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3D MICROSTRUCTURING OF GLASS BY FEMTOSECOND LASER DIRECT WRITING AND APPLICATION TO BIOPHOTONIC MICROCHIPS

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Abstract—Three-dimensional (3D) microfabrication of photostructurable glass by femtosecond (fs) laser direct writing is demonstrated for manufacture of biophotonic microchips. The fs laser direct writing followed by annealing and successive wet etching can fabricate the hollow microstructures, achieving a variety of microfluidic components and microoptical components in a glass chip. One of the interesting and important applications of the 3D microfluidic structures fabricated by the present technique is inspection of living microorganisms. The microchips used for this application are referred to as nanoaquarium. Furthermore, the optical waveguide is written inside the glass by the fs laser direct writing without the annealing and the successive etching. It is revealed that integration of the microfluidic and microoptical components with the optical waveguides in a single glass chip is of great use for biochemical analysis and medical inspection based on optical sensing.

1. INTRODUCTION

In the last decade, it has been realized that miniaturization of the "field" is urgently demanded in chemical reaction, biological analysis and medical inspection, which emphasizes the use of microcomponents three-dimensionally (3D) integrated in the microchips. Examples of the miniaturized microchip devices are the so-called Lab-on-a-chip devices and micro total analysis system (μ-TAS). By shrinking a roomful of laboratory equipments and packing them into a palm-size chip, such microchip devices are capable of performing chemical and biological analyses with great reduction of reagent consumption, waste production, analysis time and labor cost. It has been shown that femtosecond (fs) laser is a promising tool for manufacture of integrated

microcomponents in glass due to its ability of internal modification of transparent materials using multiphoton absorption. So far, a broad variety of photonic microcomponents, such as waveguides, couplers, gratings, and Fresnel zone plates have been fabricated inside the glass [1–3]. In addition, fs laser is also a good tool for fabrication of microfluidic structures embedded in the glass, like 3D microfluidic channels [4, 5]. Recently, we developed the technique that directly forms 3D hollow microstructures with smooth internal surfaces inside glass by fs laser direct writing followed by post annealing and successive wet etching [6–13]. This technique can fabricate both 3D microfluidic and microoptic components in a single glass chip by a single procedure. In the present paper, fabrication of 3D hollow microstructures embedded in the glass is demonstrated by the fs laser direct writing followed by the annealing and the successive wet etching. The microchips with 3D microfluidic structures fabricated by this technique, that are referred to as nanoaquarium, are applied for dynamic observation of microorganisms. More recently, we also succeeded in forming optical waveguides inside the same glass chip by the fs laser direct writing [14]. Then, microfluids, microoptics, and optical waveguides are integrated in a single glass chip for photonic biosensing.

2. EXPERIMENTAL

Experiments were carried out by a commercial fs laser workstation [6]. The laser wavelength, pulse width and repetition rate were 775 nm, 150 fs and 1 kHz, respectively. The focusing system was a 20× microscope objective with a numerical aperture (N. A.) of 0.46. The substrate used in this study for 3D microstructuring is photostructurable glass that is commercially available under the trade name of Foturan from Schott Glass Corporation [15]. Samples under fabrication were translated by a PC controlled xyz stage for 3D microstructuring. For fabrication of the 3D hollow microstructures, the typical irradiation condition of fs laser was a fluence of 78 mJ/cm^2 and a scanning speed of 510 mm/s. After the fs laser exposure step, the sample was subjected to a programmed annealing, and then soaked in a 10% HF solution with an ultrasonic bath at variable period of etching time, depending on the size of the hollow structures. The laser-exposed regions can be preferentially etched away with a contrast ratio of ca. 50 in etching selectivity. Lastly we baked the photostructurable glass sample again to smooth the etched surfaces. The details of procedures and mechanism of selective etching of photostructurable glass are described elsewhere [6, 8, 16–18]. In the meanwhile, for the optical

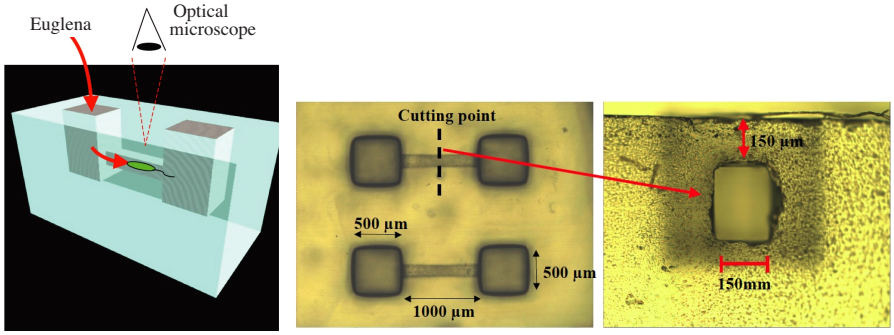
waveguide writing, the tightly focused fs laser beam was scanned inside the samples by moving the samples perpendicularly to the laser beam axis using a PC-controlled xyz stage without the annealing and the successive wet etching [14]. The typical writing condition is $0.5\,\mu$J/pulse of laser power and $200\,\mu$m/s of scanning speed.

3. INSPECTION OF MICROORGANISMS

One of the interesting and important applications of 3D microchips, that are referred to as nanoaquarium, fabricated by the present technique is inspection of microorganisms. To inspect movement of Euglena's flagellum is of great interest for biologists due to application to biomotors and clarification of the fertilization process. Currently, many biologists are trying to observe microorganisms placed in a Petri dish by an optical microscope with high-speed camera. However, the high numerical aperture objective lens used for the observation limits the field of view to a very narrow region and also limits the depth of focus to a very shallow region, thereby making it difficult to capture images of moving microorganisms. Consequently, it takes very long time to take significant images. To shorten the observation time is strongly demanded for biologists due to not only cost-effectiveness and time-effectiveness but also due to limited PC memory for taking movies using the high-speed camera. To overcome these problems, we propose to use the microchip for the observation of microorganisms. The microchip can scale down the observation site, namely, it can 3D encapsulate microorganisms in a limited area, so that it makes much easier to capture the images of moving microorganisms.

Figure 1 shows (a) a 3D schematic illustration and (b) a top view and (c) a cross-sectional view of optical microscope images of a microchip fabricated for the inspection of Euglena. The top wall of the channel is flat and parallel to the glass surface. Such a flat surface was achieved by multiple scanning of the laser beam with lateral shifts. In addition, post thermal treatment after wet etching realized very smooth surfaces [7]. These results are important for taking clear images. For the observation, Euglenas are introduced into one of reservoirs filled with water and then Euglenas swim into the channel. At this moment, we can see the movement of Euglena using a microscope from the top of the glass surface.

Figure 2 shows a picture of living Euglena taken using the microchip. We also succeeded in taking a movie that revealed that Euglena coils his flagellum around his body and rotates it with high-speed to get a driving force when he moves straight. The observation using the microchip has several advantages over the conventional



(a) (b) Top (c) Cross-section

Figure 1. (a) 3D schematic illustration and (b) a top view and (c) a cross-sectional view of optical microscope images of a microchip fabricated for the inspection of Euglena.

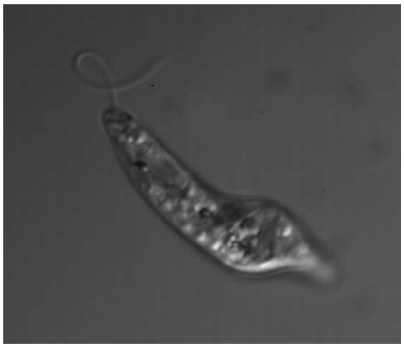


Figure 2. Picture of living euglena taken using the microchip.

observation method: (1) analysis time can be greatly reduced. For example, it takes only a few seconds to take a one shot picture while more than 10 min even in lucky case by the conventional method. Furthermore, it takes several minutes to take a movie while very long time or even quite difficult by the conventional method, (2) 3D observation is possible, (3) motion of Euglena can be controlled, and (4) the living microorganisms can be kept very fresh for long time since the microchannel three-dimensionally confined in the glass can avoid vaporization of water.

4. INTEGRATED MICROCHIP FOR PHOTONIC BIOSENSING

The present technique fabricating 3D hollow microstructures can be also used for embedding some microoptics such as micromirrors [8] and microlenses [15] in a glass chip. Furthermore, the optical waveguide can be written inside the photostucturable glass by fs laser direct writing without annealing and successive wet etching due to refractive index increase of the laser exposed regions [14]. Figure 3 shows an example of near field pattern of He-Ne laser beam guided by the written optical waveguide, showing a single-mode pattern. The propagation loss was evaluated to be approx. 0.5 dB/cm.

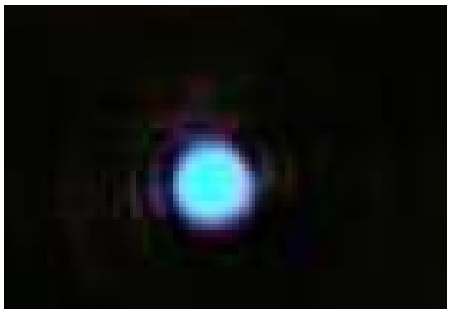


Figure 3. Near field pattern of He-Ne laser beam guided by the 10 mm long optical waveguide written by the fs laser direct writing. Pulse energy and scanning speed were 0.5 μJ and 200 μm/s, respectively.

The microfluidics, microoptics and optical waveguide can be easily integrated in a single glass chip. Figure 4 shows the schematic illustrations of the 3D integrated microchip for photonic biosensing, in which one waveguide of 6 mm length is connected to a microchamber of $1.0 \times 1.0 \times 1.0\,\mathrm{mm}^3$ volume, and two microlenses of 0.75 mm curvature radius are arranged at the left for fluorescence measurement and opposite sides from the optical waveguide across the microchamber for absorption measurement at a distance of 200 μm.

For fluorescence analysis of a liquid sample, the microfluidic chamber was filled with laser dye Rh6G of 0.02 mol/L dissolved in ethanol. A pump laser beam of the 2ω of Nd:YAG laser was guided by the optical waveguide and introduced into the microfluidic chamber. The emission spectra from the laser dye solution were collected in the detector through the plano-convex microlens I. The head of the spectrometer was placed at the end of the glass chip, i.e., behind microlens I, to detect the fluorescence. We measured the emission

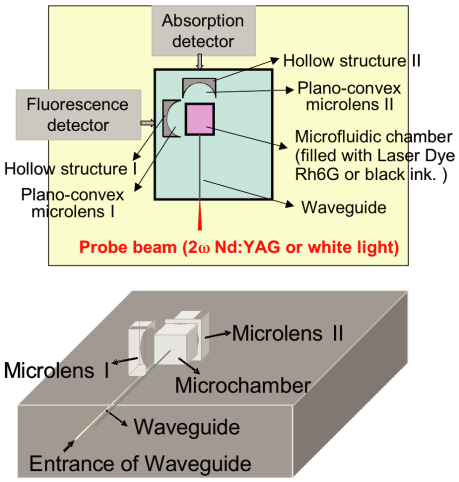


Figure 4. Schematic illustrations of the 3D integrated microchip for photonic biosensing.

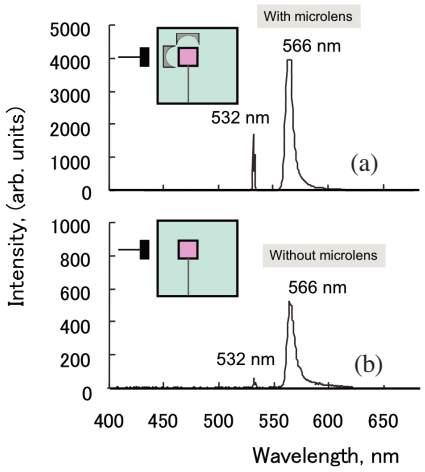


Figure 5. Fluorescence spectra from laser dye Rh6G analyzed by using microchips integrated (a) with and (b) without a microlens.

spectra from the laser dye in the microfluidic chamber at different pump energies. A typical emission spectrum with a central wavelength of 566 nm (the peak at 532 nm is from the pump laser), corresponding to the maximum emission of the dye, was obtained, as shown in Fig. 5. For comparison, the emission measurement was also performed for a

microfluidic chamber integrated only with a waveguide but without a microlens. Clearly, the enhanced emission intensity was achieved when a microoptical plano-convex lens was integrated. The enhancement of light intensity by a factor of 8 was realized. Furthermore, for the optical absorption analysis of a liquid sample through the plano-convex microlens II, the black ink at different concentration from 0.1 to 1.0% diluted with water was filled in the microchamber, so that the sensitivity was enhanced by a factor of 3 compared with the microchip without a microlens. These results indicate that the 3D integrated microchip fabricated by our techniques is highly efficient for optical analysis of biochemical samples such as fluorescence and absorption measurement.

5. CONCLUSIONS

We have demonstrated 3D microfabrication of photostructurable glass by femtosecond laser direct writing. By fabrication of the hollow microstructures, a variety of microfluidic components and microoptical components like a micromirror, a microlens are successfully integrated in a glass chip. One of the interesting and important applications of microchips, that are referred to as nanoaquarium, fabricated by the present technique is inspection of living cells and microorganisms that has several advantages over the conventional inspection method. The optical waveguide can be further integrated into the identical glass chip after the hollow microstructure fabrication. Such an integrated microchip is of great use for biochemical analysis and medical inspection based on photonic sensing. Finally, we conclude that the technique presented here is very promising for manufacture of highly integrated biophotonic microchips.

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