

# Direct fabrication of a microfluidic chip for electrophoresis analysis by water-assisted femtosecond laser writing in porous glass

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We report the direct fabrication of a microfluidic chip composed of two high-aspect ratio microfluidic channels with lengths of 3.5 cm and 8 mm in a glass substrate by femtosecond laser micromachining. The fabrication mainly consists of two steps: 1) writing microchannels and microchambers in a porous glass by scanning a tightly focused laser beam; 2) high-temperature annealing of the glass sample to collapse all the nanopores in the glass. Migration of derivatized amino acids is observed in the microfluidic channel by applying electric voltage across the long-migration microchannel.

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In the past decade, significant efforts have been exerted on the development of micro total analysis systems ( $\mu$ -TASs) and lab-on-a-chip (LOC) systems for chemical, biological, and medical applications. At the heart of most  $\mu$ -TAS and LOC devices are integrated microfluidic systems that can perform various functions, such as mixing, separation, and transport<sup>[1,2]</sup>. Traditional methods for fabrication of microfluidic systems typically rely on two-dimensional (2D) lithography technique<sup>[3,4]</sup>. In such cases, the open channel structures are first fabricated on substrate surfaces and then sealed with a flat cover layer. As a 2D approach, the development of three-dimensional (3D) structures with lithography-based fabrication technique is difficult because of the multilayer stacking and bonding requirements. Frequently, the extra bonding process causes contamination and clogging in the tiny channel.

To solve these problems, femtosecond laser fabrication techniques have been developed to create 3D microfluidic structures in various transparent materials<sup>[5–13]</sup>. A recently developed technique has enabled direct fabrication of microfluidic structures embedded in glass with nearly arbitrary sizes and geometries. This fabrication process consists of two procedures: 1) femtosecond laser direct writing in porous glass immersed in water to create hollow fluidic channels and chambers; 2) high-temperature post-annealing of fabricated samples to collapse all nanometer-sized pores; otherwise, the microfluidic channels and chambers will leak through the surrounding pores that form a connective network in the substrate. Using this technique, we have successfully created a long-running square wave-shaped channel<sup>[14]</sup>, a large volume microchamber<sup>[15]</sup>, and a 3D microfluidic mixer with complex geometry inside the glass chip<sup>[16]</sup>. In addition, this technique has extended the fabrication

resolution of fluidic structures in glass from micrometer to sub-100-nm scale<sup>[17]</sup>.

In this letter, we demonstrate the fabrication of a fully functional capillary electrophoresis (CE) analysis chip in glass using this relatively new technique. Electrophoresis is an important procedure in chemical and biological analyses because it can separate charged analytes with different size-to-charge ratios by accelerating them at different velocities in a conductive liquid in thin capillaries under the influence of an electric field. Currently, CE experiments in microfluidic channels fabricated on microchips are becoming increasingly popular because the microfluidic CE chips typically consume only picoliters of samples and produce less waste<sup>[2]</sup>. Further developments in the microfluidic chip CE technique require the integration of preparation and analysis functions into the microchips. Toward this goal, femtosecond laser 3D micromachining holds great promise and has already been demonstrated for creating a variety of functional components, including microfluidics, micro-optics, and microelectrodes<sup>[18,19]</sup>. For instance, the integration of optical waveguides into a commercially produced CE device has been demonstrated by femtosecond laser direct writing, showing enhanced functionality and ease of operation<sup>[20]</sup>. Nevertheless, in this example, the CE device is prefabricated using traditional lithography technology, primarily because of difficulties in forming homogeneous large-scale microfluidic networks embedded inside the glass with traditional femtosecond laser micromachining techniques. As we will show later on, the difficulty can be solved by writing directly on the porous glass using tightly focused femtosecond laser pulses. We also test the functionality of the femtosecond laser-fabricated CE device by observing the motion of three amino acids (L-Histidine, L-Lysine, and DL

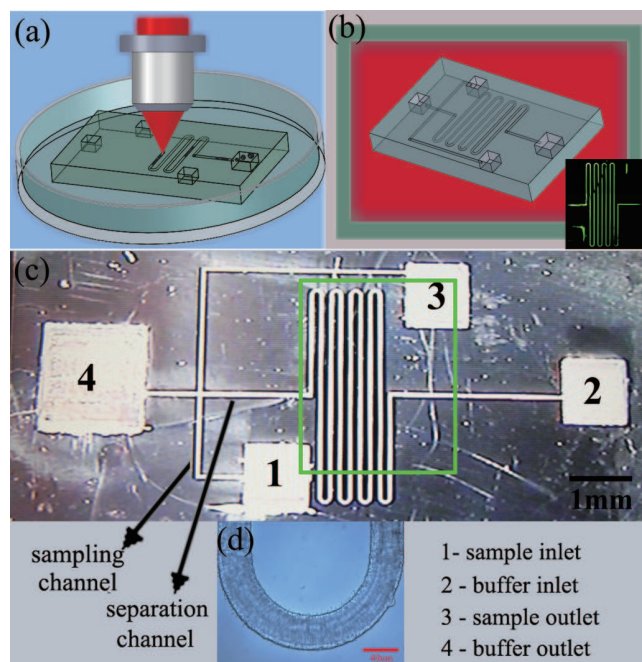


Fig. 1. (Color online) Flow chart of fabrication procedures: (a) laser direct writing inside porous glass and (b) annealed in a furnace. Inset: fluorescence microscopy image of the microchannel filled with a solution of fluorescein. (c) Overview optical micrograph of the fabricated electrophoresis microchip. (d) Close-up view of the turning area of the microchannel. The green box indicates that the areas where the microscope images in Fig. 2 are captured.

-Alanine) in the microfluidic channels.

To fabricate 3D microfluidic structures inside the glass, a high-silica porous glass is used as a substrate for femtosecond laser direct writing. The porous glass samples are obtained by removing the borate phase from phase-separated alkali-borosilicate glass in hot acid solution<sup>[21]</sup>. The composition of the porous glass is about 95.5SiO<sub>2</sub>-4B<sub>2</sub>O<sub>3</sub>-0.5Na<sub>2</sub>O (wt.%). Pores with 10 nm mean size are distributed uniformly in the glass to form a 3D connective network occupying 40% of the whole glass volume. The connected pores allow liquid to infiltrate into the glass from all directions. For this reason, ablation induced by femtosecond laser irradiation actually occurs in the liquid, which leads to bubble generation in the channel. The bubbles can then help remove the debris by carrying them out of the microchannel<sup>[15]</sup>.

We first fabricated four microchambers, as illustrated in Fig. 1. A regeneratively amplified mode-locked Ti:sapphire laser (Coherent, USA.) was used to produce the microchambers on the surface of the porous glass. The laser was operated at a central wavelength of 800 nm, with a minimum pulse duration of 40 fs after the compressor and repetition rate of 1 kHz. The laser beam energy was set to 70 mW and then focused onto the porous glass through a 50× objective with a numerical aperture (NA) of 0.50. After completing the four microchambers, two microchannels were written using another femtosecond laser with a pulse duration of 100 fs, wavelength of 800 nm, and repetition rate of 250 kHz. The microchannels were buried at an approximate depth of 200 μm beneath the surface. This channel depth was controlled by initially focusing the focal spot on the top

surface of the sample and then shifting it downward by a 160-μm distance. Considering the refractive index of porous glass immersed in water and the shrinkage of the glass sample after the post-annealing process, the final depth of the channels was approximately 200 μm. In fabricating the channels, laser pulses with 1.5-μJ energy were focused into the porous glass through an oil immersion objective, with a NA of 1.10. The microchambers were fabricated using a 1-kHz laser because of its high ablation efficiency. The femtosecond laser direct writing scheme is presented in Fig. 1(a).

After laser direct writing, the porous glass was annealed inside a furnace at 1 100 °C for 2 h to transform it into consolidated glass, as schematically illustrated in Fig. 1(b). The completed microchip is shown in Fig. 1(c), and a close-up view of the corner area of the microchannel is shown in Fig. 1(d). The smooth edge of the channel indicates that the ablation debris generated during laser writing has been efficiently removed. The lengths of the longer and shorter microchannels are 35 and 8 mm, respectively. In addition, the channels have a depth of about 10 μm and a width of approximately 36 μm. The channel width can be controlled by scanning the femtosecond laser line-by-line<sup>[15]</sup>.

Three amino acids, L-Histidine, L-Lysine, and DL-Alanine (Sigma-Aldrich), were used as analytes to demonstrate usage of the femtosecond laser-fabricated CE device. Fluorescein isothiocyanate isomer I (FITC), also purchased from Sigma-Aldrich, was applied to derivatize the amino acids. Borax (disodium tetraborate-decahydrate) and pyridine were obtained from Qiang-Sheng Chemistry Industry (Jiangsu, China) and used in preparing the buffer solution. Water was purified through a triple-distillation apparatus. All the said materials were of reagent grade. Each amino acid was dissolved in borax buffer (10 mmol/L, pH 10.0) at a concentration of 10 mmol/L. A 2-ml FITC (10 mmol/L) solution was prepared with acetone and then 10-μL pyridine was added. Each amino acid solution (20 μL) was mixed with 20 μL of the FITC solution and stored overnight in the dark. Furthermore, external platinum wires with diameter of 0.1 mm were used as electrodes to provide high voltage on the solutions in the reservoirs. Two power suppliers (DW-P252, Dongwen High Voltage Power Supply Co., China) were used to introduce high voltages over the microchannels.

After the derivatized amino acids were added into the inlet, a voltage of 200 V was applied across the sampling channel. The voltage applied across the migration channel was 100 V. After 2 s, the amino acids were driven into the junction of the two microchannels. With a migration voltage of 620 V applied across the migration channel and 200 V across the sampling channel, movement of the amino acids was observed under a fluorescence microscope (IX71, Olympus, Japan). Fluorescence excitation of the derivatized amino acids was achieved with a white light source. Only a limited spectral portion (i.e., 490 to 495 nm) of the white light can be used for fluorescence excitation, so a band-pass filter with a center wavelength of 490 nm was placed after the white light source. To observe the fluorescence (525 nm) from the amino acids, another filter was placed before the charge-coupled device (CCD). Images of the derivatized amino acids were

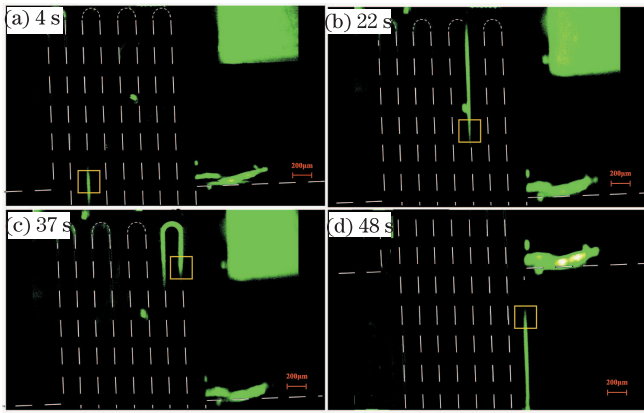


Fig. 2. (Color online) Fluorescence microscopy images (green box in Fig. 1) of the derivatized amino acids at different times, with voltage of 620 V applied across the migration channel and 200 V across the sampling channel. In all of the panels, the yellow boxes indicate the moving fronts of the migrating amino acids. In addition, the main parts of the migration microchannels are not visible due to the lack of fluorescence emission, so white dashed lines are artificially added to indicate the migration channels.

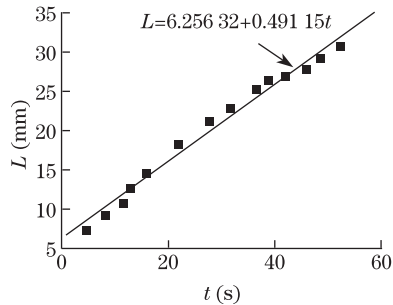


Fig. 3. Migration distances of the derivatized amino acids at different times fitted with a linear function.

captured at different times as the constant migration voltage was being applied across the migration channel, as shown in Fig. 2. The moving fronts of the amino acids are indicated by the yellow box at different times (Fig. 2). The amino acids are observed to migrate in the microfluidic channel. The migration distances of the derivatized amino acids at different times can be evaluated from the images presented in Fig. 2. The velocity of the migration is 0.49 mm/s, as observed by fitting the data with a linear function, as shown in Fig. 3. The linear increase in migration distance with respect to time confirms the homogeneity of the fabricated migration channel, as reported previously<sup>[14]</sup>.

Currently, most CE microchips used to separate analytes are fabricated on polymer/glass substrates based on lithography approaches. Polymer CE chips are easy to fabricate and the inner wall of the microchannel can be easily modified. However, the electro-osmotic flow can be unstable in polymer channels, which will decrease the separation efficiency. In addition, for CE chips fabricated using planar lithography techniques, sealing of the open channels fabricated on the upper substrate surfaces always poses a problem because complete sealing of tiny channels on the micrometer or even nanometer scales frequently causes clogging and/or leakage. We stress that

these problems can be well addressed via our technique, as we have shown above.

We employed a fluorescence microscope in observing the motion of the amino acids. Therefore, separation of the three amino acids is not observed because of insufficient temporal resolution and high background noise. Higher temporal resolution can be achieved by replacing the fluorescence microscope with a photo multiplier tube, which has much higher sensitivity and temporal resolution. Furthermore, the inner walls of the microchannels in the microchip used in this experiment are not coated to avoid adsorption of ions. Nevertheless, in our experiment, migration of the amino acids is observed. Thus, the microfluidic chip fabricated by femtosecond laser can fulfill all the requirements for CE analysis.

In conclusion, we demonstrate an electrophoresis microchip directly fabricated inside the glass by femtosecond laser direct writing. By applying voltage across the migration channel, movement of derivatized amino acids is induced through CE. Our result shows that femtosecond laser direct writing can be employed in fabricating large-scale microfluidic chips in glass substrates, which require high-aspect ratio channels with lengths at centimeter scale and diameters at micrometer scale. In addition, the CE separation function of the femtosecond laser-fabricated microchip is demonstrated by observing the migration of derivatized amino acids in the microfluidic channels with constant velocity. The expansion of the applicability of femtosecond laser microfluidic fabrication to large-scale microfluidic structures will benefit the development of highly integrated LOC systems and devices.

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