ABSTRACT

This paper reports a nano-optofluidic device using evanescent wave sensing for single molecule detection and sorting based on hydrodynamic focusing and total internal reflection (TIR). Nano-sized samples in liquid can be measured and counted even their size is smaller than the diffraction limit. We demonstrated 200-nm nanoparticle detecting which is beyond the diffraction limit. As compared to the conventional TIR microscopy, the nano-optofluidic device can measure and count all samples in real time. It has wide range applications in the single molecule detection, imaging and counting.

INTRODUCTION

Single molecule/nanoparticle detection and sorting using optical techniques have great potential in biological and chemical applications [1-4]. It provides more in-depth information as compared to the average characteristics in bulk molecule detection. Although single molecule detection is an urge for today’s research, it is still a big challenge in conventional optical techniques. For example, the diffraction limit has imposed the hindrance in detecting nano-sized particles .

In quantum optics, light consists of photons that carry angular momentum, which can be transfer to objects with finite mass by the pressure of the photons, namely optical force [5]. Many optical devices such as optical tweezers [6], optical lattice filter [7] and waveguides [1, 8] use optical force to manipulate microscopic bio-samples and particles ranging in size from atom level to hundreds of micrometers. Optical force is a significant tool for research in the field of biology, chemistry and physics. Among them, the near-field optical manipulation has become research forefront with prime interest on single molecule/nanoparticles detection and sorting by evanescent field whose size is sufficiently smaller than the wavelength of the light.

A total internal reflection (TIR) microscopy is based on the principle of the evanescent field illumination [9, 2], which is created by the total internal reflection between two medium with different refractive indices, such as glass and water. A TIR microscopy has higher sensitivity and signal-to-noise ratio than conventional microscopy. Therefore, it is one of the most widely used technologies in single molecules detection. Besides, the evanescent electromagnetic field decays exponentially, where the optical gradient is strong enough to hold and sort a single molecule/nanoparticle at the interface of two media [10]. TIR microscopy has certain advantages in single molecule/nanoparticle detection and sorting. However, it still limitations and gaps to meet the needs of today’s research and technology. Firstly, the penetration depth into the sample medium is limited with less than 200 nm. Thus, only samples near the interfaces of the two media can be detected. Secondly, compared to the pure microfluidic system, the solid-liquid interface cannot be fully exploited as the platform for complex chemical and biological processing and analysis. Finally, a pure liquid system is an advantage for real time detection and sorting which is difficult for the solid-liquid hybrid [11-13]. In this paper, a pure liquid-based TIR nano-optofluidic device is demonstrated for single molecule detection and sorting.

Hydrodynamic focusing is a pure microfluidic technology by building up the walls of the tunnel using the effects of fluid dynamics [13, 14]. The sample is injected into the middle of the sheath flow. The extreme central flow stream can be experimentally controlled down to 50 nm [14]. Two fluids do not mix and form a two-layer stable flow when they differ enough in their velocity or density. This paper reports a nano-optofluidic device using evanescent wave sensing for single molecule detection and sorting based on hydrodynamic focusing and total internal reflection (TIR). Nano-sized samples in liquid can be measured and counted even their size is smaller than the diffraction limit. As compared to the conventional TIR microscopy which can only detect the samples near the solid-liquid interface, all samples are focused in the extreme centre flow and can be measured and counted in real time. It has wide range applications in single molecule detection, imaging and counting.
DESIGN AND THEORY

Figure 1 shows the schematic of the nano-optofluidic evanescent wave sensor. It is designed to form a TIR between liquid interfaces with different refractive index. The nano-fluidic system consists of three flow streams in a micro-channel. The refractive index of the nano-core flow stream is lower than that of the sheath flow streams. The centre flow stream can be compressed by the sheath flow streams by hydrodynamic technology. The central flow streams can be controlled with the width smaller than wavelength to make the molecules/nanoparticles suspended in the centre flow stream one by one like a string of pearls by controlling the flow rates and the flow rate ratio. Different from the solid-liquid interface, the liquid-liquid interface is naturally smooth to avoid optical scattering, which is import to prevent the disturbance of the evanescent field. Furthermore, the refractive index of the sheath flow can be the same as the PDMS micro-channel to build a uniform medium for light input by using a mixture solution. The light is input by integrating an optical fiber in the micro-channel. The input light is focused and collimated by an on-chip lens to ensure that the incident angle is just slightly larger than critical angle. The evanescent wave is generated and decays along the vertical direction of the central flow stream. As a result, small nanoparticles are kept in the nano-core flow stream and detected when they are illuminated by the evanescent wave. The evanescent field is achieved by TIR in the liquid-liquid interface. The evanescent field can be designed to cover the whole nano-core flow stream to ensure that all nanoparticles can be illuminated efficiently. The nanoparticles can be further sorted by their sizes by controlling the intensity of the input laser.

In a TIR system, the penetration depth refers to the distance of electromagnetic radiation can penetrate into a material. It is defined as the depth at which the intensity of the radiation inside the material falls to $1/e$. The penetration depth with the incident angle in the TIR can be expressed as

$$d = \frac{\lambda_0}{4\pi n_i} \left[ \sin^2 \theta_i - n^2 \right]^{1/2}$$  \hspace{1cm} (1)

where $n_i$ and $n$ are the refractive indices of the sheath flow streams and the centre flow stream, respectively. In this case, $n_i = 1.410$ and $n = 1.404$, the penetration depth is infinite for propagating waves whose angle of incidence are below the critical angle. The penetration depth decrease as the angle increases. Consequently, the optical forces decrease as this angle increases. The penetration depth is nearly 6 µm as shown in Fig. 2 (a), when the incident angle is 85°. As a result, all the samples can be covered under evanescent field combined with hydrodynamic focusing technology.

The intensity of the evanescent field for both parallel and perpendicular polarizations as a function of the incident angle can be expressed as:

$$I_p(P) = I_p(S) \frac{4\cos^2 \theta (2\sin^2 \theta - n^2)}{n^4 \cos^2 \theta - n^2}$$  \hspace{1cm} (2a)

$$I_p(S) = I_p(S) \frac{4\cos^2 \theta}{1 - n^2}$$  \hspace{1cm} (2b)

Figure 2(b) shows the analytical solution of the evanescent wave intensity of the evanescent field for both parallel and perpendicular polarizations as a function of the incident angle when the refractive index contrast is fixed at 0.006. It is clearly shown that the effect of the polarization is negligible when the incident angle is 85°. Fig. 2(c) shows the simulation result in the micro-channel, the evanescent field has a strong local confinement. The light intensity in the interface between liquid media can be enhanced approximately 4 fold and then decay exponentially to form a gradient field.
RESULTS AND DISCUSSIONS

For the experimental study, the microchannel system was fabricated using soft photolithography processes. Firstly, photoresist-on-silicon mold was prepared in a clean room facility with photolithography (Micro-Chem, SU-8) using transparent glass masks (CAD/Art Services, Inc. Poway, CA). Secondly, micro-channels were molded using PDMS and sealed against flat PDMS sheets after plasma oxidation. After fabrication, the micro-channel has the width of $W = 40 \, \mu m$ and height of $H = 50 \, \mu m$. The flow streams are pumped into the micro-channel from the centre and the outer inlets using syringe pumps (Genie, Kent Scientific Corporation, CT). In the experiment, the chosen liquids are the ethylene glycol mixture as they do not swell PDMS. The refractive index of the sheath flow streams is $n_{1} = 1.410$ (75 % (CH$_2$OH)$_2$ 25 % (CH$_3$OH) in mass), which is equal to that of PDMS. The refractive index of outer flow stream is $n_{2} = 1.404$. The refractive index of the liquids and PDMS are measured by the refractometer (Reichert, AR200 digital hand-held).

Figure 3 shows the fabricated PDMS chip. The incident light is coupled from a fiber with a collimation lens with the incident angle $85^\circ$. The nano-core flow stream, which hold samples, can be focused by two sheath flows with the width controlled less than 1 µm.

Figure 4 shows the fluorescent micro-image of TIR at the liquid-liquid interface between the sheath flow and the core flow streams. The refractive index of the sheath flow streams is $n_{1} = 1.410$ (75 % (CH$_2$OH)$_2$ 25 % (CH$_3$OH) in mass), which is equal to that of PDMS. The refractive index of outer flow stream is $n_{2} = 1.404$. The critical angle is $84.7^\circ$, which is slightly smaller than the incident angle. The low refractive index contrast between the core flow stream and the sheath flow streams can reduce the smearing and keep a smooth liquid-liquid interface.

To observe the signals by the evanescent wave, we used the Andor’s EMCCD as the detector for dynamic single molecule imaging. The extraordinary signal-to-noise (S/N) is significantly greater than that of conventional CCD cameras even when operated at fast readout speeds. The exposure time can be down to 10 µs, exhibiting frame rates that are ideally suitable for dynamic acquisition of transient single molecules and their interactions.

Figure 5 shows the microscopic images of the micro/nano particles by the evanescent wave illumination in the nano-core flow stream from a Nd : YAG laser with the diameters: (a) G0500B, 5µm; (b) R0200B, 2µm; (c) F13081, 1µm; (d) F8813, 500 nm; (e) F8811, 200 nm.
CONCLUSIONS

In this paper, we report a nano-optofluidic device using evanescent wave sensing for single molecule detection and sorting based on hydrodynamic focusing and total internal reflection (TIR). Nano-sized samples in liquid can be measured and counted even their size is smaller than the diffraction limit. We have demonstrated successful imaging of 200-nm nanoparticle. As compared to the conventional TIR microscopy, which can only detect the samples near the solid-liquid interface, all samples are focused in the extreme central flow and can be measured and counted in real time. It has wide range applications in single molecule detection, imaging and counting.

ACKNOWLEDGMENTS

This work was supported by the Environmental and Water Industry Development Council of Singapore (Grant No. MEWR C651/06/171).

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