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Real-Time Measurement of Single Bacterium's Refractive Index using Optofluidic Immersion Refractometry

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Abstract

This paper presents a biophysical method to characterize single bacterium in water by using an on-chip optofluidic immersion refractometer. Water safety is a major factor in the well-being of people, but the presence of bacteria such as *Escherichia coli* (*E. coli*), *bacillus subtilis*, *Shigella flexneri* and *vibrio cholera* in drinking water can lead to infectious diseases such as typhoid fever. Hence, it is crucial to detect and identify bacteria to prevent bacterial outbreaks. In this paper, an optofluidic immersion refractometer is developed to measure three biophysical parameters, i.e. size, shape and refractive index. The refractive index of a single bacterium is measured in high sensitivity of 0.005 RIU. This system is an innovative method to allow on-site real-time detection of single bacterium in water. It significantly reduces the amount of detection time and do not require trained personnel or additional chemical and biological reagents.

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Keywords: Optofludics, immersion refractometer, waterborne bacteria

1. Introduction

Water safety is a main concern especially in heavily populated cities due to the presence of pathogenic microbial in drinking water may cause fatal outbreaks [1]. One of the main sources of pathogenic contaminants in drinking water is bacterial contamination such as *Escherichia coli* (*E. coli*), s*higella flexneri*, *vibrio cholera* and *Salmonella enterica* etc [2]. Other bacterial caused diseases include typhoid by *Salmonella enterica* and also hepatitis [3-4]. The

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infection is severe and may be fatal for children, elderly and immune-compromised individuals such as HIV positive patients. Hence, it is crucial to detect and identify bacteria to avoid bacterial outbreaks [5-6].

This paper presents a biophysical method to characterize single bacterium in water by using an on-chip immersion refractometer. Three biophysical parameters are measured: size, shape and refractive index. The refractive index of a single bacterium is measured based on the null-method in immersion refractometry.

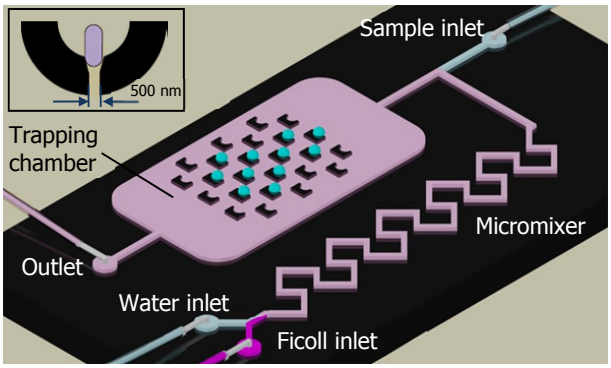

Figure 1: Schematic illustration of the optofluidic chip for biophysical measurement of single bacterium by using null-method in immersion refractometry. Inset shows the trapping structure with a gap of 500 nm.

2. Working principles and chip design

Figure 1 shows the schematic illustration of the optofluidic immersion refractometer. Samples are loaded into the microchannel and trapped in the sample trapping area which consists of an array of trapping sites. Each trapping site has a U-groove structure with a small gap of 500 nm [7-9]. The working principle of immersion refractometry is illustrated in Fig. 2. When the external buffer medium has a refractive index higher than the one of the bacterium, the bacterium appears to be darker (Fig. 2a) [10-11]. Whereas when the external buffer medium has a lower refractive index, the bacterium appears to be brighter (Fig. 2c). Once the refractive index of the buffer medium is equal to the one of the bacterium, the bacterium appears to be invisible (Fig. 2b) [12]. Hence, this null method can be employed to measure the refractive index of the bacterium. To tune the refractive index (RI) of the buffer, a microfluidic mixer is used to mix deionized water and Ficoll liquid (RI = 1.333 to 1.446).

3. Experimental results and discussion

The sizes, shapes and refractive indices of *E. coli*, are studied. Figure 3a and 3b illustrate the distribution of the

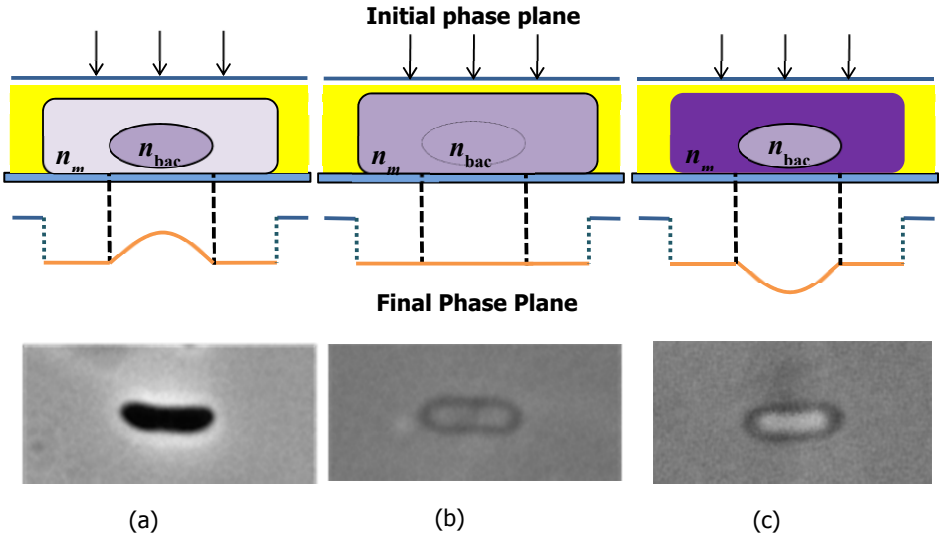

Figure 2: Phase transformation and the phase-contrast microphoto of E. coli cell being immersed into a medium with refractive index (a) lower than, (b) same as, and (c) higher than the one of the cell.

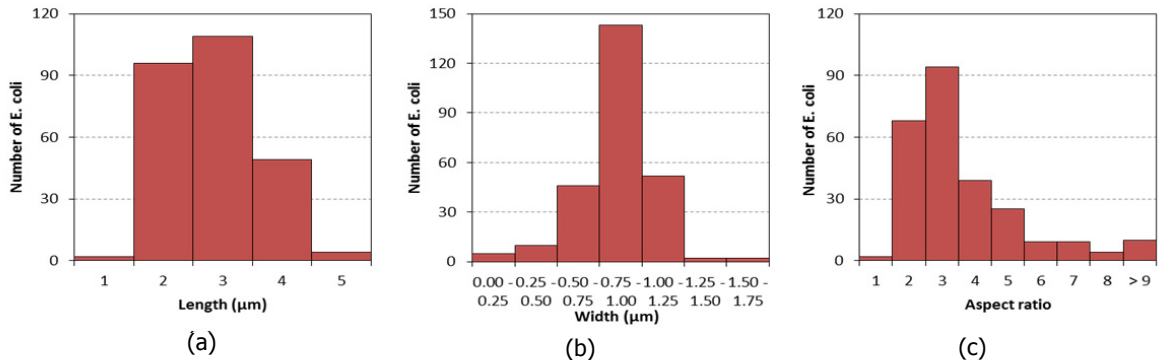

Figure 3: Morphological measurements of E. coli with sample size of 260. The length and width is measured to the nearest 1 and 0.5 μm, respectively. (a) length, (b) width,(c) aspect ratio.

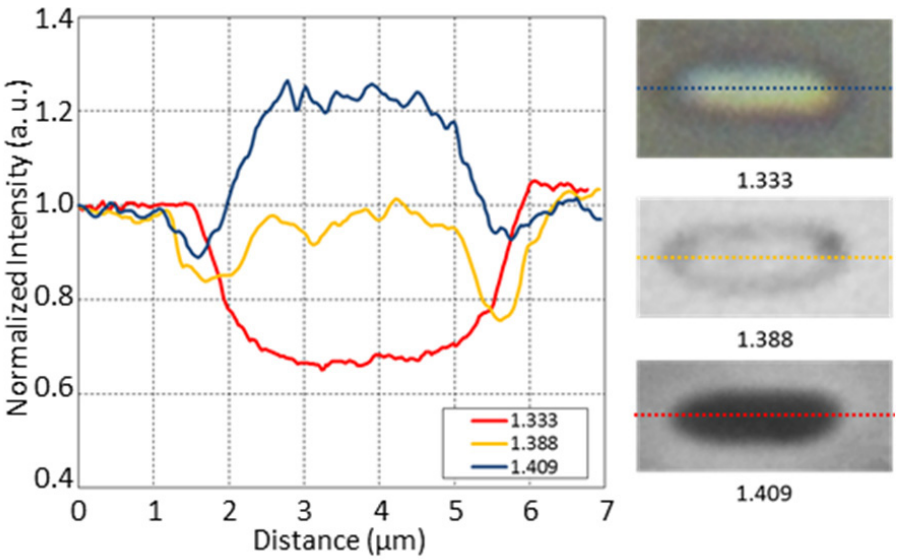

Figure 4: Pixel intensity analysis of an individual E. coli cell when the external medium is tuned. The bacteria cell appears to be invisible when the refractive index of the external medium is 1.388.

length and width of individual *E. coli*, while Fig. 3c illustrates the aspect ratio distribution of individual *E. coli*. It shows that *E. coli* has a rod shape with a mean length and width of 2.83 and 0.86 μm (aspect ratio 3.87). Figure 4 illustrates pixel intensity profiles of an individual *E. coli* when the external medium is tuned. The *E. coli* appears to be invisible when the external medium has a refractive index of 1.388, which is also the refractive index of *E. coli*.

4. Conclusions

In conclusion, an on-chip optofluidic immersion refractometer is developed and demonstrated by measuring the size, shape and refractive index of single *E. coli*. The results show that *E. coli* has a rod shape with a mean length and width of 2.83 and 0.86 μm, respectively. Its aspect ratio is 3.87 (length-to-width ratio). By using the on-chip optofluidic immersion refractometer, *E. coli* is measured with a mean refractive index value of 1.388. In future, the

biophysical database of bacteria will be expanded to revolutionalize the water quality monitoring industry by adapting the optofluidic immersion refractometer for label-free waterborne bacteria detection.

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