A SINGLE LIVING BACTERIUM'S REFRACTIVE INDEX MEASUREMENT BY USING OPTOFLUIDIC IMMERSION REFRACTOMETRY

Y. Liu[1], L. K. Chin[1]*, W. Ser[1], T. C. Ayi[2], W. M. Ho[2], P. H. Yap[2], Y. Leprince-Wang[3] and T. Bourouina[3]

[1]School of Electrical and Electronic Engineering, Nanyang Technological University, SINGAPORE 639798

[2]Defence Medical & Environmental Institute, DSO National Laboratories, Singapore 117510

[3]ESIEE, University of Paris Est, Paris 93162, FRANCE

ABSTRACT

This paper presents a biophysical method to characterize single bacterium in water by using an on-chip immersion refractometer. The working principle is based on immersion refractometry, whereby the refractive index of a single bacterium is measured using the difference between the various refractive indices. The preliminary results show that *E. coli* is larger in term of width and has a lower refractive index value as compared to *bacillus subtilis*. The database for protozoa will be expanded by measuring different kinds of bacteria using the on-chip immersion refractometer.

KEYWORDS: Optofluidic immersion refractometry, *Bacillus subtilis*, *Escherichia coli*, Refractive index

INTRODUCTION

This paper presents a biophysical method to characterize single bacterium in water by using an on-chip immersion refractometer. Water safety is a major factor in the well-being of people, but the presence of bacteria such as *E. coli* and *bacillus subtilis* in drinking water can lead to infectious diseases as typhoid fever, hepatitis and diarrhea etc [1] that spread through unclean water. Hence, it is crucial to detect and identify bacteria to avoid bacterial outbreaks. 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 PRINCIPLE

The working principle of immersion refractometry is illustrated in Fig. 1. The bacterium in the medium is imaged by a phase contrast microscope. When the external buffer medium has a refractive index higher than the one of the bacterium, the bacterium appears to be darker (Fig. 2a). 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). Hence, this null method can be employed to measure the refractive index of the bacterium.

Figure 2 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 [3]. To tune the refractive index (RI) of the buffer, a microfluidic mixer is used to mix deionized water and Ficoll liquid. By varying the flow rate ratio between the water and ficoll solution, the refractive index of the mixed solution can be tuned from 1.333 to 1.446.

EXPERIMENTAL RESULTS AND DISCUSSION

Figure 3 shows the size and shape measurements of two bacteria species, i.e. *E. coli* and *Bacillus subtilis*. *E. coli* has

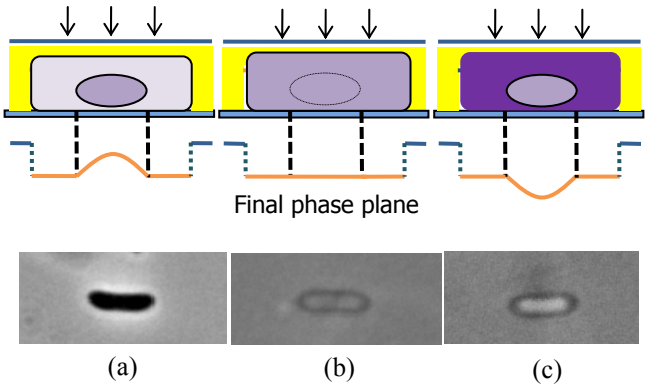


Figure 1: Phase transformation and the phase-contrast microphoto of E. coli cell being immersed into a medium with refractive index (a) lower than, (b) same as, and (c) higher than the one of the cell.

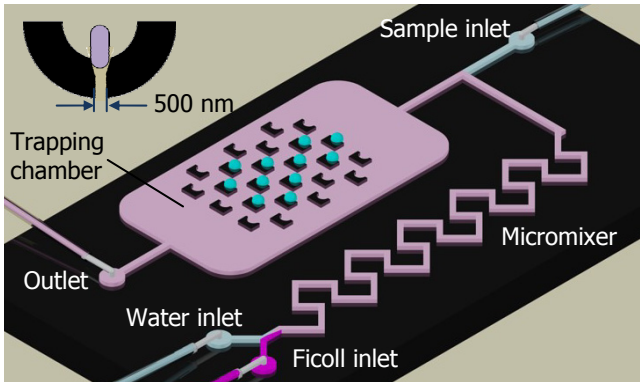
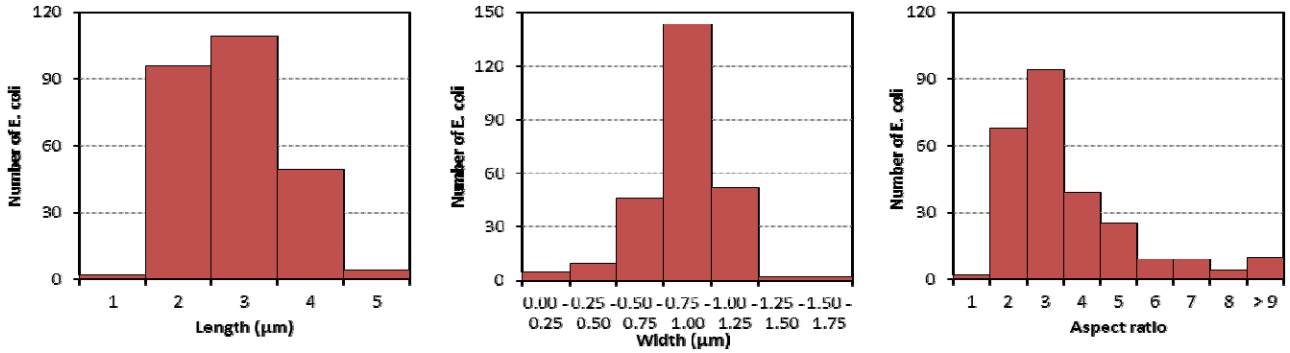
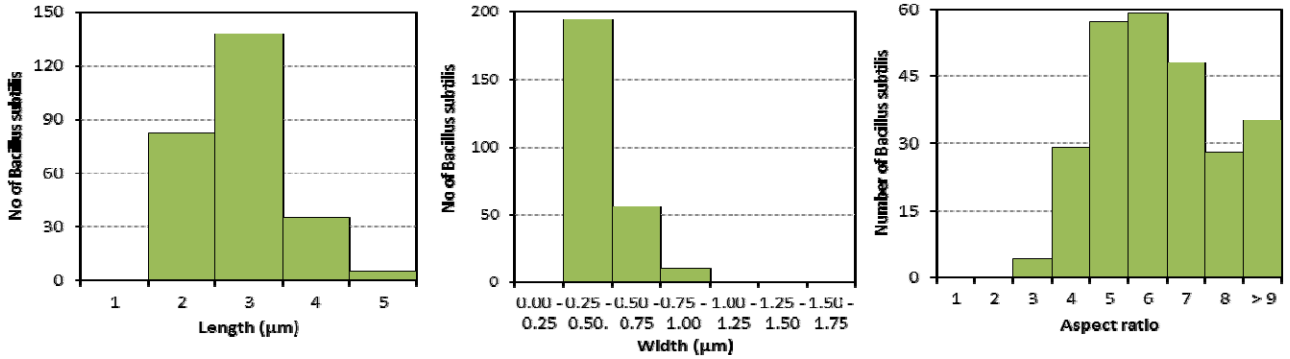


Figure 2: Schematic illustration of the optofluidic chip for biophysical measurement of single bacterium by using null-method in immersion refractometry. Inset shows the trapping structure with a gap of 500 nm.



(a)



(b)

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

a mean length and width of 2.83 and 0.86 µm, respectively. *E. coli* has a rod shape with a mean aspect ratio of 3.87. For *Bacillus subtilis*, its mean length and width are 2.86 and 0.47 µm, respectively. Similar to *E. coli*, *Bacillus subtilis* has a rod shape but with a mean aspect ratio of 6.47.

Figure 4 shows the refractive index measurement result of *E. coli*. The pixel intensity of the *E. coli* is analyzed when the external medium is tuned. The *E. coli* appears to be dark in pure DI water with a refractive index of 1.333. The *E. coli* appears to be invisible when the external medium has a refractive index of 1.388. When the external medium is further tuned to a refractive index of 1.409, the *E. coli* appears to be bright. This shows that the refractive index of *E. coli* is 1.388 measured by the optofluidic immersion refractometer.

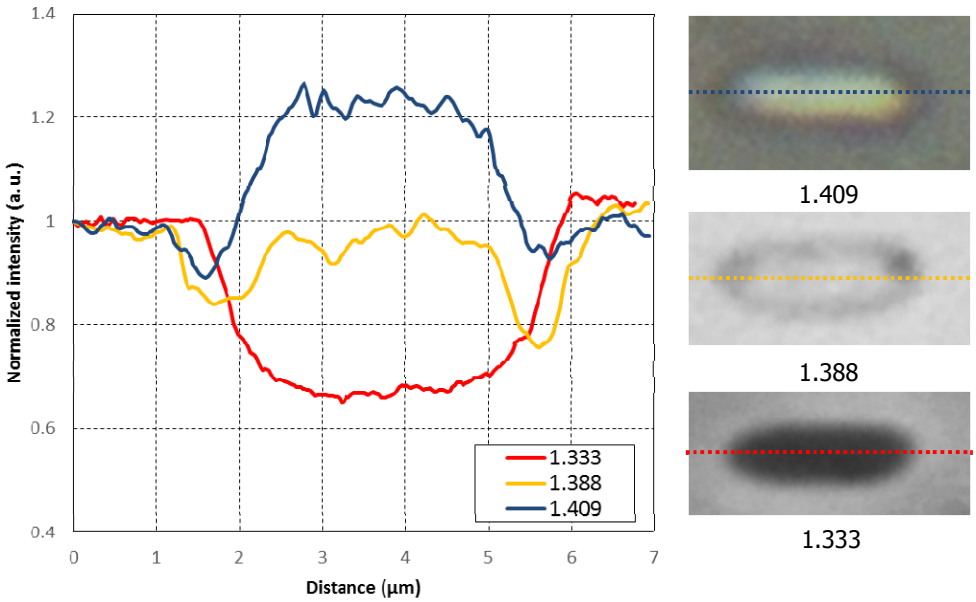


Figure 4: Pixel intensity analysis of E. coli cell when the external medium is tuned. The bacteria cell appears to be invisible when the refractive index of the external medium is 1.388.

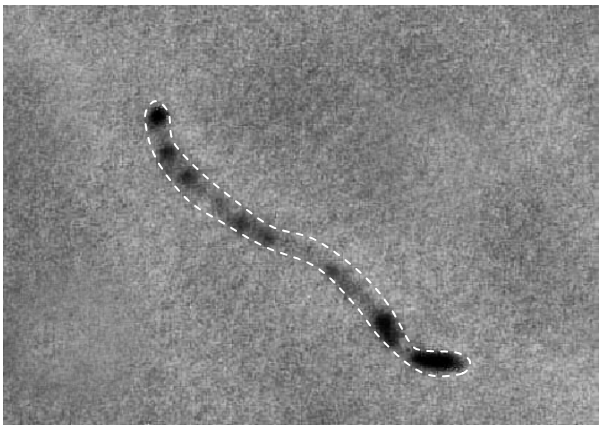


Figure 5: Phase contrast microphoto of bacillus subtilis when the external medium has a refractive index of 1.446. The bacillus subtilis appears partly invisible and partly dark.

Figure 5 shows the phase contrast microphoto of *Bacillus subtilis* when the external medium has a refractive index of 1.446. Part of the bacterium appears to be invisible, but part of it still appears to be dark. This shows that the refractive index of *Bacillus subtilis* is slightly higher than 1.446.

CONCLUSION

In conclusion, the size, shape and refractive index of *E. coli* and *Bacillus subtilis* are measured by the optofluidic immersion refractometer. The preliminary results show that *E. coli* is larger in terms of width and has a lower refractive index value as compared to *Bacillus subtilis*. *E. coli* has a mean length and width of 2.83 and 0.86 μm, respectively. *E. coli* has a rod shape with a mean aspect ratio of 3.87. For *Bacillus subtilis*, its mean length and width are 2.86 and 0.47 μm, respectively. Similar to *E. coli*, *Bacillus subtilis* has a rod shape but with a mean aspect ratio of 6.47. The biophysical database of bacteria will be expanded by measuring different kinds of bacteria using the on-chip immersion refractometer.

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CONTACT

*Chin Lip Ket, Tel: +65-6790 6532; Email: lkchin@ntu.edu.sg