Electronic Supporting Information

Versatile Microfluidic Total Internal Reflection (TIR)-based Devices: Application to Microbeads Velocity Measurement and Single Molecule Detection with Upright and Inverted Microscope

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Glass microchannel fabrication.

A PDMS microchannel (30 mm in length, 500 µm in width and 80 µm in height) as mask for HF etching of a cover glass (NEO micro cover glass, 24 x 60 mm, No. 1: 0.12-017 mm in thickness, Matsunami, Japan) since PDMS is resistant to repetive immersion of diluted HF under 20% (Fig. 1(a)).[1,2] Since only the channel region of the PDMS was etched, the glass channel dimensions would be 30 mm in length, 500 µm width (Fig. 1(c)). The depth of this glass channel substrate was controlled by etching time. Using HF solution of 5%, the etching depth can be 16 µm after 15 min etching time. The etched glass channel was sealed with a coverslip using water glass bonding technique.[3,4] The through holes on the coverslip were etched using another PDMS mask with more concentrated HF solution of 49% (Fig. 1(b)). Typically, it took 15-20 min to etch through hole of the

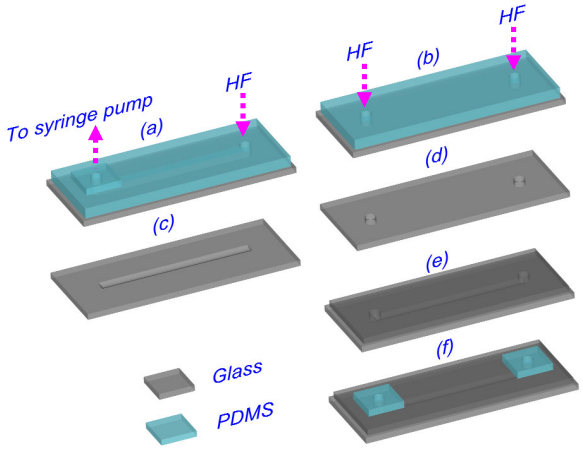

Fig. 1 Fabrication process of glass microchannel (a) glass slide etching using HF (5%) (b) coverslip through holes etching using HF (49%) (c-d) clean etched glass channel and coverslip after PDMS removals (e) coverslip and etched channel were brought into contact, pressed and cured in an oven (f) inlet/outlet PDMS connectors were bonded using PDMS glue

coverslip using HF 49%.

The water glass bonding was done as follows. At first, the etched channel and coverslip were cleaned in acetone ultrasonication for 5 min then rinsed and dried with DI water and N_2. Next, they were hydrolyzed in $NH_4OH/H_2O_2/H_2O$ (2:1:4) solution at 80°C for 15 min (Fig. 1(c-d)). Sodium silicate (contains 35-58 wt% SiO_2, 17-19 wt% Na_2O and Fe 0.02 wt%, Kanto Chemical Co., Japan) diluted in DI water at 10 wt% was prepared as water glass solution. Before spincoating, the water

glass solution was filtered through 0.22 μm pore size filter (MN Sterilizer PES, Macherey-Nagel, Germany) to eliminate the any precipitated particles. The filtered water glass solution was spincoated on the coverslip at 2000 rpm in 30s (Fig. 1(d)). After spincoating, the coverslip was immediately brought into contact with the etched channel, placed on a flat ceramic plate and manually pressed all over by using a roller. Curing was done in an oven at 90^0C in 1h (Fig. 1(e)). Finally, the inlet and oulet connectors made in PDMS were attached using liquid PDMS as glue (Fig. 1(f)). There was no leaking observed although we pumped the red dye back and forth several minutes. With our process, we could achieve bonding efficiency of over 90% and the whole fabrication process can be completed within 6 hours.

Observation of TIR spots with multimode and singlemode fibers

We compared the TIR spots generated by microfluidic TIR-based devices integrated with multimode and singlemode fiber using upright microscope by imaging TMR fluorescent dye (C-1171, Molecular Probes Inc., USA) in dimethylsulfoxide (DSMO) at a concentration of 0.5 mM. 10 μl of TMR sample was injected into the inlet of the PDMS microchannel and the syringe pump was used to load the sample inside the channel.

Since singlemode fiber only allow one mode of transmission with smaller NA. It is expected that singlemode fiber would result in more coherent and more uniform TIR spots. The uniformity of the TIR spot is very important to guarantee all fluorescent molecules in the field of view are equally excited. Fig. 2(a) and (c) show the fluorescent image of the TIR spots of TMR dye at 10X magnification and 150 ms exposure using multimode and singlemode fibers, respectively. Although TMR dye was filled all over the microchannel substrate, only within the TIR spot that TMR dye was excited by evanescent wave and fluoresced. Fluorescent intensity scan profiles of Fig. 2(a) and (c) are shown in Fig. 2(b) and (d), respectively. As can be clearly seen, the singlemode fiber has resulted in a more uniform and more coherent TIR spot compared to that of multimode fiber.

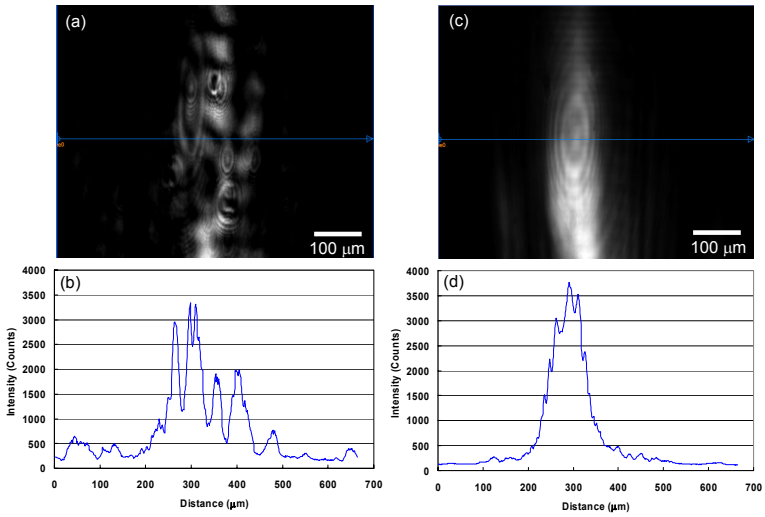

Fig. 2 (a) and (c) are TIR spot fluorescent images of TMR dye (0.5mM) captured by using upright configuration with multimode fiber and singlemode fiber, respectively. (b) and (d) are fluorescent intensity scan profiles of (a) and (c), respectively. For both cases, the magnification was 10X, exposure time was 150 ms and laser diode power was adjusted to 10 mW.

References

1 I. Rodriguez, P. Spicar-Mihalic, C. L. Kuyper, G. S. Fiorini and D. T. Chiu *Anal. Chim. Acta*, 2003, **496**, 205–215

2 R. Yokokawa, S. Takeuchi and H. Fujita *Analyst*, 2004, **129**, 850-854

3 H. Y. Wang, R. S. Foote, S. C. Jacobson *Sens. Actuators B*, 1997, **45**, 199-207

4 T. Ito, K. Sobue and S. Ohya *Sens. Actuators B*, 2002, **81**, 187-195