Measuring the Refractive Index and Its Two-Dimensional Distribution of Picoliter Microfluidics With the Scanning White Light Interference Microscopy Method

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ABSTRACT Measurements on refractive index and its distribution of microfluidics are widely applied in the field of biological detection, chemical analysis, material researches, medical diagnostics, and lab on a chip. Since the volume of the microfluidics is ultra-small, such as nanoliters or even picoliters, measuring the refractive index of microfluidics is challenging. We here presents an effective method to measure the refractive index and its two-dimensional distribution of picoliter microfluidics by the SWLI (scanning white light interference) microscopy technology. This method has the advantages of simplicity, low cost, non-contact, wide measurement range and high precision. We conduct the experiment to measure the refractive index of 2.4 picoliters liquid. The resolution is $10^{-4}$RIU (refractive index unit), and the relative error is $10^{-4}$ compared with the Abbe refractometer. We obtained the relationship between refractive index and concentration of glucose solution. In addition, we can measure the 2D (two-dimensional) distribution of RI (refractive index) of liquids. We can observe the liquid interface between deionized water and liquid paraffin clearly. Besides, we can observe the process of solute dissolution in the liquid and the change of refractive index.

INDEX TERMS Microfluidics, picoliters, refractive index measurement, scanning white light interference, two-dimensional distribution of refractive index.

I. INTRODUCTION

Studies of nanoliters or picoliters liquids are crucial for microfluidics technology [1], [2]. In recent years, more and more researchers are studying microfluidics, because of the low agent consumption of a single test specimen, low cost, fast analysis speed, and precise control of reactions. [1]–[5]. For example, nanofluidics play a key role in DNA sequencing [6]. Therefore, it is of great significance to study ultra-small liquids. This kind of research has great application prospects in the fields of biology [6]–[9], chemical analysis [10], and medical area [11], [12].

For a small volume of liquid, many biochemical reactions will not undergo easy-to-observe morphological changes or obvious color changes [3], [13]. Although it can be detected by fluorescent labeling [14], additional labels are required. Optical methods can be used for microfluidics detection without physical contact with the sample. The RI is important optical properties. We can determine the type, composition, concentration, and even the thermo-optic coefficients [15] of the liquid through it. Hence, the measurement of RI plays a key role in the analysis of physical and chemical properties [15], [16]. For example, we can detect different solutes from simple monoatomic ions to complex proteins without the addition of external labels or contrast agents [17], [18]. Therefore, the RI measurement of microfluidics has been extensively applied in chip-based analysis [19]–[21].
Measuring RI of ultra-small volumes of liquid is challenging [3], [22]. When the volume of the liquid is as small as picoliters, some classic measurement ways are difficult to apply. In addition, new methods are constantly being proposed. Such as Surface Plasmon resonance sensors [23]–[26], most of them are based on the surface Plasmon polarization phenomenon of Au. The cost is high and the equipment is complicated. Besides, they can only measure a small volume of liquid and it is extremely time-consuming to measure the distribution of RI point by point. Digital holographic microscopy [27]–[29] also can be used to measure the RI of microfluidics. But it is affected by the limitation of the resolution of the CCD camera and the insufficient digital reconstruction algorithm. The later demodulation algorithm is not only complicated, but also time-consuming. In addition, the optical system is extremely complex and the equipment is expensive. Moreover, some optical fiber-based RI sensing ways have also been proposed. Such as fiber grating [30], interferometric fiber sensors [31]–[33], sensor based on Brillouin scattering [34], and FWM-based RI sensor [35]. Those sensors have high accuracy, but they need put the fiber into liquid to measure its RI.

To adjust the limitations of the above methods, here we propose a method to measure the RI and its 2D distribution of ultra-small volume of liquids by scanning white light interference imaging technology. It is easy to construct the measuring system, and the cost is very low. The proposed method does not need physical contact with the sample. Besides, it has high precision and wide measurement range. It also has the advantages of fast measurement rate, only takes few seconds. We can measure the RI of many objects at the same time. We added a compensation plate to the reflector of the white light interference system to overcome the short coherence length limitation of white light and improve the contrast of interference fringes. We have measured the RI of liquids at 2.4pL. The resolution of RI measurement is 10^{-4} RIU (refractive index unit), and the relative error is 10^{-4} compared with the Abbe refractometer. We have also measured the 2D distribution of RI, and clearly observed the interface of different liquids. Besides, we achieve the observation the process of solute dissolution in the liquid and the change of RI. This method is expected to be applied in biological detection, chemical analysis and chip analysis fields. Such as the detection of cell growth status, chemical reaction detection, fluids mixing process, and microfluidics properties.

II. PROPOSED APPROACH

The white light interference system built in this experiment is shown in Figure 1(a). It is well known that the refractive index is sensitive to temperature. We put the entire measurement system into a thermostat. We can control the ambient temperature to 298 ± 1K, which has negligible impact on the precision of measurement. Here we first consider the case of sufficiently small temperature variations such that a linear dependency of the refractive index as function of the temperature is a good approximation. This assumption is justified as long as the temperature increase does not exceed 20 K. The change in refractive index (δRI) caused by the change in temperature (δT) can be calculated approximately by the following formula [36]:

$$\delta RI = -1.0514 \times 10^{-4} \delta T$$

When δT is 1K, δRI is only −1.0514×10^{-4}. This has little effect on our final measurement results.

Our system includes a halogen tungsten lamp with a center wavelength of $\lambda = 580$nm as a white light source, a 50/50 beam splitter prism, a Piezoelectric Transducer (PZT), a reflection Mirror, Thorlabs camera (sCMOS camera), and computer. The light is emitted from the light source and enters the beam splitting prism through the condenser lens, and it is divided into two beams here. One beam passes through the compensation mirror and then enters the reference mirror, and is finally reflected to the beam splitter. Another beam enters to the sample mirror through the microchannel and is reflected to the beam splitting prism. When the optical path length is equal, the reflected light from the two arms will recombine and interfere. When scanning the entire optical system vertically, Thorlabs camera records a series of images of the sample and transmits them to a computer for further signal processing.

We utilize beads with a diameter of 5 $\mu$m and transparent UV-curing glue to support a microchannel on the Si substrate, and cover the upper surface with a quartz cover sheet. The structure is shown in Figure 1(b). We put the compensation quartz plate closed to the reference mirror to adjust the reference light. In this way, the optical path difference between the reference beam and the measurement beam caused by the microchannel quartz cover can be offset indirectly. Upon that, the two beams meet the condition of the short coherence length of white light, and the contrast of interference fringes becomes higher.

Following the general principle of white light interference, emitted by the same light source, the reference beam reflected by the reference mirror interferes with the measurement beam passing through the sample. The general white light interfer-
The intensity distribution of white light interference fringes is shown in Figure 2(a). The interference fringe pattern is bright and dark, the center fringe is the brightest, and it gradually darkens to both sides. White light interference measurement uses broadband light as the light source, so white light interference can be regarded as the superposition of multiple monochromatic light interference [37]. The relationship between light intensity and optical path difference is shown in Figure 2(b), when the optical path difference is zero, the zero-order fringes of each wavelength are completely overlapped, and the interference light intensity is the largest at that time, that is, there is the brightest interference fringe here. The contrast of white light interference fringes decreases with the increase of the optical path difference, and the interference light intensity at the zero optical path difference position is the largest.

The intensity distribution of white light interference fringes can be written as [38]:

$$I = I_0[1 + m^*v^*\cos(\theta + 2\pi \delta/\lambda)]$$  \hspace{1cm} (2)

$I_0$ represents the background light intensity, which can be regarded as the direct current component of the light intensity, reflecting the background light intensity in the interference image. $m$ is the light intensity contrast, reflecting the amplitude of the interference fringes, $\theta$ is the initial optical path difference, $\delta$ is the optical path difference, $\lambda$ is the center wavelength of the white light source, and $v$ is the coherent envelope, $m^*v^*$ is the coherent modulation degree of the white light source. Because it is the superposition of the interference light intensity of different wavelengths, the peak envelope of the white light interference fringe presents a Gaussian distribution. When the optical path difference is zero, $m^*v^*$ reaches the maximum value. Therefore, when performing surface measurement, the position of zero optical path difference can be converted into the peak position of the envelope, that is, the position of the maximum degree of modulation.

In order to obtain the position of the zero optical path difference of the white light interference signal, we analyzed the white light interference fringe pattern. Using both the seven-step phase shift method [39] and the Morlet wavelet transform method [40]. The seven-step phase shift method has a fast processing speed and better calculation effect but poor anti-interference ability. The Morlet wavelet transform method has a slower processing speed but strong anti-interference ability. After comparing the two means, we adopted the Morlet wavelet transform method finally.

For the white light interference signal, by extracting its envelope, the peak value of the interference signal can be obtained, thereby obtaining the position of zero optical path difference. Morlet wavelet is a complex modulation signal with a Gaussian envelope, which is very similar to the white light interference signal envelope, as shown in Figure 2(c). Morlet wavelet transform can be used to obtain the signal peak.

$$\Psi_{a,b}(n) = \frac{1}{\sqrt{a}}\Psi\left(\frac{n-b}{a}\right)$$  \hspace{1cm} (3)

$\Psi_{a,b}(n)$ is the Morlet wavelet. $a$ is the scale parameter, which is related to the frequency domain characteristics of the signal. If the value of $a$ is changed, the subwavelets will have different center frequencies and bandwidths. $b$ is the position parameter (Frame number), by setting a different $b$ will realize the translation of the position of the sub-wavelet, so that the region of interest in the signal can be extracted in the time domain or the space domain.

We perform wavelet transformation on the white light interference signal, the optical path difference (d) caused by phase of the corresponding pixel point is obtained as follows:

$$d = \frac{\lambda}{8}b_{\text{max}} - \frac{\lambda}{4\pi}\varphi_{\text{b}_{\text{max}}}$$  \hspace{1cm} (4)

$d$ is optical path difference, $b_{\text{max}}$ is the number of frames at the peak of the signal, and $\varphi_{\text{b}_{\text{max}}}$ is phase at signal peak. If $d_{\text{empty}}$ is the optical path difference of empty microchannel and UV-curing glue, $d_{x}$ is the optical path difference of UV-curing glue and the liquid filled in microchannel to be tested. They have the following relationship with the refractive index:

$$d_{\text{empty}} = RI_{UV} h - h$$  \hspace{1cm} (5)

$$d_{x} = RI_{UV} h - RI_{x} h$$  \hspace{1cm} (6)

where $h$ represents the actual height of the microchannel, $RI_{UV}$ is the refractive index of UV-curing glue, and $RI_{x}$ is the refractive index of the liquid to be measured. Combining formulas (7) and (8) can get the refractive index of the liquid to be measured:

$$RI_{x} = RI_{UV} - \frac{d_{x} \ast (RI_{UV} - 1)}{d_{\text{empty}}}$$  \hspace{1cm} (7)
III. EXPERIMENTAL RESULT AND DISCUSSION

We detected the empty channel, the channel filled with water, and the channel filled with 95% alcohol solution respectively, and the obtained interference fringe image is shown in Figure 3(a)-(c). It can be seen that the fringes are obviously bent at the channel. And when the medium in the microchannel is different, the degree of deformation of the interference fringes obtained is also different. This is due to the RI of different media is different, resulting in different optical path differences of the interference beam. The result obtained after Morlet wavelet transform is shown in Figure 3(d)-(f). Then we take 200 pixels and measure the RI of water and 95% alcohol solution respectively.

We have measured the RI of different species. Each value of RI is the average of 100 pixels. The system temperature is 25°C. The results are shown in Table 1. The resolution is 10^{-4} RIU, and the measurement uncertainty is from ±0.0002 to ±0.0005 respectively, the relative error is about 10^{-6} compared with the Abbe refractometer, achieving high-precision measurement of refractive index of picoliter liquid.

By measuring the RI of glucose solution with different concentrations, we have obtained the empirical formula of the relationship between RI and concentration of glucose solution. We prepared a glucose solution with gradient concentration, and the concentration increased from 0 g/l to 100 g/l, a total of 6 concentrations. For each concentration, we selected 50 points for RI calculation, and the result is shown in Figure 4. The degree of convergence is 0.9996.

### TABLE 1. Measurement results of refractive index.

| SPECIES                  | Abbe refractometer/RI | SWLI/RI  | relative error |
|-------------------------|-----------------------|----------|----------------|
| deionized water         | 1.3333                | 1.3330   | 2.25×10^{-4}   |
| 95% alcohol solution    | 1.3626                | 1.3622   | 2.94×10^{-4}   |
| 60% glucose solution    | 1.3424                | 1.3420   | 2.98×10^{-4}   |
| 20% NaCl                | 1.3712                | 1.3717   | 3.65×10^{-4}   |
| liquid paraffin         | 1.4768                | 1.4763   | 3.59×10^{-4}   |
| 60% sucrose solution    | 1.5318                | 1.5312   | 3.92×10^{-4}   |

The relationship between RI and C (concentration) is

\[
RI = 1.3331 + 1.502 \times 10^{-4} C \tag{8}
\]

We can measure the concentration of blood by a similar way, which can be applied to the field of medical analysis.

In lab on a trip system, microfluidics control can be better realized by detecting the distribution of microfluidics. It is also of great significance to observe the interface conditions of different fluids [41]. In order to verify the feasibility of the system to measure the 2D distribution of RI of liquids, we injected deionized water and liquid paraffin into the microchannel. Since both liquids are transparent and have small volumes, no obvious liquid separation phenomenon can be seen, as shown in Figure 5(a). However, the interface between the two liquids can be clearly detected in the interference fringe diagram, as shown in Figure 5(b), the interference fringes are found to be significantly bent at the water-liquid paraffin and liquid paraffin-UV glue interfaces, and the dividing line between deionized water and liquid paraffin can be clearly seen in the interference fringe diagram. After calculation, we can get the 2D distribution of
refractive index, as shown in Figure 5(c), the distribution has a stepped shape.

We utilize the above system to detect the dissolution process of anhydrous glucose in deionized water, and reduce the dissolution rate of glucose by refrigerating deionized water for observation. The camera captures interference fringes every two seconds, a total of 4 shots. The interference fringe changes captured by the camera are shown in Figure 6(a). It can be clearly observed that the volume of glucose gradually decreases, and the interference fringes are constantly changing with the dissolution of glucose. Surface reconstruction of glucose solubilization by wavelet transform is shown in Figure 6(b). The distribution and change of RI during the glucose dissolution process are shown in Figure 6(c). The RI of the liquid near glucose decreases as the distance between the liquid and glucose increases, and with the continuous dissolution of glucose, the distribution of RI of the solution gradually becomes uniform.

IV. CONCLUSION

In summary, a simple measurement method of RI of microfluidics based on scanning white light interference is applied to achieve high precision measurement of picoliter liquid. This method has the advantages of simplicity, low cost, non-contact, fast measurement rate, and wide measurement range. Our system is extremely simple, only includes white light source, beam splitter, PZT, condenser, mirror, camera and computer. A compensation plate is utilized on the reflector to overcome the limitation of short coherence length, and improves the contrast of interference fringes. We have measured the RI of 2.4pL liquids. The resolution of measurement is $10^{-4}$ RIU, and the relative error is $10^{-4}$ compared with the Abbe refractometer. At the same time, we have measured the 2D distribution of RI of different liquids, and clearly observed the interface. Furthermore, we achieve the observation the process of solute dissolution in the liquid and the change of RI. This method has great application prospects in biological detection, chemical analysis and chip analysis fields, such as the detection of cell growth status, chemical reaction detection, fluids mixing process, and microfluidics properties.

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**M. Xia et al.: Measuring the Refractive Index and Its Two-Dimensional Distribution of Picoliter Microfluidics**

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