Electronic Supplementary Information (ESI)

Direct laser writing of sub-50nm nanofluidic channels buried in glass for three-dimensional micro-nanofluidic integration

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I. Materials and Methods

Porous glass preparation

The porous glass was fabricated by removing the borate phase from a phase-separated alkali-borosilicate glass in hot acid solution. Reagent-grade chemicals of $Na2CO_3$, SiO_2, and $H3BO_3$ were used as starting materials. The chemical composition of initial glass was $9Na_2O$-$26B_2O_3$-$65SiO_2$ (mol%). After a thorough mixing, the powders were melted in a platinum crucible at 1400 °C for 90 min. A subsequent heat treatment was carried out at 580 °C for 40 h for the development of phase separation. The obtained phase-separated alkali-borosilicate glass was cut to pieces with a size of 10 × 10 × 3 mm, and polished, afterwards they were leached by 1 mol/L HNO_3 at 100 °C for 72 h and then the porous glass samples were obtained. The composition of the porous glass obtained was $95.5SiO_2$-$4B_2O_3$-$0.5Na_2O$ (wt.%). The pores with a mean size of ~10 nm are distributed uniformly in the glass, which occupy 36% in volume of the glass. Particularly, these nanopores in the porous glass form a 3D connective network which allows liquid to flow through.

Fabrication of 3D micro-nanochannel networks

A regeneratively amplified mode-locked Ti:sapphire laser (Coherent, Inc.) with a pulse duration of ~100 fs, a central wavelength of 800 nm and a repetition rate of 250 kHz was used to produce the microchannels inside the mesoporous glass. The energy

of the beam was controlled using a combination of polarizer and waveplate and a set of neutral density filters. The polarization direction of the linear-polarized laser was controlled with a λ/2 waveplate. The initial 8 mm diameter of the femtosecond laser beam was reduced to 3 mm by passing through a circular aperture so as to guarantee a high beam quality (i. e., the central part of the beam was used to produce a more uniform energy distribution). The porous glass sample was then fixed in a petri dish filled with distilled water, which can be translated by a computer-controlled XYZ stage with a resolution of 1 μm. For fabrication of reservoirs, a 20× objective (N.A.=0.45) was employed for focusing the beam onto the surface of sample. For fabrication of microchannels and nanochannels, a 100× water-immersed objective (Nikon, CFI Plan Achromat, NA=1.10) with a long working distance of 2.5 mm was employed for focusing the beam into the sample. (See Figure S1 in Supporting Information)

After the laser direct writing, the glass sample was annealed at ~1120 °C for 120 min, and then naturally cooled to the room temperature, by which the nanopores (i. e., average diameter ~10 nm) in porous glass can be collapsed. However, the fabricated nanochannels survived due to their relatively larger size (i. e., ~40 nm width). To verify that the porous glass had been completely consolidated and the nanochannels enclosed in glass could function as a through structure, we filled the nanochannels with Rhodamine B solution with a concentration of 1 mg/ml in water. The fluorescence microscopy image of the nanochannels are shown in Figure 2. It is clear

that the Rhodamine solution can be observed over the entire length of the nanochannels. (See Figure S2 in Supporting Information)

Nanochannel Imaging and Characterization

To measure the cross-sectional dimensions of the nanochannels after postannealing, several nanochannel arrays were fabricated under same conditions inside one glass substrate, and then the substrate was mechanically cleaved to expose the cross sections of some of the nanochannels. The cross-sections of nanochannels were directly characterized using a scanning electron microscope (Zeiss Auriga 60). The top-view bright-field optical images and fluorescence microscopy images of the nanochannels were taken using an inverted optical microscope (Olympus IX71) equipped with a cooled CCD camera (Lumenera INFINITY2-1R).

Stretching λ DNA in Nanochannels

The λ DNA (Takara Co., Code No. 3010) of 48.5 kb (contour length ~16.5 μm) is labeled with SYBR@ Gold (Molecular Probes, S11494) at a saturated concentration of 1 dye molecule per 4 base pairs in 0.5× TAE buffer (20 mM Tris-acetate, 1 mM EDTA, pH 8.0). The sample solution was pipetted into one reservoir, whereas the other reservoirs were kept empty to induce a capillary force in the nanochannels. The full contour length of the stained DNA is expected to have an increase of 30% due to the saturated intercalating dyes, which corresponds to a length of ~21.5 μm. In our experiment, the average stretched length of the DNA molecules and its standard deviation in the nanochannels were calculated from the measured end-to-end lengths of at least 10 extended single DNA molecules.

II. Figures S1- S2

Figure S1

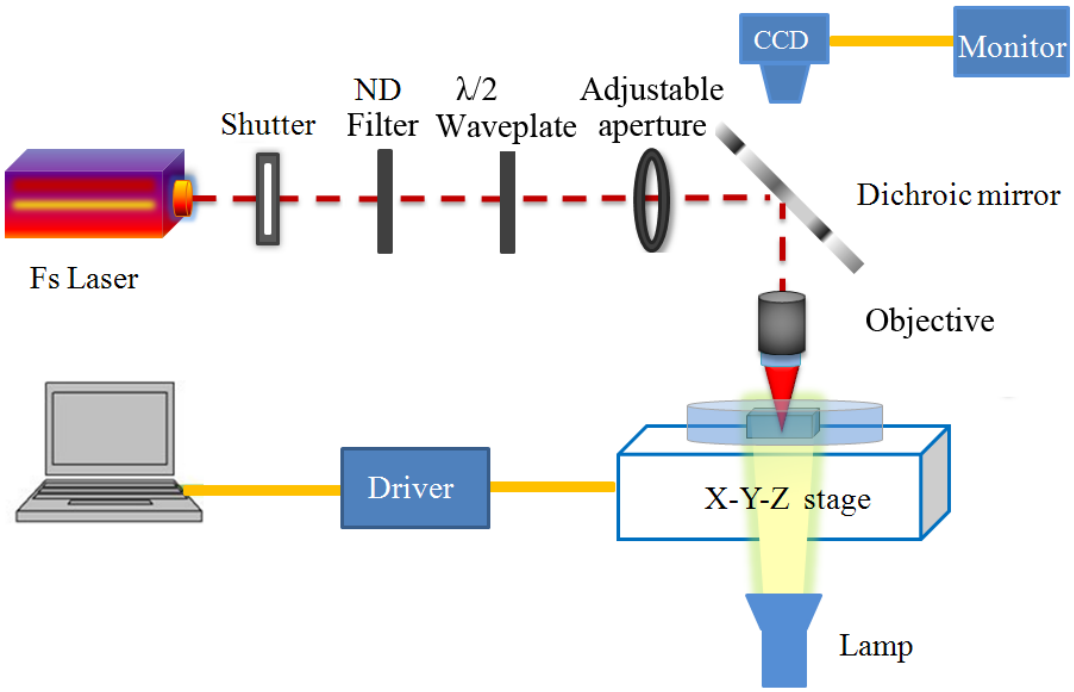

Figure S1. Schematic diagram of 3D femtosecond laser machining system.

Figure S2

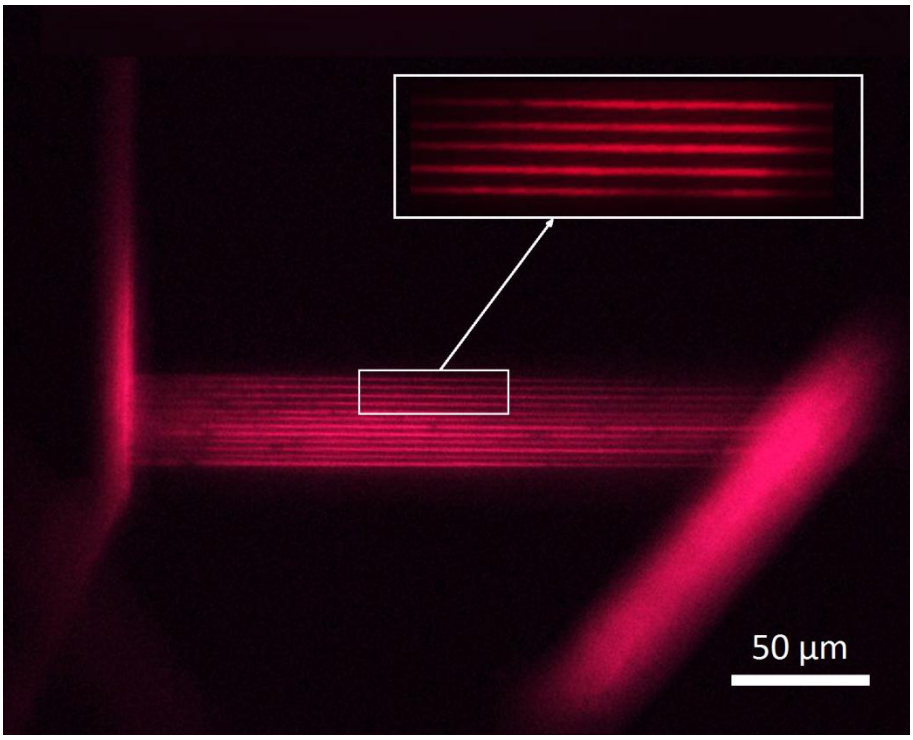

Figure S2. Fluorescence microscope image of the postannealed nanochannels filled with Rhodamine B solution, showing that the nanopores have been collapsed (i. e., no leakage was observed) while the nanochannels all have survived after postannealing.

III. Movie M1

Movie 1. Real-time video clip showing the process of direct writing of nanochannel in porous glass immersed in water. The nanochannel was written with the pulse energy of ~132 nJ at a translating speed of 5 μm/s. The continuous ejection of bubbles provides a clear evidence that an unclogged nanochannel with a length of ~ 50 μm has been formed with a single scan.