Technique of Micro-Lens/Microsphere Imaging for Simple Easy and Rapid Real Time In-Situ Biosensing

Abstract

This mini review describes the technique of micro-lens/microsphere imaging and presents information about its advantages and applications in various aspects of biosensing.

Keywords: Micro-lens; Microsphere; Imaging; Biosensing

Mini Review

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Introduction

The currently developed technique of micro-lens/microsphere imaging has attracted many attentions and applied to a variety of biosensing [1-4]. For the technique can in one hand measure the refractive index (RI) of a single micro-lens/microsphere or even simultaneously measure the RIs of tens of micro-lens/microspheres in the same field of view. In the other hand, when the RI of a micro-lens/microsphere is known, the micro-lenses/microspheres can be used as sensors to measure the local refractive index anywhere in spatially inhomogeneous media or in living specimens. As the refractive index of a solution is a function of the solute concentration and temperature [5], micro-lenses/microspheres therefore can be used as sensors of local concentration or temperature measurement.

Principle & Applications

When a micro-lens or a microsphere with a radius of R is immersed in a medium of n_1 and illuminated by a plane light wave propagating along its optical axis. If the refractive index n_2 of the micro-lens or microsphere is greater than n_1, due to the refraction effect, no parallel paraxial ray incident on the lens' spherical surface or the microsphere will emerge to the periphery of its second surface, thus a dark ring appears in the image of the micro-lens or microsphere as shown in Figure 1. The radius r of the central bright spot in the image was proven to be a function of R, n_1, n_2 [1] and the height h of the cylindrical part for micro-lens [2]. For microsphere, the relation takes the form:

$$r = R\left[2k^2\left(\sqrt{\frac{4k^2-1}{3k^2}}\right)^3 - \sqrt{\frac{4k^2-1}{3k^2}} + 2k\sqrt{1-k^2\frac{4k^2-1}{3k^2}}\sqrt{\frac{4k^2-1}{3k^2}}\sqrt{\frac{1-k^2}{3k^2}}\right] \quad (1)$$

For micro-lens:

$$r = R\sin\alpha - \left[R\cos\alpha + h\right]\frac{\sin\alpha\sqrt{1-k^2\sin^2\alpha} - k\sin\alpha\cos\alpha}{\cos\alpha\sqrt{1-k^2\sin^2\alpha} + k\sin^2\alpha} \quad (2)$$

Where $k = n_1/n_2$, α is the incident angle of the light to the spherical surface of the lens. Therefore, by immersing a micro-lens or microsphere into a liquid medium and measuring r in the image, one can easily use either eq.(1) or (2) to determine the refractive index n_1 of its surrounding medium, or inversely determine the refractive index n_2 of the micro-lens or microsphere using a medium with known n_1. Since the images of the central bright spot and the lens' periphery are located at the same focal plane, the technique of micro-lens imaging has better image optical quality than the technique of microsphere imaging (Figure 1). By using a 14 M pixels camera for imaging, the standard deviation in RI determination of the micro-lens technique was proven to be 2×10^{-6} which can satisfy the requirement of high sensitivity for many measurements of biosensing.

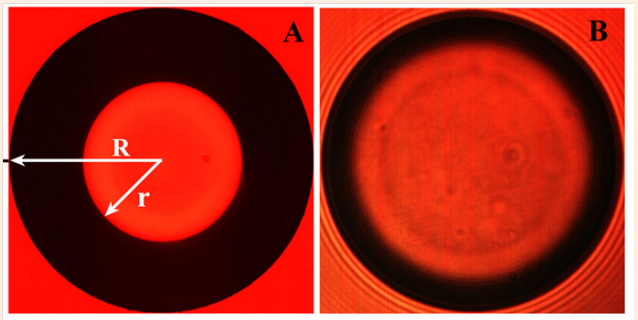

Figure 1: The images of (A) micro-lens and (B) microsphere in solution.

As we know that optical refraction takes place at the speed of light, any instant variation of the RI/concentration in the surrounding medium of a micro-lens or microsphere can immediately induce a change in the radius r of the central bright spot. Therefore, the method can catch up instantaneous RI or concentration variation by using a high speed camera for the imaging, while it is not influenced by disturbances which could ruin the measurements by many other methods, such as the movement of a living specimen and its surrounding medium, the slight vibration of the microscope, etc. On the other hand, by

putting several micro-lenses or microspheres at different places, the RI/concentration distribution in various sites of a spatially inhomogeneous medium can be simultaneously measured. Therefore, the method can be applied to different kinds of biosensing measurements. The most immediate application of the technique is local concentration measurement in inhomogeneous media. It was applied to monitor the concentration varying with time in a liquid mixture and the dynamic process of glycerol diffusing in water [1]; the continues on-site concentration variation monitoring of NaCl solutions in a microfluidics bioreactor during micro-perfusion; and simultaneous measurement on the concentrations in different regions in a bioreactor by putting several micro-lenses/microspheres in the places [1,4]. It was even used to monitor the local molecular composition varying with time in a living fish egg by inserting microspheres into the egg [1]. The method has an obvious advantage of without artifacts such as photobleaching and interference on the normal physiological activities of a living specimen typically present with chemical staining when using fluorescent probes. Since the only needed operation in the method is to capture the image at any desired time, it is able to perform easy and rapid real time in-situ monitoring analysis on biological specimens.

The micro-lens/microsphere was also used as a temperature sensor. Unlike the conventional methods with thermo/electrical probes for temperature detection, the method can easily determine the local temperature with a precision of ± 0.2 °C anywhere in a solution without disturbance on it [1]. The method can simultaneously monitor the temperature variations at different places of a solution by using several micro-lenses and has a spatial resolution as small as a micron for temperature gradient measurement when using micron scale micro-lens/ microspheres. Since during the process of antigen-antibody reaction, the refractive index of an Ag/Ab solution changes with time, the method of micro-lens/microsphere imaging can be used for easy and rapid multi-pass detection of Ag/Ab. It was demonstrated by various Ag/Ab systems that the method has the following advantages over the conventional methods for Ag/Ab detection [2]: (1) the detection is performed directly in Ag/Ab solution, so it is simple and easy, while without any requirement of labeling, expensive enzymes, pre-immobilization/modification, and post-washing; (2) The detection is objective, both qualitative and quantitative with high accuracy, reliability, repeatability, and its detection limit is as low as pg/mL; [3] It just requires very low sample volume (several μL) for detection and can be finished within two minutes; [4] .It is also able to continually monitor the dynamic process of the Ag-Ab reaction in real time to provide the kinetic and thermodynamic parameters about the reaction; [5] It can even perform Ag/Ab detection on colored and severely hemolyzed clinical samples and with excellent capability to against interference; [6] The device for the detection is also simple and can be portable for on-site analysis. Moreover, since many molecular reactions can induce RI changes in solution [6-8], the technique of micro-lens/microsphere imaging can be also used to detect the interactions of different biological systems from proteins, oligosaccharides, oligonucleotides, and lipids to small molecules, and viral particles.

Due to its capability of simultaneously monitoring the refractive index variation of several polymer microspheres together with temperature, the technique of microsphere/ micro-lens imaging can determine the glass transition temperatures (T_g) of several different polymers in a single experiment. The measurement is performed by preparing the polymer under test into microsphere for imaging. The method has sensitivity about six fold better than the conventional methods such as Differential scanning calorimetry (DSC), differential thermal analysis (DTA) [9,10], and thermo-mechanical analyzer (TMA) [11], and can eliminate most thermal lag at the same time [3]. It not only can measure the glass transition temperature with better accuracy and the determined Tg has less variation with cooling/heating rate, but is also simple and easy in performance while saving much time and energy. The technique of microsphere/micro-lens imaging can be further improved by using dual-wavelengths in the imaging to eliminate the thermal noise for accurate RI/concentration detection [4]. By the method, the measured concentrations can be just 0.005% - 0.01% deviating from true values for one centigrade temperature fluctuation which is just one tenth of that by single wavelength imaging. This would be very useful for on-site real time and continues monitoring the instant concentration/ refractive index variation in an inhomogeneous medium or fluid mixture for the separation and reaction processes and microfluidics processes. Because during the processes, though the processing unit can be under thermostatic control, temperature fluctuation of 1~2 °C would often happen and the stream or micro fluid sometimes gets into the unit for mixing so fast that it has no enough time to be in equilibrium with the temperature of the unit. Therefore, dual-wavelength micro-lens imaging technique can help to get accurate information about the compositions of the stream or micro fluid entering into processing units so that undesirable variation in product stream composition can be avoided.

Discussion

We have described the technique of micro-lens/microsphere imaging and some of its applications. We can see that the technique has several advantages over the conventional methods of refractive index detection: (1) it is simple and easy, RI is determined simply by immersing the micro-lens/microsphere in a medium and analyzing its images. (2) It can simultaneously perform measurements on the refractive indices of several micro-lenses/ microspheres, or in-situs measurement on several local refractive indices anywhere in spatially inhomogeneous media or even in living specimens. [3] It can perform rapid real time accurate measurement on the refractive index of different samples or monitor the variation of RI with time while without disturbance on the sample solutions [4]. The RI measurement is objective, Of high reliability and repeatability. Since refractive index of a solution is a function of concentration, temperature, and can reflect the state of antigen-antibody reaction in an Ag-Ab solution, the methods can be used as sensors for local concentration, temperature, glass-transition temperature and antigen-antibody detection. They can be also potentially applied to many other fields of studies including material analysis, environmental monitoring, biomedical researches and disease diagnosis.

Compared with microsphere imaging, micro-lens imaging has much better image optical quality so that its accuracy and precision of RI detection can reach magnitude of ± 2×10^{-6}. While for microsphere imaging, it would be just approximately $\pm10^{-5}$. So micro-lens imaging has priority in accurate RI/concentration measurement over microsphere imaging. However, microsphere is easier to be prepared and more convenient for people to find commercial products; for example, polystyrene microsphere and silicone microsphere are readily available anywhere. Moreover, since its symmetry, it can be more manageable for some specific cases such as inserting into biological specimens for molecular concentration monitoring. Therefore, both of the imaging methods have their advantages and can be applied to actual measurements according to different requirements. Moreover, the material of the micro-lens and microsphere can be varied from silica, glass to polymers according to the application and the convenience of preparing the micro-lens and microsphere. Usually, to avoid molecular adsorption on the surface of the micro-lens/microsphere which may disturb the imaging in biomedical samples, inert optical materials such as silica, BK-7 glass, polystyrene and polymethyl methacrylate (PMMA) are recommended to be used as their material. Furthermore, hydrophilic treatment of their surfaces is also suggested. Their imaging is preferably performed using a specific device with autofocus system and intelligent image recognition and analysis system as described in reference [2] to ensure fast and objective imaging and measurements of *r* and *R*. However, for those unable to have such a device, it can be substituted with a phase contrast microscope which also uses parallel light for illumination, while during the imaging and the measurements of *r* and *R*, care must be taken to avoid the interference from subjective factors. We believe that by having the aforementioned marvelous advantages, the technique of micro-lens/microsphere imaging will promote more and more interesting applications for biosensing in the future.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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