Studies on Photothermal Effect in Nanofluidic Channel Toward Ultrasensitive Detection of Nonfluorescent Molecules Using Differential Interference Contrast Thermal Lens Microscope

H. Shimizu*, K. Mawatari** and T. Kitamori***

*School of Engineering, The University of Tokyo, Tokyo, Japan, shimizu@icl.t.u-tokyo.ac.jp
**School of Engineering, The University of Tokyo, Tokyo, Japan, kmawatari@icl.t.u-tokyo.ac.jp
***School of Engineering, The University of Tokyo, Tokyo, Japan, kitamori@icl.t.u-tokyo.ac.jp

ABSTRACT

Thermal lens effect in nanofluidic channel was investigated by calculation and experiment. Recently, analytical chemistry has been rapidly miniaturized to micrometer and nanometer scale. In such small spaces, high sensitivity and versatility are required for detection techniques. Previously, our group has developed a differential interference contrast thermal lens microscope (DIC-TLM) to detect nonfluorescent molecules in nanofluidic channels smaller than wavelength of light. The DIC-TLM, which was based on light absorption and nonradiative relaxation followed by a change in refractive index, realized a sensitive determination of concentration of nonfluorescent molecules to date. However, heat transfer was remained as a problem to decrease sensitivity significantly. In this paper, the heat transfer was closely investigated by a computational method and the simulation results were compared with experimental result to discuss causes of the signal decrease and strategies to improve sensitivity..

Keywords: nanofluidics, nanochannel, photothermal spectroscopy, nonfluorescent molecules

1 INTRODUCTION

The field of analytical chemistry has rapidly been miniaturized as represented by single cell analysis. Especially, microfluidic technologies using micrometer-scale space has greatly developed and applied to various analyses as micro total analysis systems (μTAS) [1]. Contrary, nanotechnology has developed to use quantum effects of materials in molecular-scale space and produced various functional materials and devices. Recently, an intermediate region of microspace and nanospace which is 10-1000 nm scale has been focused from both areas of microfluidics and nanotechnology. In this region, most liquids act as fluid but have strong influences from surface and specific physical and chemical properties. For example, our group found high viscosity [2], low dielectric constant [3] and high proton mobility [4] of water confined in silica nanochannels and named this region *extended-nano space*. In addition, our group established fabrication and fluidic control techniques for extended-nano space and proposed novel analytical devices including chromatography overcoming the limitation of conventional packed columns [5]. Furthermore, the surface dominant properties of extended-nano space have been exploited for novel functions such as sample concentration, separation, rectification [6] and a novel research field called nanofluidics has emerged.

In such small spaces, detection techniques are quite important. First of all, high sensitivity is required because the number of target molecules decreases with decreasing sample volume. Moreover, detection of nonfluorescent molecules is also important for versatility. Therefore, our group developed thermal lens microscope (TLM) based on light absorption and nonradiative relaxation followed by a change in refractive index [7]. To date, ultrasensitive detection of nonfluorescent molecules (0.4 molecules in 7 fL) [8] and single nanoparticle counting [9] were achieved using the TLM. Nevertheless, the conventional TLM could not been applied to extended-nano space, which makes impossible to detect nonfluorescent molecules. This is because the detection principle of the conventional TLM is based on refraction that is geometric optics unavailable in a space shorter than wavelength of light. Therefore, our group developed differential interference contrast thermal lens microscope (DIC-TLM) to detect the change in refractive index using interference that is wave optics available if a space is smaller than wavelength [10]. Using the DIC-TLM, sensitive determination of concentration (390 molecules in 0.25 aL) was demonstrated in an extended-nano channel of 500-nm deep [11].

In the determination of concentration in extended-nano space, however, heat transfer from sample solution to glass nanochannel remained as a problem to decrease sensitivity significantly. In particular, generated heat in the nanochannel was inevitably lost due to fast thermal diffusion in glass and the change in refractive index of glass caused a cancellation with the change of a sample solution. In this paper, heat generation and transfer in extended-nano channel was closely investigated by computational and experimental methods for more sensitive detection using DIC-TLM.

2 THEORY

2.1 Principle of DIC-TLM

The principle of DIC-TLM is explained in another literature [10]. Briefly, a probe beam is separated into two beams and integrated again using a pair of DIC prisms. Contrary, an excitation beam is not separated to lap over one of the probe beams. Then, a sample absorbs the excitation beam and produce heat by nonradiative relaxation, which causes a local change in refractive index called thermal lens effect for the one of the probe beams. As a result, phase contrast appears between the two probe beams and detected as a small intensity change of an interfered probe beam. To detect the small change, the amplitude of the excitation beam is modulated to detect a synchronous component of the interfered probe beam.

2.2 Heat Transfer in Nanochannel

When the DIC-TLM is used for extended-nano channel, generated heat transfers from the sample to the nanochannel because the physical size of the thermal lens effect is larger than the nanochannel. Generally, the physical size of the thermal lens effect is regarded as a thermal diffusion length l which is calculated using equation 1.

$$l = \sqrt{\frac{D}{\pi f}} \tag{1}$$

Here, f is modulation frequency of excitation beam and D is thermal diffusion constant of solvent. From experiments using the conventional TLM, it is confirmed that f around 1 kHz gives the highest signal-to-noise ratio for signal processing. Then, l is ~6 μm when a solvent is water, which means a large part of generated heat transfers from the sample solution to nanochannel. Therefore, the heat transfer phenomena between the solution and nanochannel should be investigated closely to optimize the sensitivity of DIC-TLM. The amount of generated heat Q is expressed using equation 2 [12],

$$Q = \frac{2P\alpha}{\omega_{ex}^{\,2}} \exp(-\alpha z) \exp\left(\frac{-2r^2}{\omega_{ex}^{\,2}}\right)\left[1+\cos(2\pi f t)\right] \tag{2}$$

where P is excitation power, α is extinction coefficient of sample and ω_{ex} is beam waist radius of excitation beam. Then, diffusion of the generated heat is subjected to the thermal diffusion theory expressed as equation 3.

$$\nabla^2 T - \frac{1}{D}\frac{\partial T}{\partial t} = -\frac{Q}{\kappa} \tag{3}$$

using thermal diffusion constant D and thermal conductivity κ.

On the other hand, the phase contrast between two probe beams could be calculated using following equations [13].

$$\Delta\phi = \frac{2\pi}{\lambda_{pr}}\left[\Delta n(r=0) - \Delta n(r=d)\right] \tag{4}$$

$$\Delta n = \left(\frac{dn}{dT}\right)\int_z \Delta T \Big/ \left(1+\frac{z^2}{z_{r,pr}^{\,2}}\right) dz \tag{5}$$

where λ_{pr}, is wavelength $z_{r,pr}$ is Rayleigh length of probe beam and *(dn/dT)* is temperature gradient of refractive index. The value of *(dn/dT)* is negative for water and positive for glass, which should be noted because the changes in refractive indices could be cancelled with each other. The d in equation 5 is shear value which means distance of the separated probe beams determined by DIC prisms.

3 EXPERIMENTAL

3.1 Experimental Setup of DIC-TLM

Figure 1 is an experimental setup of DIC-TLM. An Ar^+ laser (λ = 488 nm) was used for excitation beam and a He-Ne laser (633 nm) was used for probe beam. Polarization planes of the laser beams were controlled to -45 and 0 degree respectively, which allowed the excitation beam not separated and probe beam separated by a DIC prism. The separated probe beams were focused on a sample in parallel keeping the distance of shear value, d = 5.3 μm. The separated probe beams were integrated again by another DIC prism and made interference using a polarization filter. Finally, the interfered probe beam were detected by an avalanche photodetector and its output is fed into a lock-in amplifier to extract a synchronous component of the modulation frequency of the excitation beam as a signal.

A microchip with an extended-nano channel was fabricated by electron-beam lithography and dry-etching process on a fused silica substrate. Here, channels with depths of 0.5, 1, 2, 8 and 100 μm were fabricated and used. The nanochannel substrate was laminated with a top substrate and fused into one chip at 1080° C in a vacuum furnace. A nonfluorescent dye, Sunset Yellow FCF was used as a sample and its aqueous solution was introduced into the nanochannel using a pressure-driven nanofluidic control system. A pressure controller was used to generate air pressure (~0.5 MPa) in a sample vial and the sample solution is pushed out from the vial into the nanofluidic chip.

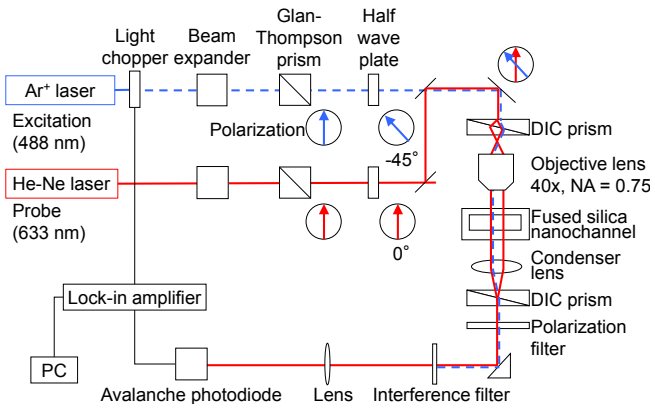

Figure 1: Experimental setup of DIC-TLM.

3.2 Finite Element Analysis

The heat transfer phenomena was simulated by a finite element analysis software (COMSOL Multiphysics). Equation 2-5 are used for the simulation to calculate the phase contrast $\Delta\phi$ as a signal of DIC-TLM. A geometry used for the calculation is shown in figure 2.

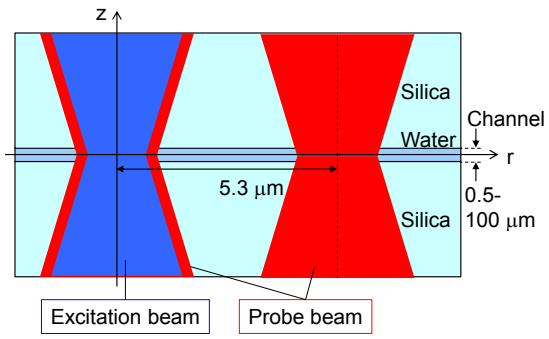

Figure 2: Geometry of channel and laser beams used for finite element analysis.

4 RESULTS AND DISCUSSIONS

A temperature distribution of the thermal lens effect in the 500-nm channel was simulated using equation 2 and 3 is shown in figure 3. Through the heat transfer from water to silica, a steep temperature increase was induced due to a smaller thermal conductivity of water compared to silica. The size of the temperature-increasing region was almost one or two nanochannel depths. This temperature distribution couldn't be estimated from equation 1 and was firstly investigated by this finite element analysis.

Next, refractive index change was integrated using equation 5 and phase contrast was calculated using equation 4. Figure 4 is a comparison of the simulated phase contrast and experimental results of DIC-TLM signal plotted against depth of channels. The experimental results of the signal rapidly decreased with decreasing channel depth when

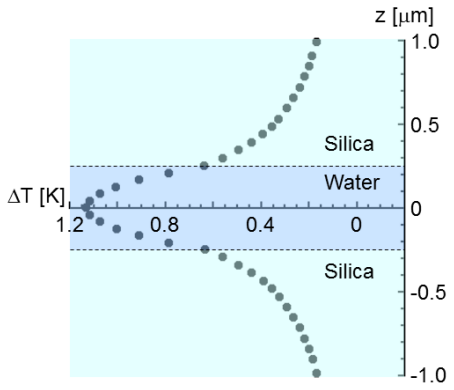

Figure 3: Simulation of thermal lens effect in 500-nm deep nanochannel.

channel depth was smaller than the confocal length of the excitation beam (2.0 μm). In order to investigate causes of the signal decrease, the simulation results were decomposed into three components. Firstly, if thermal diffusion was not considered, thermal lens signal decreased proportional to channel depth because detection volume of DIC-TLM was proportional to channel depth as shown by a dotted line in figure 4. Next, phase contrast was calculated from a change in refractive index of water. The calculated phase contrast was shown by a dashed line in figure 4, although the rapid signal decrease could not be explained only by the thermal diffusion. Finally, a change in refractive index of silica was taken into account to calculate phase contrast, which was well accorded with the experimental results as shown by red solid line in figure 4. This was because the negative change in refractive index of water was cancelled by the positive change of silica.

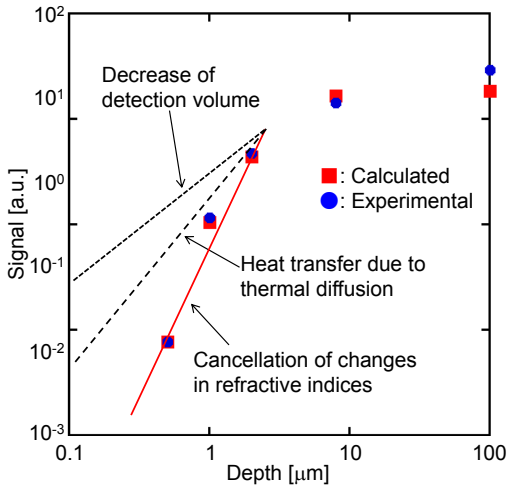

Figure 4: Comparison of experimental results and simulation. Three components of rapid signal decrease are separately shown below 2-μm depth. Dotted line: decrease of detection volume, dashed line: heat transfer and solid line: cancellation of change in refractive index.

According to the simulation, the largest problem among the three is the cancellation of the changes in refractive indices. Therefore, the sensitivity is expected to be recovered largely if a special material with a negative change in refractive index is used as nanochannel material as shown in figure 5 [14].

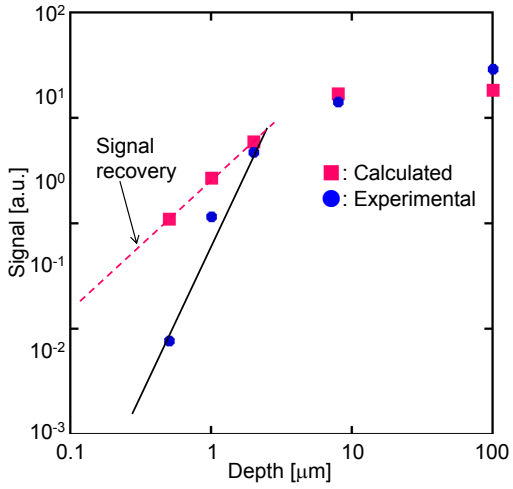

Figure 5: Simulation of signal recovery using optical glass (Schott N-PK51) with negative refractive index change

5 CONCLUSION

To summarize, thermal lens effect detected in extended-nano channel was investigated by computational and experimental methods using a DIC-TLM. The thermal lens effect in nanochannel had significant influences of thermal diffusion and cancellation of changes in refractive indices. Therefore, controls of thermal diffusion and a change in refractive index of nanochannel material are critical for sensitive detection using the DIC-TLM. Because the DIC-TLM is the only method to detect absorbance in nanospace, this study would greatly contribute to development of nanofluidics and creation of novel functional devices.

ACKNOWLEDGEMENTS

This work was supported by Grant-in-Aid for Specially Promoted Research (21000007) of Japan Society for the Promotion of Science (JSPS).

REFERENCES

[1] D. R. Reyes, D. Iossifidis, P. A. Auroux and A. Manz, Anal. Chem. 74, 2623, 2002.
[2] A. Hibara, T. Saito, H. B. Kim, M. Tokeshi, T. Ooi, M. Nakao and T. Kitamori, Anal. Chem. 74, 6170, 2002
[3] T. Tsukahara, A. Hibara, Y. Ikeda and T. Kitamori, Angew. Chem. Int. Ed. 46, 1180, 2007.
[4] Y. Chinen, K. Mawatari, Y. Pihosh, K. Morikawa, K. Kazoe, T. Tsukahara, and T. Kitamori, Angew. Chem. Int. Ed. 51, 3573, 2012.
[5] R. Ishibashi, K. Mawatari and T. Kitamori, Small 8, 1237, 2012.
[6] M. Napoli, J. C. T. Eijkel and S. Pennathur, Lab Chip 10, 957, 2010.
[7] K. Uchiyama, A. Hibara, H. Kimura, T. Sawada and T. Kitamori, Jpn. J. App. Phys. 39, 5316, 2000.
[8] M. Tokeshi, M. Uchida, A. Hibara, T. Sawada and T. Kitamori, Anal. Chem. 73, 2112, 2001.
[9] K. Mawatari, T. Kitamori and T. Sawada, Anal. Chem. 70, 5037, 1998.
[10] H. Shimizu, K. Mawatari and T. Kitamori, Anal. Chem. 81, 9802, 2009.
[11] H. Shimizu, K. Mawatari and T. Kitamori, Anal. Chem. 82, 7479, 2010.
[12] W. B. Jackson, N. M. Amer, A. C. Boccara and D. Fournier, Appl. Opt. 20, 1333,1981.
[13] A. Y. Luk'yanov and M. A. Novikov, Tech. Phys. 70, 99, 2000.
[14] T. H. Jamieson, Opt. Eng. 20, 156, 1981.