NOVEL DETECTION OF NON-ABSORBING MOLECULES BY OPTICAL NEAR-FIELD INDUCED THERMAL LENS MICROSCOPY

T. H. H. Le *1, K. Mawatari1, H. Shimizu1, T. Yatsui2, T. Kawazoe2, M. Naruse2, M. Ohtsu2, and T. Kitamori1

1Department of Applied Chemistry and
2Department of Electrical Engineering and Information Systems, University of Tokyo, JAPAN

ABSTRACT

This study reports a novel detection method of Thermal Lens Microscopy (TLM), in which the excitation of analytes is manipulated by Optical Near-Field (ONF) generated on nanostructures. Owning to the property of apparent higher energy than incident photons, herein the ONF allows the electronic excitation of molecules even though the energy of incident light is lower than absorption energy of molecules. As a result, the detection of UV-absorbing molecules has been realized with visible excitation. Our result promises important implications in detection of biomolecules, which usually have absorption in UV range. It is also worthy to note that the present work is novel in using ONF on nanostructures of non-metal materials, which is conceptually different from the plasmonic field enhancement on metal nanoparticles. This study is expected to open a new perspective for ONF-induced spectroscopies realized by top-down nanofabrication.

KEYWORDS: Differential Interference Contrast TLM (DIC-TLM), Optical Near-Field (ONF), detection of non-absorbing molecules, nanofabrication

INTRODUCTION

In integrated micro chemical system, the scaling down of detection volume to ~fL has led to the rise in demand for detection of non-fluorescent molecules in ultra-small volume. We have developed the TLM based on the refractive index change following the thermal relaxation to detect non-fluorescent molecules. We have also introduced the differential interference contrast TLM (DIC-TLM) based on the photothermal interferometry principle, and succeeded in detection in extended-nano (100~1000nm) channel[1]. However, like other laser-induced spectroscopies, TLM is restricted to molecules that have absorption at the excitation wavelength, consequently, it is difficult to apply conventional DIC-TLM with visible excitation for UV-absorbing molecules.

On the other hand, the ONF generated on nanostructures has been reported to have the property of apparent higher energy, or it is possible to excite a molecule with incident photon energy lower than that of its absorption[2]. We have confirmed the UV-induced photo chemical reaction with visible excitation by using ONF generated on nanostructures[3]. This concept has inspired us a novel way of using ONF to manipulate the molecular excitation for detection. The objective of this work is to develop a new DIC-TLM using the ONF generated on nanostructures for detection of non-absorbing molecules in UV region. For that purpose, (1) we have developed a new DIC-TLM that allows the detection in 50nm-depth channel, which is equivalent to the ONF field. (2) We have also succeeded in verifying the signals of non-absorbing molecules induced from ONF effect for the first time in 50nm-depth channel.

PRINCIPLE

Fig. 1 shows the concept of ONF-induced DIC-TLM. The nanopillars are integrated into the extended-nano channel for ONF generation by top-down fabrication. As molecules with absorption wavelength shorter than excitation wavelength (488nm) are introduced, at nanopillars area, molecules are excited by ONF, meanwhile in flat area there is no ONF generated and molecules cannot be excited. As a result, the signals from molecules are detected at the nanopillars only. However, according to previous studies and calculation model, the localized field exists in the very thin layer adjacent to the surface(<100nm), thus it is of great importance to carry out the DIC-TLM in <100nm-depth channel to effectively utilizing the ONF effect. In the present work, we have introduced new chip structure and realized the DIC-TLM detection in <100nm-depth channel.
EXPERIMENTAL

For the ONF generation, nanopillars $180 \times 180 \times 50\text{nm}^3$ are fabricated onto a fused-silica substrate by Electron Beam Lithography. Then it is deposited with a $100\text{nm}$-thick layer of rutile TiO$_2$. Herein TiO$_2$ is introduced to improve the sensitivity of DIC-TLM for detection in <100nm-depth channels as explained in the results below. The structure of extended-nano channels (W $100\mu \text{m} \times D 50\text{nm}$) which consists of the upper SiO$_2$ wall and the lower TiO$_2$ wall is shown in Figure 2.

RESULTS AND DISCUSSION

1. Improvement of DIC-TLM for detection in ONF field (<100nm-depth channels)

The principle of DIC-TLM is based on the change of refractive index following the temperature increase inside the materials, consequently DIC-TLM signal is very sensitive to $dn/dT$ property of materials. In channels made of SiO$_2$ (figure 3(a)), the thermal generated by the excitation of analytes results in the temperature increase of solvent inside the channel, yet the thermal diffusion towards the inner-wall leads to the temperature distribution in SiO$_2$ substrate as well. However, while most of solvents have negative $dn/dT$ value, that of value in SiO$_2$ is positive, consequently, the cancellation effect occurs and it leads to the significant decrease of sensitivity as decreasing channel depth. The dependency of sensitivity on channel depth is investigated in flat channel with the analyte that has absorption at 488nm. Thus the excitation can be considered as the normal one without ONF effect. Figure 3(a) shows the dependency of apparent sensitivity of 1 Sunset Yellow on the depth of SiO$_2$ channel. The result indicates that it is very difficult to realize the detection in <100nm-depth channel. We propose the introduction of a new material with negative $dn/dT$ value to overcome the problem of cancellation, and herein the rutile TiO$_2$ ($dn/dT = -1.2 \times 10^{-4}$) is chosen. The channel structure is shown in Figure 2(b). The dependency of apparent sensitivity on channel depth has confirmed no significant change of sensitivity as decreasing depth. We also succeeded in detection of Sunset Yellow in 50nm-depth channel as shown in Figure 3(c). The detection limit of ~1100 molecules in a detection volume of ~25aL has been achieved. It is concluded that the introducing of TiO$_2$ has solved the cancellation problem in conventional DIC-TLM. In the next step, we are going to apply this TiO$_2$ structure for the detection of ONF-induced DIC-TLM in 50nm-depth channel.

![Figure 2. The structure of detection channel with $180 \times 180 \times 50\text{nm}^3$ pillars integrated](image)

![Figure 3. (a) Dependency of apparent sensitivity of 1 Sunset Yellow molecule on channel depth in SiO$_2$ chip and (b) in TiO$_2$ chip (c) Calibration curve of Sunset Yellow in 50nm-depth TiO$_2$ channel](image)
2. ONF-induced DIC-TLM detection on nanopillars

The TiO\textsubscript{2} nanopillars 180×180×50nm\textsuperscript{3} are integrated into channels as shown in Figure 2. As molecules with absorption wavelength shorter than excitation wavelength (488nm) are introduced at nanopillars, molecules are excited and detected by ONF. Fig. 4(a) shows the absorption spectra of 3 dyes, which have no absorption at 488nm, yet DIC-TLM signals are detected at nanopillars. Although, the sensitivity decreases drastically as the absorption peak of dye is far from the excitation wavelength, we have succeeded in detection of near-UV absorbing molecules by 488nm-excitation (Figure 4(b)). The DIC-TLM signal is detected and it is proportional to absorbance or concentration of dyes. For example, in Figure 5, the calibration curve of dye B (absorption peak at 424nm) clearly shows the significant enhancement signals at nanopillars compared to the flat area. While signal detected at flat area originates from band tail absorption at 488nm of dye B, the signal at nanopillars can be attributed to the ONF generated at nanopillars that induces the excitation of dye. The detection limit of 0.3mM has been achieved. The sensitivity is expected to be improved by changing the size and structure of nanopillars for better ONF generation.

CONCLUSION

In conclusion, we have demonstrated a novel DIC-TLM detection of non-absorbing molecules by using ONF generated on nanostructures. It is conceptually novel in the way of using ONF on non-metal materials to manipulate the electronic excitation of molecules. Our method can be applied to other spectroscopies, thus it is expected to open a new perspective in spectroscopy realized by nanofabrication. From the practical viewpoint, our result promises important impacts in detection of biomolecules, which usually have absorption in UV-range. Furthermore, in the present study we have shown the improvement of DIC-TLM that enable the detection in below 50nm-channel for the first time. The new DIC-TLM has helped us to further investigate the mechanism of molecular excitation induced by ONF. It should be an effective spectroscopy to study surface phenomena or thermal-related phenomena in restricted small space (~aL).

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CONTACT *T. Kitamori, tel: +81-3-58417232; kitamori@icl.t.u-tokyo.ac.jp