DETECTION OF ZEPTOMOLE NONLABELED PROTEIN IN
EXTENDED-NANO CHANNEL USING UV EXCITATION
DIFFERENTIAL INTERFERENCE CONTRAST THERMAL LENS
MICROSCOPE (DIC-TLM)

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ABSTRACT
Nonlabeled protein was detected at zeptomole (zmol) order with an optical path length of 900 nm using UV differential interference contrast thermal lens microscope (UV-DIC-TLM). The principle of DIC-TLM, which is based on wave optics, allowed sensitive detection in a nanofluidic channel comparable to the wavelength of light. By introducing UV excitation into the DIC-TLM, detection of nonlabeled protein was realized in a nanofluidic channel. This technology is promising for analytical devices using nanospace including chromatography and extremely small volume analysis such as single cell analysis.

KEYWORDS: Photothermal spectroscopy, Nanofluidics

INTRODUCTION
The field of analysis has been rapidly downsizing from microscale to nanoscale, which makes possible the addition of various novel functions such as analysis of stretched DNA molecule and preconcentration of charged biomolecules. Previously, our group has developed analytical devices including chromatography [1] using 100 nm scale channel which we call extended-nano channel, and realized high separation efficiency of 450,000 plates/m (theoretically 7,000,000 plates/m). The extended-nano chromatography also realized a sample injection of 180 attoliter (aL) at minimum, which is promising for analysis of ultrasmall volume sample such as single cell. However, detection is difficult for nonfluorescent molecules due to the extremely small number of analyte molecules (e.g. the volume of a 100 nm cube is 1 aL and the molecular number of 1 mM solution in the cube is 1 zmol). Therefore, in order to detect nonfluorescent molecules in extended-nano channel, we have developed a DIC-TLM based on wave optics that made possible detection in nanospace smaller or comparable to the wavelength of light [2]. To date, we have succeeded in detection of 390 molecules in 250 aL and combination with chromatography using the DIC-TLM [3]. Nevertheless, separation analysis of nonlabeled biomolecules has not been achieved, which is partly because the DIC-TLM is not available for UV excitation. Thus, we have recently reported the design, fabrication and verification of a UV-DIC-TLM [4]. In this paper, detection of a nonlabeled protein in extended-nano channel was investigated and the results revealed the UV-DIC-TLM has a potential to detect at least 6.6 zmol bovine serum albumin (BSA).

THEORY
The theory of DIC-TLM was reported in a previous literature. Briefly, a linearly-polarized probe beam is separated by a DIC prism into two beams and focused by an objective lens. The probe beams are collected and integrated again using another DIC prism and made a negative interference. Next, an excitation beam, whose polarization is controlled not to separate, is introduced coaxially with the probe beam. Then, a sample is heated by the excitation beam and refractive index and phase of probe beam are changed only for the one of the probe beam. Finally, the phase contrast is detected as a signal by interference.
EXPERIMENTAL

The experimental setup of the UV-DIC-TLM is shown in figure 1. Second harmonic generation of an argon ion laser (257 nm) and a He-Ne laser (633 nm) were used for an excitation and probe beam, respectively. Polarization planes of both beams were controlled to separate only the probe beam. The microscope was custom-built with a symmetric structure of DIC prisms and UV objective lenses. The pair of DIC prisms were made of an artificial crystal Li$_2$B$_4$O$_7$ which has high UV transparency and birefringence. In addition, the DIC prisms were designed that the probe beam has a separation distance of 5 μm under the objective lens. The interfered probe beam was detected by an avalanche photodiode and its output was processed by a lock-in amplifier with a modulation frequency of 2.5 kHz.

RESULTS AND DISCUSSION

Figure 2 shows a fused silica chip and micro/nanochannels used for measurements of protein. The nanochannel was 100 μm wide and 900 nm deep, while the excitation beam had a waist diameter of 3.9 μm and a confocal length of 3.8 μm. The detection volume could be calculated from the beam waist size and channel depth (optical path length) to be 440 aL.
Figure 3 shows a calibration curve of BSA protein in phosphate buffer solution. The detection limit was 15 µM that corresponds to 6.6 zmol in the detection volume of 440 aL.

In order to investigate this detection limit of molecular number thoroughly, the relationship of channel depth and signal was studied. Figure 4 shows sensitivity (gradient of calibration curve) plotted against channel depth. If the signal of DIC-TLM is proportional to the generated heat, the gradient of the sensitivity in figure 4 must be 1 because the detection volume is almost proportional to the depth when the depth is smaller than the confocal length. Nevertheless, the gradient of sensitivity was ~3 and signal was not detected when the depth was 500 nm. This result suggests that a mechanism of sensitivity decrease we have proposed previously [2] occurred also in UV-DIC-TLM. The mechanism, which is shown in figure 5, is coming from rapid heat transfer from water to glass and cancellation of the change in refractive index (RI) due to the opposite temperature gradient of RI for water and glass. This result also suggests that the detection performance of UV-DIC-TLM could be improved further if a material which has negative RI change is used for nanochannel.

**CONCLUSION**

In conclusion, nonlabeled protein molecules could be detected in a nanofluidic channel using UV-DIC-TLM. The detection limit was 15 µM which corresponds to 6.6 zmol in the detection volume of 440 aL. The optical path length, which is the channel depth in this experiment, was 900 nm. Furthermore, the dependence of sensitivity on channel depth strongly suggested the cancellation of the refractive index change, which indicates a possibility of sensitivity improvement by materials.

**REFERENCES**


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