NULL-METHOD IN IMMERSION REFRACTOMETRY FOR BIOPHYSICAL MEASUREMENT OF CRYPTOSPORIDIUM AND GIARDIA LAMBLIA

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ABSTRACT

This paper presents an on-chip null-method immersion refractometer for the measurement of size, shape and refractive index of protozoa in treated water. The key features of the on-chip immersion refractometer include (1) multiple single cyst trapping sites, which enable the measurement of multiple sample per test; and (2) zig-zag micromixer, which enables the refractive index variation of external medium as the key component of immersion refractometry. The results show that *Cryptosporidium parvum* oocysts have size of 3 to 7 μm, spherical with ovality lower than 0.3, and refractive index of 1.418. *Giardia lamblia* cysts have size of 8 to 12 μm, oval with ovality higher than 0.3, and refractive index of 1.433. The refractive indices of the samples are measured with high precision of $< 10^{-3}$.

KEYWORDS: Immersion refractometer, Protozoa, *Cryptosporidium*, *Giardia lamblia*

INTRODUCTION

Water quality is critical as clean sustainable water is one of the important resources and may cause fatal outbreak especially in densely-populated mega-city if health-affected contaminants exist in the treated water. One of the main sources of contaminants in water resource is protozoan parasites, which normally transmitted through the oral-fecal route. Among the protozoa, *C. parvum* and *G. lamblia* are two commonly found waterborne protozoan parasites. In 2001, an outbreak occurred in Saskatchewan of Canada had reported about 6,000 cases of cryptosporidiosis [1]. The source of contamination is the drinking water, which shows that it is vital to ensure the absence of *C. parvum* oocyst in drinking water. In 1998, *G. lamblia* outbreak was reported in Sydney, Australia due to the mis-measurement of the concentrations of microbes in the water supply [2]. Therefore, it is essential to monitor the concentration of *C. parvum* oocysts and *G. lamblia* cysts in treated water.

Current widely accepted monitoring protocol employed for *C. parvum* and *G. lamblia* identification is the USEPA Method 1623. The protocol incorporates the collection of 10L water sample, sample filtration, immunomagnetic separation, and immunofluorescence assay microscopy. The protocol is laboratory based with long processing time. Since protozoan parasites commonly found in treated water supply are limited in species, large in size (several to tens μm), regular in shape (cyst), resistance to chemical treatment, it may be feasible to identify different protozoa based on their morphologies (size and shape) and biophysical properties (refractive index). Refractive index of a biological sample is correlated with the mass density of its internal constituents such as protein concentration, nucleus contents and cellular sub-organelles [3]. In protozoan parasites, the contents inside the cysts for different species are distinctive. For example, *C. parvum* ocysts contain up to 4 sporozoites, which are bow-shaped and *G. lamblia* cysts contain 4 nuclei, clearly visible axostyles and lack of mitochondria. Based on these differences, their refractive index values might be different. Using these biophysical parameters, new detection system can be developed for real-time and on-site measurement.

WORKING PRINCIPLE AND CHIP DESIGN

Immersion refractometry refers to the measurement of refractive index of a sample by immersing it into a liquid and observing the optical properties of light passing through the substance and the liquid. In this paper, a null method is employed on the phase-contrast microphoto to determine the refractive index of the sample as shown in Fig. 1. When a sample is immersed into a medium with refractive index lower than the one of the sample (Fig. 1a), the light passing through the sample is experiencing phase delay as compared to the one passing through the medium. The phase delay experienced by the light can be expressed as $\Delta\phi = \left(n_{\text{cyst}} - n_{\text{medium}}\right) t_{\text{cyst}}$ where t_{cyst} is the thickness of the sample, n_{cyst} and n_{medium} are the refractive indices of the sample and the medium, respectively. By observing the phase-contrast microphoto, the sample appears brighter than the medium. By slowly increasing the refractive index of the medium, the brightness difference is reduced; and at one point, the sample appears to be invisible in the medium as shown in Fig. 1b. The refractive indices of the sample and medium match equally, at this instance. Subsequently, when the refractive index of the medium is further increased, the sample appears darker than the medium (Fig. 1c). Therefore, in the experiments, the samples are exposed to external medium varying from low to high refractive index. At the instance when the samples become invisible, the refractive index of the sample is obtained by measuring the refractive index of the respective medium. Once a sample-matched immersion medium has been found, the refractive index of the medium is measured using a handheld refractometer (PAL-RI, Atago) with resolution of 10^{-4} is used, such that it is not the limiting factor in the measurement.

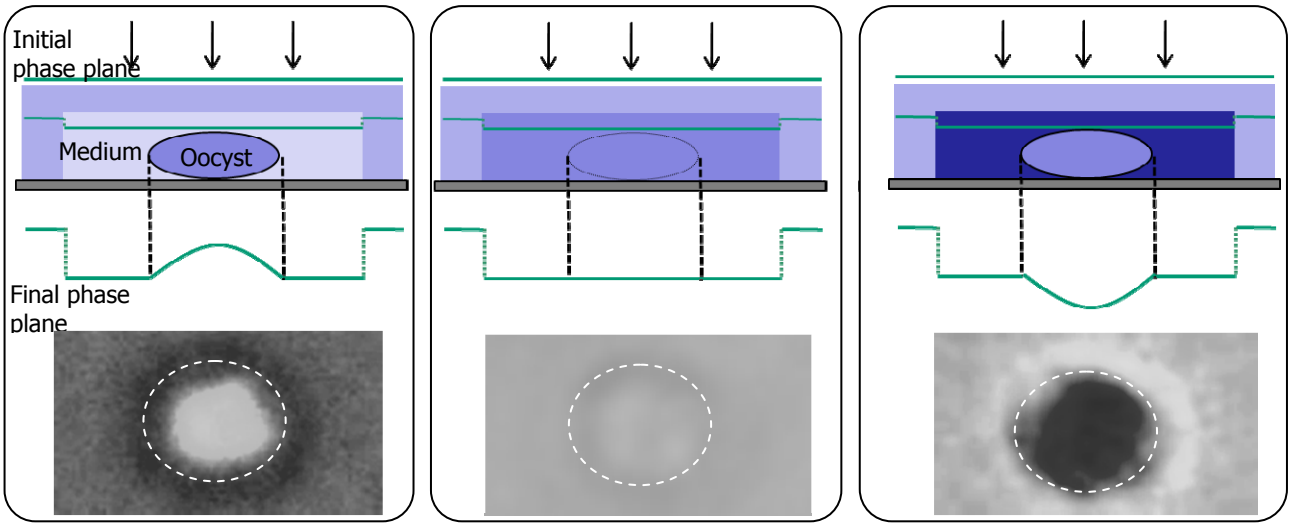


Figure 1: Working principle of the null method in immersion refractometry: The phase transformation and the phase-contrast microphoto of the oocyst being immersed into a medium with refractive index (a) lower than, (b) same as, and (c) higher than the one of the oocyst.

The design of the on-chip immersion refractometer is shown in Fig. 2. The chip consists of two key features, i.e. trapping microchamber and integrated micromixer. The trapping microchamber consists of multiple single cyst trapping sites, in which cysts flowing through the microchamber can be trapped in the sites individually. To perform immersion refractometry as discussed, an integrated micromixer is introduced to vary the external medium from low refractive index to high refractive index. Two liquids are used in the experiments, i.e. DI water (n = 1.3326) and 99% glycerol solution (n = 1.4651). Therefore, the refractive index of the external medium can be tuned from 1.3326 to 1.4651 by tuning the flow rate ratio between the two liquid flows. The microfluidic chip was fabricated in polydimethylsiloxane (PDMS) material using the standard soft lithography process.

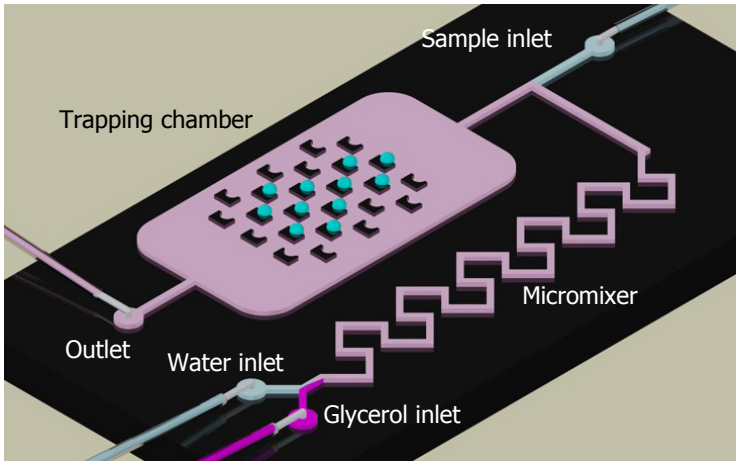
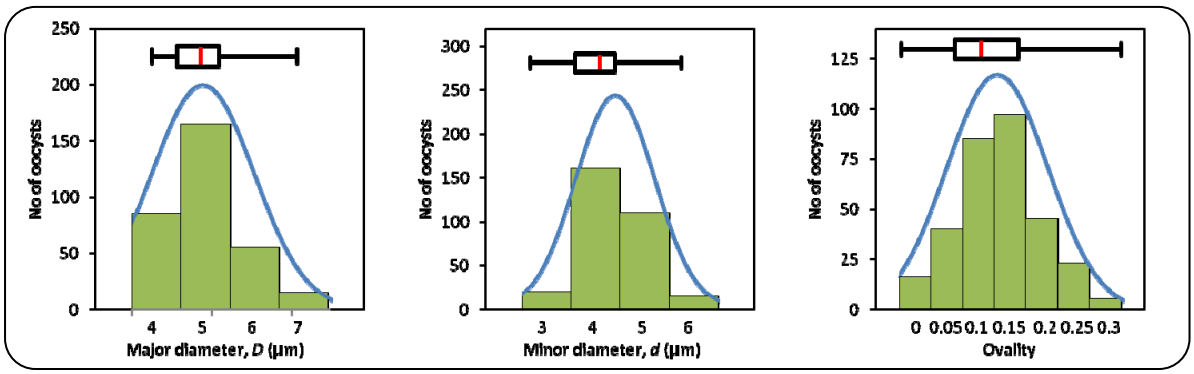


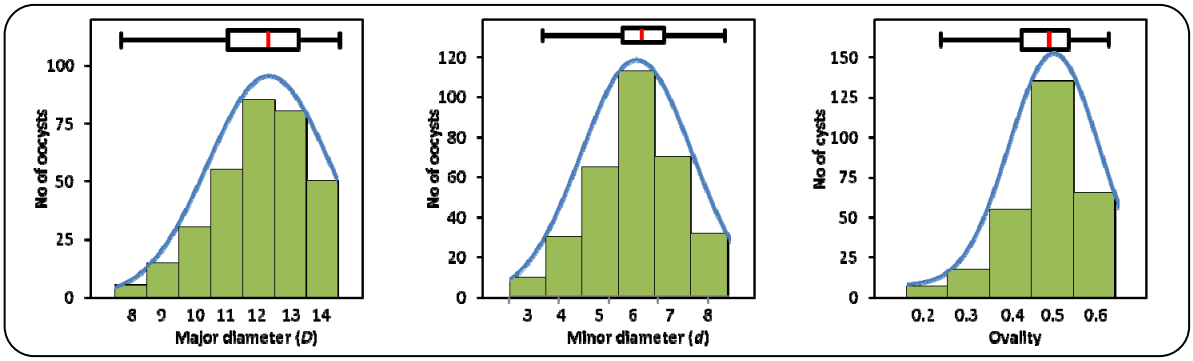
Figure 2: Schematic illustrations of the on-chip immersion refractometer.

RESULTS AND DISCUSSIONS

In the experiments, the samples and the liquids are injected into the microchannel using syringe pumps. As the samples flowing into the trapping microchamber, they are trapped in the trapping sites. Once the trapping sites are filled with samples, the major diameter, minor diameter and the ovality of the samples are measured. The ovality is defined as $O = (D - d)/D$ where D and d are the major and minor diameters of the oocyst, respectively. Fig. 3 shows the statistical results of *C. parvum* oocysts and *G. lamblia* cysts. For *C. parvum* oocysts, the major and minor diameters are ranging from 3 to 7 µm. The *C. parvum* oocysts are generally spherical in shape, such that the measured ovalities are lower than 0.3 and with a mean of 0.13. On the other hand, the major and minor diameters of *G. lamblia* cysts are measured to be ranging from 8 to 14 µm and 3 to 8 µm, respectively. Since *G. lamblia* cysts are generally oval in shape, their ovalities are higher than 0.3, with a mean value of 0.48. Fig. 4 shows the pixel intensity analysis for formalin prepared *C. parvum* oocyst by varying the refractive index of the external medium. The intensity contrast is higher than 1 when the refractive index of the oocyst is higher than the one of the external medium, and vice versa. The matching refractive index occurs when the intensity contrast is equal to 1. This occurs when the external medium has a refractive index of 1.418. Therefore, the *C. parvum* oocyst is measured to have a refractive index of 1.418. The process is repeated for formalin prepared *G. lamblia* cyst. The matching refractive index is measured as 1.433. Therefore, the *G. lamblia* cyst has a refractive index of 1.433.



(a)



(b)

Figure 3: Morphological measurements of (a) C. parvum oocysts, and (b) G. lamblia cysts. The sample size is 300. The major and the minor diameters are measured to the nearest µm.

CONCLUSIONS

In conclusions, the biophysical measurements of protozoa via the null-method using an on-chip immersion refractometer is demonstrated. The refractometer has two functions: (1) trapping of cysts (> 50) in an array of trapping sites; and (2) tuning the refractive index of the external medium to perform the null-method phase-contrast imaging. The size, the shape and the refractive index of *C. parvum* oocysts and *G. lamblia* cysts were investigated using the on-chip immersion refractometer. The results show that *C. parvum* oocysts and *G. lamblia* cysts can be differentiated using these three parameters (*C. parvum* oocyst: size of 3 to 7 µm, spherical with ovality lower than 0.3, and refractive index of 1.418; *G. lamblia* cyst: size of 8 to 12 µm, oval with ovality higher than 0.3, and refractive index of 1.433). Therefore, it is feasible to use these three parameters for the identification of the protozoan species.

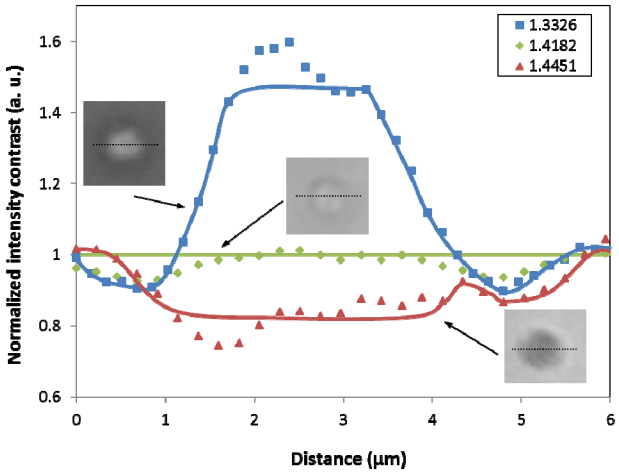


Figure 4: The oocyst appears to be invisible when the refractive index of the external medium is 1.418.

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