Notes

Using Laser Interference Lithography in the Fabrication of a Simplified Micro- and Nanofluidic Device for Label-free Detection

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Recently, we developed a label-free detection method based on optical diffraction, and implemented it in on our fabricated micro- and nanofluidic device. This detection method is simple and useful for detecting biomolecules, but the device fabrication consists of complicated processes. In this paper, we propose a simple method for fabricating the micro- and nanofluidic device; the fabrication combines laser interference lithography with conventional photolithography. The performance of a device fabricated by the proposed method is comparable to the performance of the device in our previous study.

Keywords Label-free detection, nanofluidic device, diffraction grating, laser interference lithography, photolithography

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Introduction

The development of a label-free detection method for biomolecules is important not only for fundamental scientific research, but also for practical applications, such as clinical diagnosis.[1–5] Recently, we developed a label-free detection method for biomolecules using our fabricated micro- and nanofluidic device.[6] This detection method is based on optical diffraction that occurs when the laser beam passes through nanostructures inside the micro- and nanofluidic device. The device has periodically arranged, wall-like shaped, nanostructures inside the microchannels; the spaces between these nanostructures are the nanochannels. These periodic nanochannels function as a transmission grating and the laser beam perpendicularly introduced into the device is diffracted accordingly. Here, the intensity of the diffracted light depends on the refractive index of the sample inside the nanochannels. Therefore, this device can be applied as a highly sensitive refractometer by introducing the sample into the nanochannels and measuring the change of the diffracted light intensity. In addition, the refractive index depends on the sample concentration. Therefore, simple and quantitative detection for small sample amounts can also be provided.

When we fabricated the micro- and nanofluidic device that we used in our previous study,[6] the microchannel was fabricated by photolithography and the nanochannels were fabricated by electron-beam (EB) lithography and Ni electroplating. These methods take a long time and the fabrication processes are complicated. Especially, EB lithography and Ni electroplating require expensive and dedicated facilities. Therefore, the development of a simple, low-cost fabrication method for the micro- and nanofluidic device is desired. In this paper, we report on the fabrication method for the micro- and nanofluidic device; the method combines conventional photolithography and laser interference lithography.[7–10] The micro- and nanofluidic device fabrication is simple and low cost because no Ni electroplating or EB lithography apparatuses are necessary. We evaluated the performance of the fabricated device experimentally by introducing six solvents with different refractive indexes into it, and then examined the refractive index dependency of the diffracted light intensity. The performance was comparable to that obtained in the previous study, which indicates the high utility of our simplified fabrication method.

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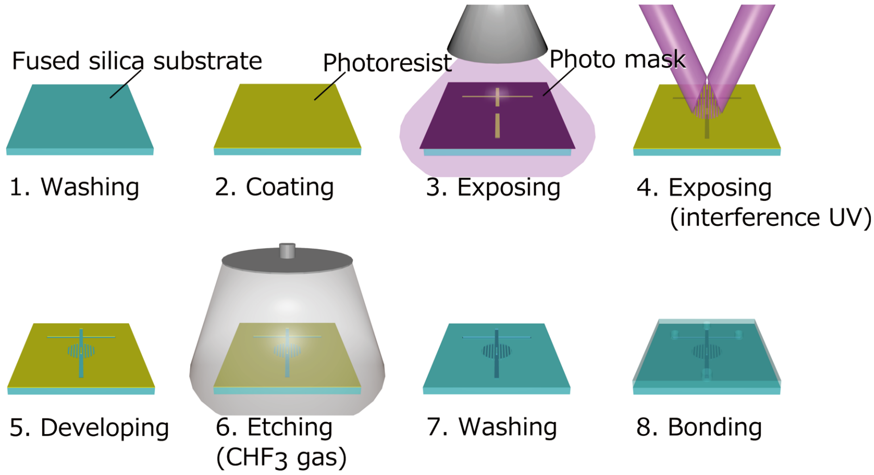

Fig. 1 Procedure for the preparation of a simple column electrode, S-CE.

Experimental

Reagents and chemicals

Ultrapure water was obtained using a Direct-Q UV system (Merck Millipore, Tokyo). Methanol (refractive index n = 1.3290), ethanol (n = 1.3614), 2-butanol (n = 1.3978), chloroform (n = 1.4459), *o*-xylene (n = 1.5018), chlorobenzene (n = 1.5241)[11] were purchased from Wako Pure Chemical Industries (Osaka). Fused silica substrates (n = 1.460118 at λ = 546.074 nm (from the company's data sheet)) were purchased from Daico MFG Co., Ltd. (Kyoto). A positive photo resist (TDMR-AR80) and developer (NMD-3) for fabricating the micro- and nanofluidic device were purchased from Tokyo Ohka Kogyo Co., Ltd. (Tokyo). An adherence agent (AZ AD promotor) and an anti-reflection reagent (AZ Aquatar) were purchased from AZ Electronic Materials Co., Ltd. (Tokyo).

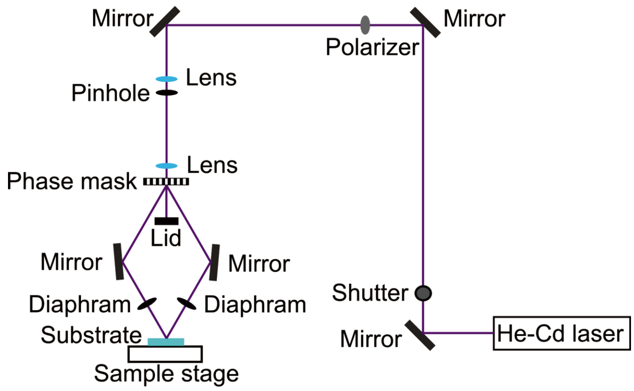

Fig. 2 Schematic illustration of the laser interference lithography setup.

Micro- and nanofluidic device fabrication

Figure 1 shows a schematic illustration of the device fabrication processes. First, the cross-shaped microchannel (width, 50 μm) was patterned as in the conventional photolithography method. After spin-coating an adherence agent and photoresist at 2500 rpm for 60 s, the fused silica substrate was pre-backed at 95°C for 20 min, and then the anti-reflection reagent was spin-coated on the top surface. The coated substrate was exposed to UV light through a photomask. This photomask was designed to close a part of the cross-shaped microchannel, and the nanochannels were patterned around this closed part using laser interference lithography after microchannel patterning. Figure 2 shows a schematic illustration of the experimental setup for laser interference lithography. A collimated light beam from a He-Cd laser (325 nm in wavelength; Model IK3401R-F; Kimmon Electric Company, Ltd., Tokyo) was separated into ±1st order diffracted beams using a phase mask (Ibsen Photonics A/S), and interfered on the sample stage. We set the incident angle so that the fringe pitch would be 900 nm, as calculated from Bragg's law. The interference beam was illuminated on the center of the substrate (ϕ5 mm area) for 70 s. After baking for 90 s at 110°C, the photoresist was developed and rinsed in the developer for 90 s and in water for 10 s, respectively. The patterned substrate was etched in a mixture gas of CHF_3 (5 cc/min) and Ar (20 cc/min) using an ICP dry etcher (ICP power, 50 W; bias power, 25 W; substrate temperature, 25°C) (RIE-101iP, Samco Inc., Kyoto). Finally, the micro- and nano-patterned substrate was bonded to another fused-silica substrate, which had inlet and outlet holes drilled with a diamond-coated drill by thermal fusion bonding (1080°C, under vacuum).[12,13] The fabricated device easily introduces the sample solution from the inlet hole and fills the channels *via* the capillary force. We dried the channels by heating before introducing the sample solution.

Device evaluation when used as a label-free detection system

We introduced six pure solvents (methanol, ethanol, 2-butanol, chloroform, *o*-xylene, chlorobenzene) with different refractive indexes into the nanochannels and measured the diffracted light intensity to evaluate the device. After filling the nanochannels with air, we introduced each solvent through the inlet hole by dropping the sample solution (2 μL) and filling the channels *via* the capillary force, before measuring the transition of the diffraction signal intensity. Five measurements were made for each solvent. The diffraction signal intensity was measured using our label-free detection system, which we reported before.[6,13] A semiconductor laser (wavelength, 532 nm; OPTO-LINE, Inc., Tokyo, Japan) was integrated into the experimental setup, which also included an inverted microscope. The incident laser beam was focused onto the micro- and nanofluidic device position on the microscope stage, and the laser beam diffracted

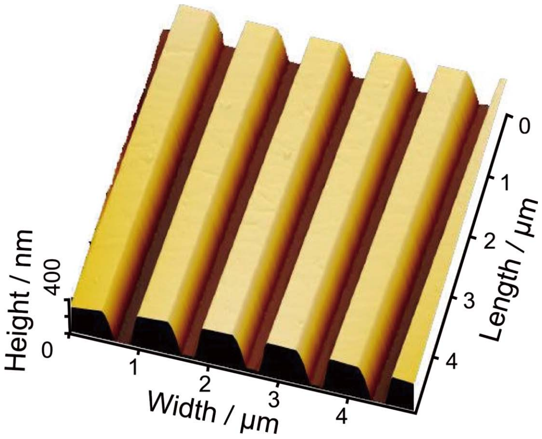

Fig. 3 AFM image of the nanochannels.

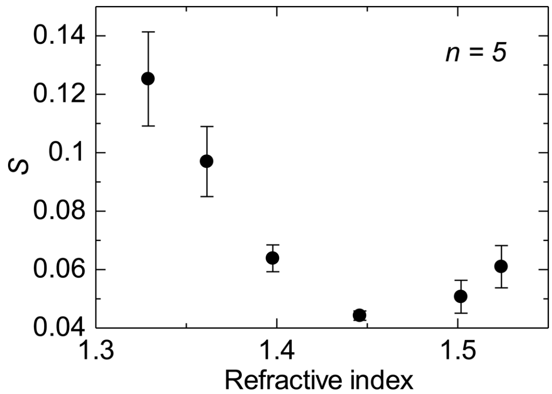

Fig. 4 Normalized signal intensity *versus* refractive index. Methanol (refractive index $n = 1.3290$), ethanol ($n = 1.3614$), 2-butanol ($n = 1.3978$), chloroform ($n = 1.4459$), *o*-xylene ($n = 1.5018$) and chlorobenzene ($n = 1.5241$) were introduced into the device and respective transitions of the diffracted light intensities were measured.

by the nanostructures was detected by a photodiode (ET-2030, Electro-Optics Technology, Inc., Traverse City, MI, USA). The signal from the photodiode was read with a data-logger (TR-V500, Keyence Corporation, Osaka) through a lock-in-amplifier (LI5640, NF Corporation, Yokohama).

Results and Discussion

Figure 3 shows an AFM image of the fabricated nanochannels. The periodic nanochannels were successfully fabricated on the fused-silica substrate by laser interference lithography. These nanochannels were 500 nm wide, 350 nm deep, and with 400 nm spaces between them. In our previous work,[6,13] fabricating the nanochannel by EB lithography took about 20 min for the patterning of a 1.2-mm square. In addition, it is not so easy to expand the patterning area. On the other hand, laser interference lithography can complete patterning in a short time (70 s), and the patterning area can be easily expanded by expanding the laser spot size. Furthermore, this method is suited to mass production because it can pattern multiple substrates at the same time if the substrate size is sufficiently small.

For device evaluation, we introduced six different pure solvents into the nanochannels and measured the diffraction signal intensity (Fig. 4). The diffraction intensity ratio, S, was defined by the following equation:

$$S = \frac{\text{Diffracted light intensity (sample)}}{\text{Diffracted light intensity (air)}}$$

S changed with the refractive index and this tendency showed good agreement with our previous evaluation results.[6,13] The numerical values, however, did not match exactly. Since we reported the size of the nanostructures affect on the signal response to the refractive index,[13] we attributed this to the size of the nanostructures and the optical properties of the fused-silica substrate not being completely identical to those of our previous study.

From the presented results, we demonstrated that the combined use of laser interference lithography with conventional photolithography is a useful method for the micro- and nanofluidic device fabrication. Furthermore, pillar-like shaped nanochannels can be easily fabricated by two-dimensional interference exposure. In the near future, we expect to realize a high-performance separation of DNA molecules using the nanopillar device fabricated by this method.

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