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<td>Author(s)</td>
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EUROSENSORS 2014, the XXVIII edition of the conference series

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

P. Y. Liu¹,², L. K. Chin², W. Ser², T. C. Ayi³, P. H. Yap³, T. Bourouina¹ and Y. Leprince-Wang¹

¹Université Paris-Est, UPEM, F-77454 Marne-la-Vallée, France
²School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore 639798
³Defence Medical & Environmental Institute, DSO National Laboratories, Singapore 117510
⁴Université Paris-Est, ESYCOM, ESIEE Paris, F-93162 Marne-la-Vallée, France

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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Peer-review under responsibility of the scientific committee of Eurosensors 2014.

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), shigella flexneri, vibrio cholera and Salmonella enterica etc [2]. Other bacterial caused diseases include typhoid by Salmonella enterica and also hepatitis [3-4]. The
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.

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 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.

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.
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 refractive index of the external medium is 1.388.

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 revolutionize the water quality monitoring industry by adapting the optofluidic immersion refractometer for label-free waterborne bacteria detection.

Acknowledgements

This work is supported by the Environmental and Water Industry Development Council of Singapore (Research project Grant No. 1102-IRIS-05-02).

References