the droplet-based optofluidic system for bacteriophage detection. Bacteriophages are mixed with the host cells (*E. coli*) and encapsulated in isolated droplets. Once the bacteriophages are amplified, the droplet scattering pattern is captured and analyzed.

Rapid detection and identification of viral contamination in drinking water sources are critical for maintaining water safety standards. Waterborne viruses, which are significantly small in size (hundreds of nanometers), are difficult to detect and treat [1-2]. Conventional methods commonly include pre-concentration process and culturing steps or molecular biological assay, which require at least 24 h before the detection results are available [3-4]. These detection techniques are not in real-time, which are not effective to avoid an outbreak due to viral contamination in drinking water. In this paper, we design a droplet-based optofluidic system for real-time and label-free bacteriophage detection with high sensitivity and low cost.

Figure 1 shows the design of the droplet-based optofluidic system for bacteriophage detection. The sample containing bacteriophages (M13KE phage), which is mixed with host cells (E. coli strain K12). This mixture solution is divided into droplets by using the microfluidic droplet generating T-junction structure. Then, the droplets are accumulated in a microchamber and incubated for bacteriophage amplification. The enrichment of bacteriophages affects the concentration of the host cells in the droplets, which is monitored by detecting the diffraction and scattering patterns of the individual droplets. The diffraction and scattering patterns are depending on the concentration of the host cells in the droplets.

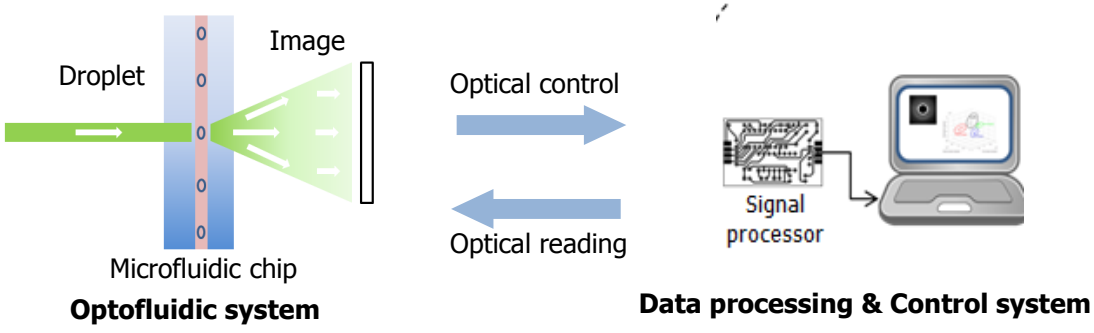
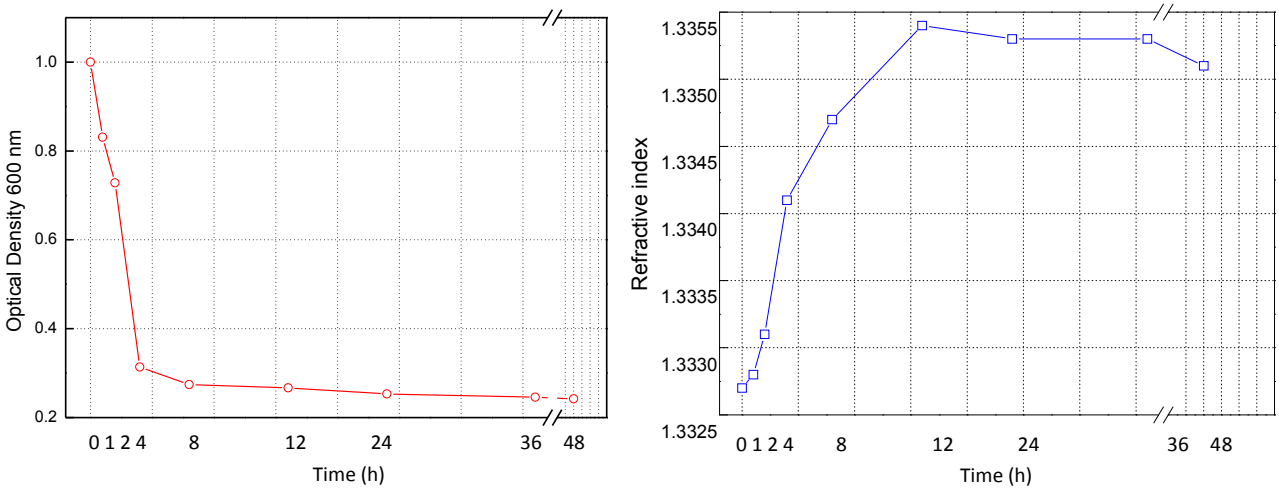



Figure 2: Droplet-based optofluidic system setup for bacteriophage detection.

## EXPERIMENTAL SETUP

The experimental setup of the droplet-based optofluidic system is shown in Fig. 2. A precision pump is used to control the flow rate of the two immiscible phases. The bacteriophages and host cells mixture are encapsulated during the microdroplets formation in mineral oil. The size of the droplets can be controlled by varying the flow rates of the two immiscible phases. The initial concentration of the bacteriophage and host cells are $10^3$ pfu/ml and $10^9$ /ml, respectively. Bacteriophage cannot be observed directly by the optical microscope because of its small size. To quantify the concentration of bacteriophages and host cells in the droplet, the scattering pattern of the droplet is captured and characterized. The optical detection system consists of a laser source and optical components for beam focusing. The scattering pattern of the bacteriophages and the host cells containing droplets is captured by the optical sensor and analyzed by the signal processing system.

## EXPERIMENTAL RESULTS AND DISCUSSIONS




(a) (b)

Figure 3: (a) Optical density 600 nm and (b) effective refractive index change of E-coli culture medium for 48 h.

Figure 3 shows the transmitted optical density and effective refractive index change of *E-coli* culture medium in 48 h. The optical density can be correlated with the *E-coli* concentration, which is the general measurement method. The concentration value is consistent with the standard growth curve of *E-coli*. Although the concentration of *E-coli* has significantly changed, the effective refractive index does not change significantly (from 1.3325 to 1.3355) due to the bacterial and culture medium has similar refractive index (1.3841 and 1.3325 for 600 nm) [5]. The detection resolution could only be $10^5$. Therefore, it is more accurate to measure the distribution of cells among culture medium by using the scattering pattern.

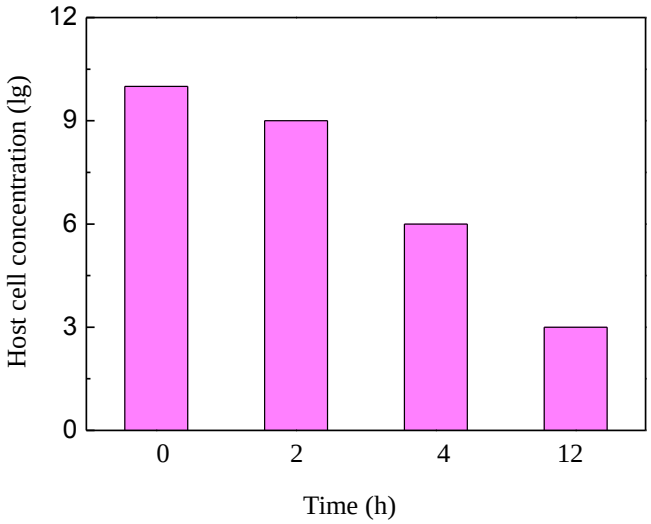



Figure 4: Relationship between bacteriophages amplification and host cell concentration change.

Figure 4 shows the relationship between the bacteriophage amplification and the change of the host cell concentration. The concentration of the host cell is calculated by counting the number of host cells in each individual droplet or measured by using cell counting chamber analysis. The results show that the host cell concentration decreases from $10^9$ /ml to $10^3$ /ml after 12 h incubation when the bacteriophages have been amplified under several amplification cycles.

Figure 5 shows the scattering pattern analysis of a single microdroplet. During incubation, the population of the bacteriophages increases and the number of host cells decreases in the mixture. The variation of concentration of the host cells in the droplet changes the distribution density and the refractive index of the droplet. As a result, the droplets containing different amounts of host cells have different scattering patterns as shown in Fig. 5(b) and (c). Fig. 5(b) and (c), which show the fluorescent intensity distribution of the scattering pattern of the microdroplet with

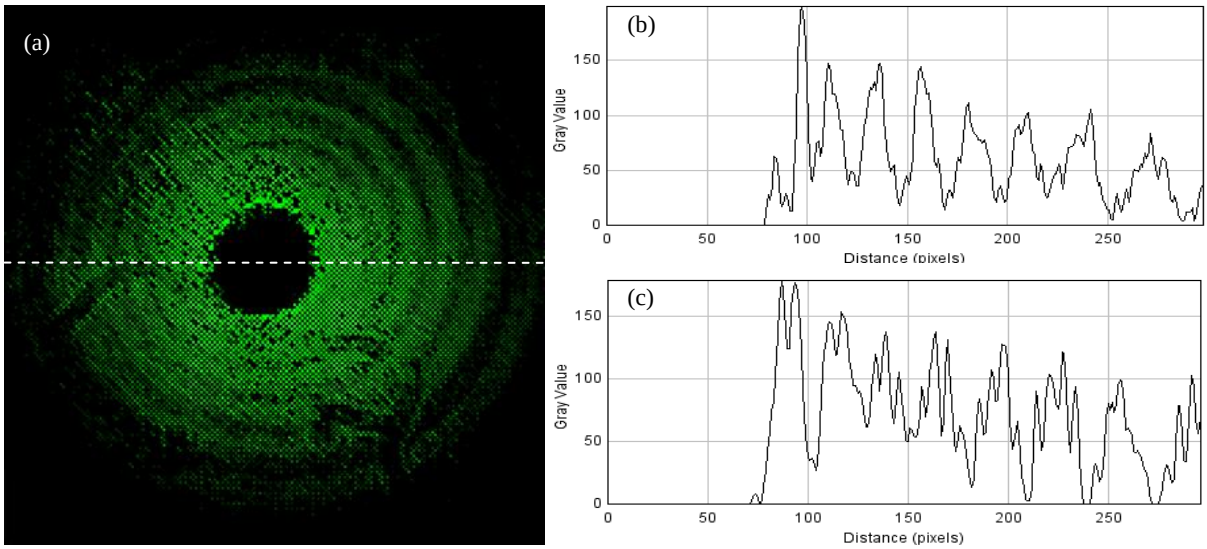



Figure 5: (a) The scattering pattern of single microdroplet (b) the fluorescent intensity distribution of the scattering pattern of *E. coli* concentration of $10^3$ and (c) the fluorescent intensity distribution of the scattering pattern of *E. coli* concentration of $10^{12}$.

an *E. coli* concentration of $10^3$ and $10^{12}$, respectively. The total intensity value is significantly reduced with higher concentration of *E. coli* because the transmission rate is reduced. The normalized integration gray value reduces by 0.2725/cell. The detection resolution is $10^3$. Furthermore, the distance of adjacent peaks increases with the increase of the host cell concentration. The pattern becomes indistinctive from a clear distribution one. More information can be analyzed by using a data processing system to get a higher resolution detection, which will be done in our future work.

## CONCLUSIONS

In summary, a droplet-based optofluidic system is developed to effectively detect and quantify bacteriophages. The existence and amplification of bacteriophages can be monitored by measuring the concentration change of host cells, which are mixed inside a single microdroplet. A real-time host cell concentration measuring system is designed based on the optical scattering pattern analysis. The optofluidic system is developed to effectively detect and quantify bacteriophages in real-time with a detection resolution of $10^3$. A higher resolution can be realized by using a data processing system to analyze the scattering pattern, which will be done in our future work.

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