A proposed paper submitted to the

Journal of Micromechanics and Microengineering

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Abstract

This paper describes a fast, low-cost but reliable process for fabrication of microfluidic systems on soda-lime glass substrates. Instead of using expensive metal or polisilicon/nitride layer as etch mask, a thin layer of AZ 4620 positive photoresist (PR) is used for buffered oxide etching (BOE) of soda-lime glass. A novel two-step baking process prolongs the survival time of the PR mask in the etchant, which avoids serious peeling problems of the PR. A new process to remove precipitated particles generated during etching process is also reported by dipping the glass substrate in 1 M HCl solution. A microfluidic channel with a depth of 35.95 ± 0.39 μm has been formed for 40-minute BOE etching in ultrasonic bath. The resulting channel has a

smooth profile with surface roughness less than 45.95 ± 7.96 Å. Glass chips with microfluidic channels are then bonded at 580 °C for 20 minutes to seal the channel while a slight pressure is applied. A new bonding process has been developed such that the whole process can be finished within 10 hours. To our knowledge, this is the shortest processing time that has ever been reported. In the present study, an innovative microfluidic device, “micro flow-through sampling chip”, has been demonstrated using the fabrication method. Successful sampling and separation of Cy5-labeled BSA and anti-BSA has been achieved. This simple fabrication process is suitable for fast prototyping and mass production of the microfluidic systems.

Keywords: Microfluidic system, Soda-lime glass, Etch mask, Fusion bonding, Glass chips

Nomenclature

BSA: bovine serum albumin

Anti-BSA: anti-bovine serum albumin

PR: photoresist

BOE: buffered oxide etch

HCl: hydrochloride

μ–TAS: micro total analysis system

DPI: dot per inch

HMDS: hexamethyldisilazane

K_{sp}: solubility product constant at 25 °C

EDAX: energy dispersive analysis X-ray

LIF: laser induced fluorescence

1. Introduction

Recently, microfabrication of miniature analytical instrument on glass substrates for chemical and biomedical applications has been widely studied. Various microfluidic devices have been fabricated on different substrates for μ–TAS (micro total analysis system) applications such as capillary electrophoresis[1-8], electrochromatagraphy[9], DNA separation[10,11], and semen testing[12]. Among them, silica-based devices are of great importance for microfluidics since they provides advantages over other materials such as high resistance to mechanical stress and chemicals, large optical transmission range, and high electrical insulation. Even though fabrication techniques for microfluidic devices on silica-based substrates are extensively investigated [13], there still exists a need for development of a simple, low-cost, and time-efficient technique. In this study, we report a fast, low-cost and reliable process for the fabrication of microfluidic devices on soda-lime substrates.

An evaporated metal film or a polysilicon/nitride layer was usually used as wet-chemical etch mask for the fabrication of glass-based

microfluidic devices. Several microfluidic devices have been demonstrated successfully[1,4]. However, deposition of the metal/polysilicon/nitride films in a vacuum system is an expansive and time-consuming process. Instead, polymer-based soft etch-mask showed potential for fabrication of glass-based microfluidic chips. Mathies[14] reported that Shipley 1400-31 positive PR could be used as etch mask for soda-lime glass in buffered oxide etching process and an 8 μm-deep channel for 15-min etching could be achieved. Similarly, Stjernström[15] used Shipley 1813 positive PR as the etch mask for soda-lime glass in buffered HF solution. In order to prolong the survival time of the PR etch mask in the etchant, both of the studies used a long baking process more than 80 min. In the present study, a novel two-step baking process was developed, in which temperature variation during baking process was relieved and residual thermal stress between the glass substrate and the PR was reduced accordingly. Subsequently, the improved adhesion of the PR on the glass substrate was achieved. Experimental data showed that AZ 4620 positive PR mask using the two-step baking process could sustain more than 50 min in ultrasonic-agitated BOE bath.

For planar microfluidic devices, surface roughness and channel geometry after etching are the major factors that would affect the performance of the devices. An excellent review article describing the details of the etching kinetics and mechanism of multicomponent glasses in acid-based solution can be found in Ref. 16. It was found that the etched surface became porous[16-18] and was covered with some precipitates for soda-lime glass substrates[17,20]. It is believed that precipitated particles generated during etching process may distort the device geometry and roughen the surface of the etched channel. Several works have reported the etching rate and etched qualities of glass substrates using different etchants[4,15]. However, quantitative data have not been shown in their studies. In this present study, a novel etching process achieving an excellent etching quality and smoother etched surface was quantified using surface profilometer and scanning probe microscope imaging methods.

Additionally, bonding process is a critical step in the fabrication of the microfluidic device. Low temperature bonding techniques, including HF bonding[21-23] and adhesive-layer methods[23], are widely used for sealing of

the microfluidic channels. The low temperature bonding yields low residual stress and makes chemical surface modification prior to bonding feasible, if necessary. However, HF bonding needs a rigorous cleaning procedure of the glass substrates prior to bonding. The existence of any un-removed particle will fail the bonding process. Besides, bonding strength could not be as large as one using other methods. The formation of precipitates after bonding also hinders the application of the chips, which makes the bonded substrates turn blurred while precipitated particles are sediment in the sealed channel. On the other hand, the use of adhesives or intermediate layers may clog the channels. Solvent extraction and analyte adsorption of the adhesives could also affect the performance of the microfluidic chips. Alternatively, anodic bonding has been reported for glass bonding. However, it is unsuitable for glass-glass bonding without any intermediate layer[24]. Instead, thermal bonding provides good bonding strength for glass (or quartz) substrates. For thermal bonding, quartz substrates need the fusion temperature above 1050 ºC and borosilicate glasses need the temperature between 650 to 800 ºC. The temperature holding time is about 6

to 8 hours, not including the sophisticate heating and cooling procedures. The long thermal process is time-consuming and may cause collapse and distortion of the channel. In the present study, we developed a new thermal process for glass-glass bonding. To the best of our knowledge, it is the shortest processing time that has ever been reported.

In this work, a fast, simple but reliable process has been reported for the fabrication of the microfluidic process. The developed two-step baking process is not only reduce the processing time but it also prolongs the survival time of positive PR etch mask in BOE solution. An excellent surface quality could be obtained by an improved etching procedure. We also described a shorter thermal bonding process without sophisticate polishing and cleaning procedures. The high bonding yield was obtained by using sintered alumina flats as the upper and underlying supports. This simple fabrication method will enable us to fabricate microfluidic structures in an efficient way.

2. Materials

Commercially-available microscope glass slides (76 x 26 x 1 mm^3, Marienfeld, Germany) and polished soda-lime glasses (300 x 400 x 1.1 mm^3, G-Tech optoelectronics Crop., Taiwan) were used in this study. Prior to microfabrication of the glass chips, the microscope glass slides were annealed at 400 °C for 4 hours to relieve the residual stress inside the slides. The polished soda-lime glasses were cut into small piece (50 x 43 mm^2) to fit the design of the microfluidic device with no need for annealing. The photomasks were generated using a layout software (AutoCAD) and were printed on a transparent film using a high-resolution laser printer (10,000 DPI). All the chemicals used in this study were regent (J. D. Baker, Philadelphia, USA) grade and processing solutions were prepared using de-ionized (DI) water.

3. Methods

The fabrication process used in this study was shown in Figure 1 and described as followed:

Substrates cleaning: Both kinds of the glass substrates were cleaned in a

boiling Piranha solution (H_2SO_4 (%) : H_2O_2 (%)= 3:1) for 10 min, then rinsed in DI water and blown-dry with nitrogen gas. The dehydration process was done by baking the glasses on a hot plate (100 °C) for 3 min to remove residual water molecules.

Lithography: The glasses were then spin-coated with HMDS (J. T. Baker) solution and baked on a hot plate (100 °C) for 3 min in order to improve the adhesion of the PR. The primer-treated substrates were coated with AZ 4620 positive PR, and a novel two-step baking process was used in this study. First, soft-baking process was done at 100 °C for 1 min, 120 °C for 1.5 min, and then maintained at 100 °C for another 1 min. This two-step process can reduce the thermal stress between the glass substrate and the PR, resulting in longer survival time of the positive PR in BOE etchant. The thickness of the PR was approximately 3 μm after soft-baking. The UV lithography was processed using a mask aligner (OAI Corp.) and exposure dose was 180 mJ/cm^2. The developing of the PR was accomplished in 70 seconds by immersing the exposed substrate into the developer (one part AZ A400K developer : three parts DI water). After rinsed by DI water and

blown-dry by N_2 gas, hard-baking of the PR was processed at 120 °C for 1 min, 145 °C for 10 min, and then returned to 120 °C for 1 min.

Glass etching: After baking of the PR, the glass substrates were immersed in the BOE (6:1) etchant. The etching was processed in an ultrasonic bath. In order to remove precipitated particles, we intermitted the etching process every 5 min and dipped the substrates in a 1 M HCl solution for 10 sec during the etching process. After the HCl dipping, the substrates were then cleaned by dipping in DI water and then immersed into BOE etchant again. The etching and de-precipitation processes were iterated until the etching process was finished.

Glass bonding: The etched glass substrate and another cover glass with drilled via-holes were cleaned using boiling Piranha solution for 10 min and then rinsed by DI water in ultrasonic bath for 3 min. The two glass flats were then carefully aligned and clung to each other by DI water. The atmospheric pressure will hold the two glasses tightly prior to bonding process. Bonding was performed by fusing the two glass in a sintering oven at 580 °C for 20 min with a ramp rate of 5 °C/min. Polished alumina flats

were stacked as upper and underlying plates which could prevent the formation of the fusion between glass substrate and alumina flats and kept the glass surface smooth. A sealed microfluidic device could be formed after bonding of the two glass plates.

4. Results and Discussions

Soda-lime glass is a multicomponent mixture of SiO_2 (72.8 %), Na_2O (13.7 %), CaO (8.8 %), MgO (4.0 %) and small amounts of Fe_2O_3 (0.12 %) and Al_2O_3 (0.1 %). The insoluble products will be present and crystalline precipitates will be formed while etching soda-lime glass in HF-containing solution. Since K_{sp} (solubility product constant at 25 °C) values of CaF_2 and MgF_2 are 4.0×10^{-11} and 6.4×10^{-9} [26], respectively, it is believed that most insoluble products are mainly CaF_2 and MgF_2. Previous work by EDAX (energy dispersive analysis X-ray) analysis of the precipitate also confirmed this surmise[15]. The formation of precipitates will result in bigger surface roughness and distort the shape of the channel. It will also hinder the etching process by preventing etchants in contact with the glass substrates.

In order to solve the problem, 1 M HCl solution was used in this study and it turned the precipitates into soluble products of $CaCl_2$ and $MgCl_2$, which could be removed easily afterwards.

In this study, two kinds of glass substrates, 10 pieces per batch, were fabricated using the methods described in the previous section. Figure 2 shows the etched glass substrate with PR etch mask. Without peeling the PR etch mask, an undercut structure can be clearly seen, which is the feature of isotropic etching of the glass substrate. An etched microfluidic channel and a reservoir structure are shown in Figure 3. A very sharp geometry and smooth etched surface structures were observed. Figure 3(b) shows a close-up image of Figure 3(a). It indicates that the edge of sidewall is well defined due to good adhesion of PR on the glass substrate. The depth and the surface roughness of the etched glass substrates were measured using a surface profilometer (Alpha-step 500). Three different locations on each glass substrate were measured. The average depth of the etched channels is 35.95 μm and the standard deviation is 0.39 μm for all 30 measured values. The etching rate is about 0.9 μm/min at room temperature and the etching is

isotropic inherently. The high etching rate and uniformity of the wet etching process was due to the fact that appropriate agitation was achieved by performing etching process in ultrasonic bath. Furthermore, ultrasonic agitation will make the insoluble precipitate, such as Al_2O_3, suspend in the solution instead of being sedimentary on the glass substrates. Figure 4 shows the surface profile of an etched glass substrate by using a scanning probe microscope (Solver P7LS). The maximum peak-to-peak roughness is 0.71 Å and the average surface roughness (R_a) is less than 0.1 Å inside a 3.0 μm x 5.0 μm scanned area. There was no observable difference regarding etching rate and surface roughness for microscope glass slides and polished soda-lime glasses However, annealing process indeed enhances etching quality of the microscope glass slides. Figure 5 shows a SEM micrograph of a BOE-etched microscope glass slide without annealing. Significant needle-liked structure was observed after etching, which blurred the glass and resulted in a rough surface. In addition, there were a lot of precipitates formed in the channel after etching and it could cause rough surface for an unannealed slide. Similar observation has been reported by Fan *et al*.[4] for

Corning 7740 Pyrex glasses.

The developed fabrication process was then used to demonstrate its capability for fabrication of microfluidic devices. Figure 6 shows a continuous flow-through sampling device fabricated on a 50 mm x 43 mm soda-lime glass substrate. The chip has a 20-mm-long separation channel, a 1.3-mm-long injection channel and 1.2 mm drilled holes for buffer and sample reservoirs. The dimension of the flow-through channel is 3 mm x 30 mm with a depth of 36 μm. In this study, we used the fusion bonding techniques to seal the channels. The 3 mm wide and 36 μm deep flow-through channel did not collapse after the fusion bonding process, while Harrison et. al.[1] reported that 1 mm-wide channel tended to collapse using similar thermal bonding process. It is believed that the developed bonding process using slighter pressure solves the problem. No permeation around the channel was observed by filling dye into the channel for leakage test. Figure 7 shows cross section view of the bonded channel. The etched channel remained its original shape and the interface between the two glass substrates disappeared after the fusion bonding process. It is found that

bonding temperature and holding time are the major factors for the fusion bonding process. Applied pressure and temperature ramping procedure only have minor effects on bonding quality. Temperature below 570 ºC caused an incomplete bonding even though two plates were held for a very long period of time. While temperature was higher than 580 ºC and holding-time is longer more than 30 min, it resulted in fusion of the glass substrates and the alumina plates, causing cracking of the device due to the difference of the thermal expansion coefficients of the two materials. In the present study, the overall time for the fusion bonding process including cooling procedure can be less than 6 hours, which is the shortest time ever reported for thermal bonding technique. The bonding process used in this study can reduce the operation time, and more importantly, preserve the original geometry of the etched channel with large area.

5. Application

In this study, a microfluidic chip for flow-through sampling and separation was fabricated and tested (Fig. 6). Detail information regarding

working principle and experimental setup was described in Ref. 25. A fluorescence dye-labeled (Cy5) BSA and anti-BSA contained buffer (20 mM Na_2HPO_4, pH=7.4) was used as the sampling flow. The concentration of the BSA and Anti-BSA in the sampling buffer was 5.0 ppm and 3.8 ppm, respectively. The flow rate was set at 2 μL/min and both of the injection and separation voltage were 1.3 kV. A continuous sampling and injection was taken at every 0.7 min with the loading time of 1 sec. The test was accomplished in 2 min. Signals were attained by a laser induced fluorescence (LIF) detection technique. The detection system was constructed through modifications of a commercial reflection microscope (Model BX40, Olympus, Tokyo, Japan). Briefly, a Helium-Neon laser beam with a wavelength of 632.8 nm (10 mW, LHR-991, Melles Gliot, Carlsbad, CA, USA) was focused within the channel using a 50x NA0.5 long working distance objective. Fluorescence was collected by the objective and passed through a beam splitter and a band-pass filter, followed by spatial filtering prior to photomultiplier detection (R928, Hamamatsu, Tokyo, Japan). Amplified photoelectron pulses were

converted to an analog signal and acquired by a commercial interface (Model 9524, SISC, Taipei, Taiwan) with a Pentium 75 MHz computer.

Figure 8 shows the testing result of a continuous flow-through sampling and separation microchip. BSA was first eluted and BSA-anti-BSA complex was eluted subsequently. These samples can be separated in less than 1 min with a continuous injection mode. The flow-through sampling microchip fabricated by this fast prototyping method could be used for biochemical analysis.

6. Conclusions

A fast prototyping fabrication process for microfluidic system on soda-lime glass was reported in this study. The fabrication technique presented here did not require expensive thin-film deposition process or mask-alignment procedure. Instead of using metal or polysilicon/nitride layers as the etch masks, a low-cost, simple but reliable positive PR was used directly as an etch mask in buffered HF solution. A two-step soft-baking and hard-baking process was developed to enhance the adhesion of the PR on the

soda-lime glass substrate. Not only does it reduce the thermal stress between the PR and the glass substrate but it also prolongs the survival time of the PR etch mask in BOE etchants. The formation of insoluble precipitates during wet etching process could be removed easily by dipping glass substrates in 1 M HCl solution for a short period of time. A very smooth etched surface could be obtained by iterating dipping and etching procedures with ultrasonic agitation. The process developed in this study could be finished within 10 hours including bonding process. In the present study, an innovative microfluidic device, "micro flow-through sampling chip", has been demonstrated using the fabrication method. Successful sampling and separation of Cy5-labeled BSA and anti-BSA has been achieved. This simple fabrication process is suitable for fast prototyping and mass production of the microfluidic systems.

Acknowledgements

Authors would like to thank NSC Southern MEMS Center for access of equipments. Dr. Gwo-Bin Lee would like to thank financial supports from

National Science Council (NSC 89-2323-B006-009).

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Figure legends

Figure1: Simplified fabrication process for fast prototyping of microfluidic chips on soda-lime glass substrates.

Figure2: AZ 4620 positive PR used as etch mask for buffered HF etching. An isotropic under-etch profile of glass substrate is formed beneath the PR etch mask. The PR etch mask adheres well on the glass substrate even at an extremely sharp convex corner.

Figure3: (a) SEM micrograph of a microfluidic reservoir after stripping the PR. (b) A close-up view of the sharp convex corner. The surface of glass substrate is well protected by AZ 4620 PR without any peeling.

Figure4: Surface profile of an etched channel in buffered HF using scanning probe microscope. The max peak-to-peak height R_{max} is 0.710 Å and the surface roughness R_a is 0.049 Å in this scanned area.

Figure5: SEM micrograph of a microscope slide etched in buffered HF

without annealing. Stress corrosion caused by residual thermal stress results in needle-liked etched patterns.

Figure6: A flow-through sampling device fabricated on a 50 mm x 43 mm soda-lime glass substrate. The channel is filled with black dye for leakage test after bonding. The width of the flow-through channel is 3 mm and the length of the separation channel is 20 mm.

Figure7: SEM micrograph of a channel (cross-section view) after fusion bonding. The channel is 36 μm deep and 102 μm wide. Note that interface of the two glass plates is not observable.

Figure8: Test results of the flow-through sampling chip. The samples were injected at every 0.7 min. It can be clearly seen that BSA and BSA-anti-BSA complexes could be separated in less 1 min.

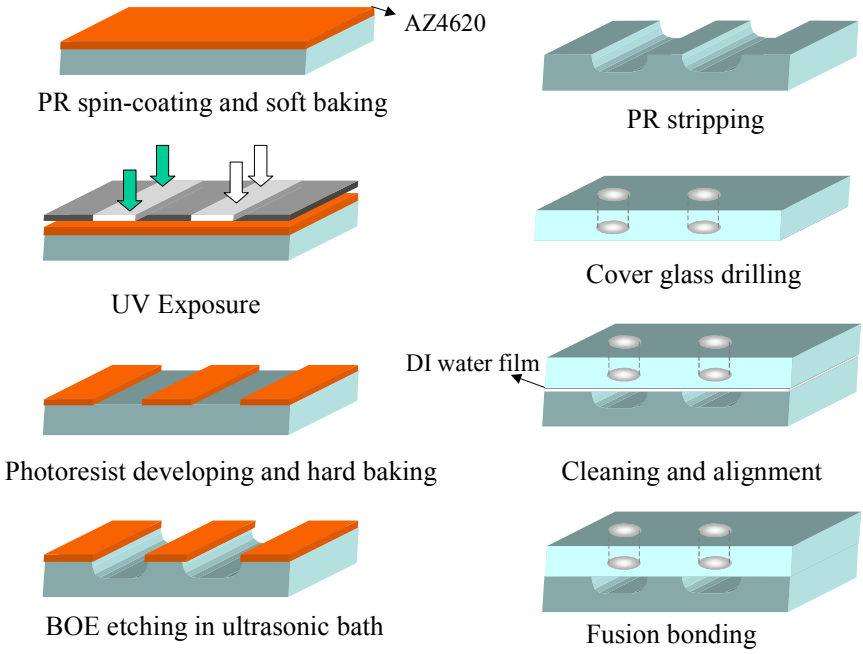
AZ4620
PR spin-coating and soft baking
PR stripping
UV Exposure
Cover glass drilling
DI water film
Photoresist developing and hard baking
Cleaning and alignment
BOE etching in ultrasonic bath
Fusion bonding

Fig 1

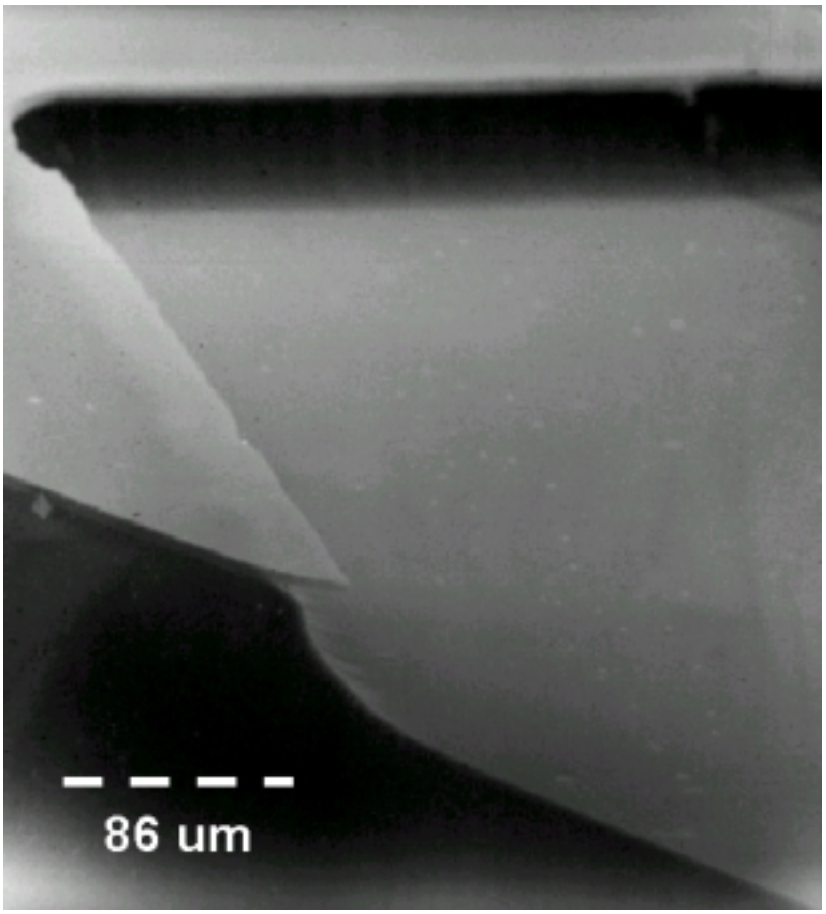

Fig 2

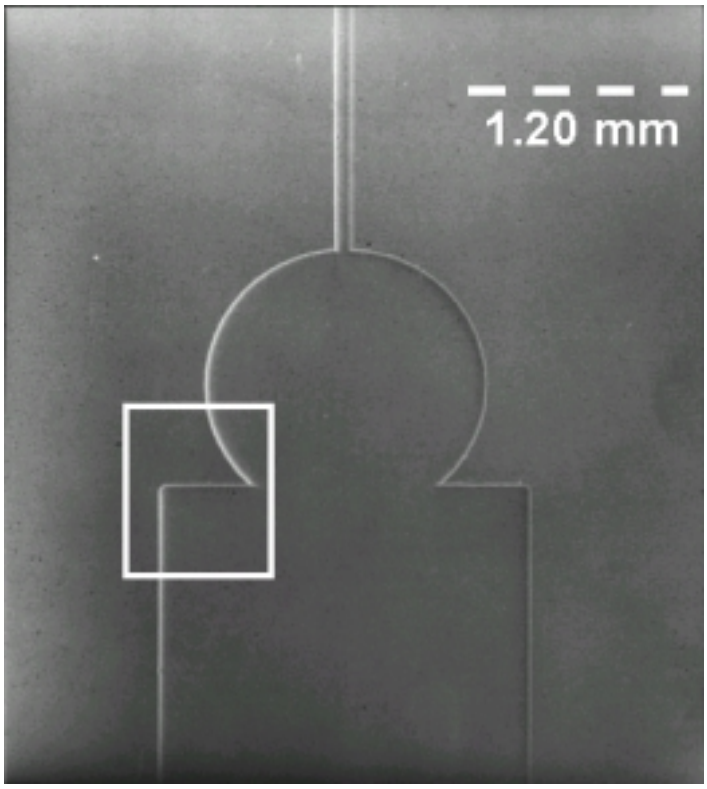

Fig 3(a)

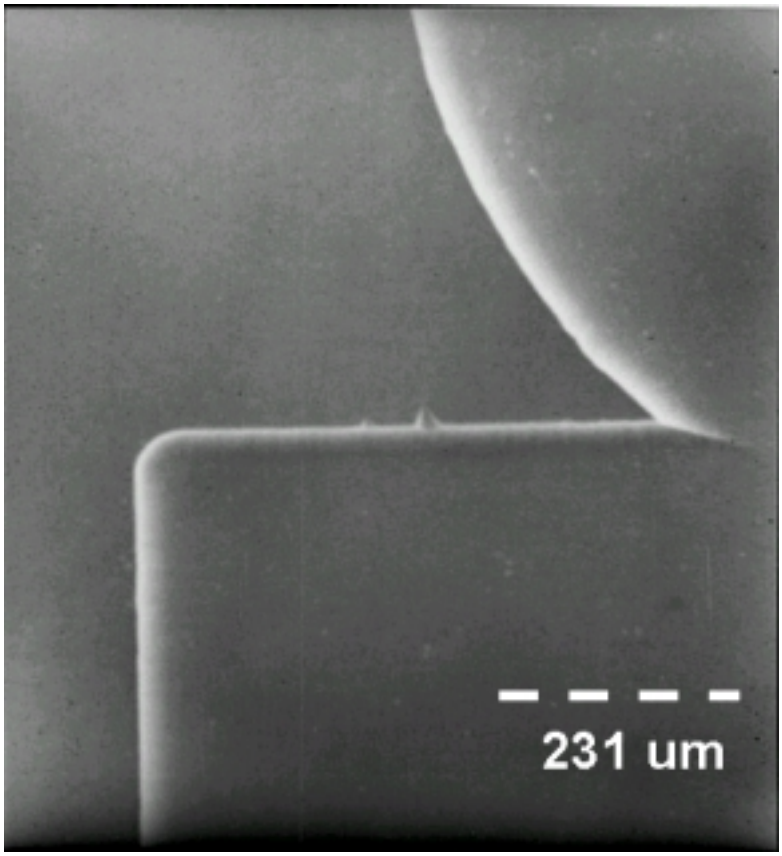

Fig 3(b)

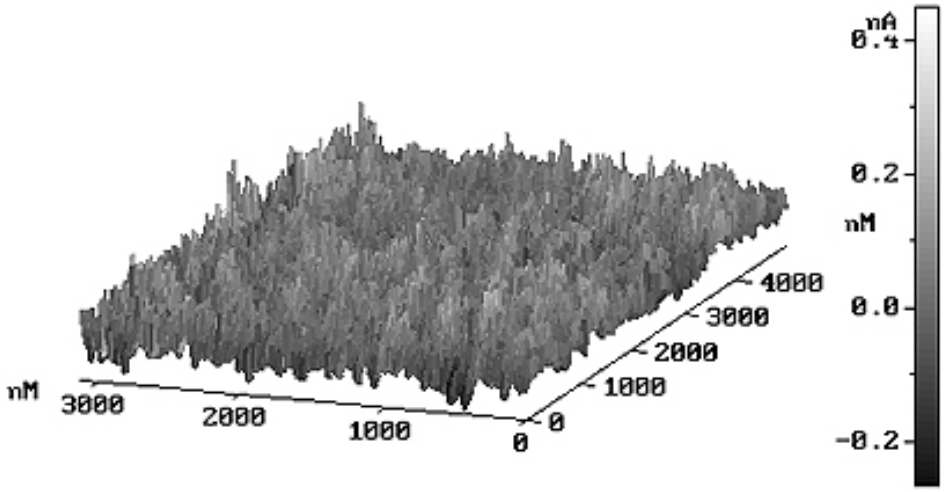

Fig 4

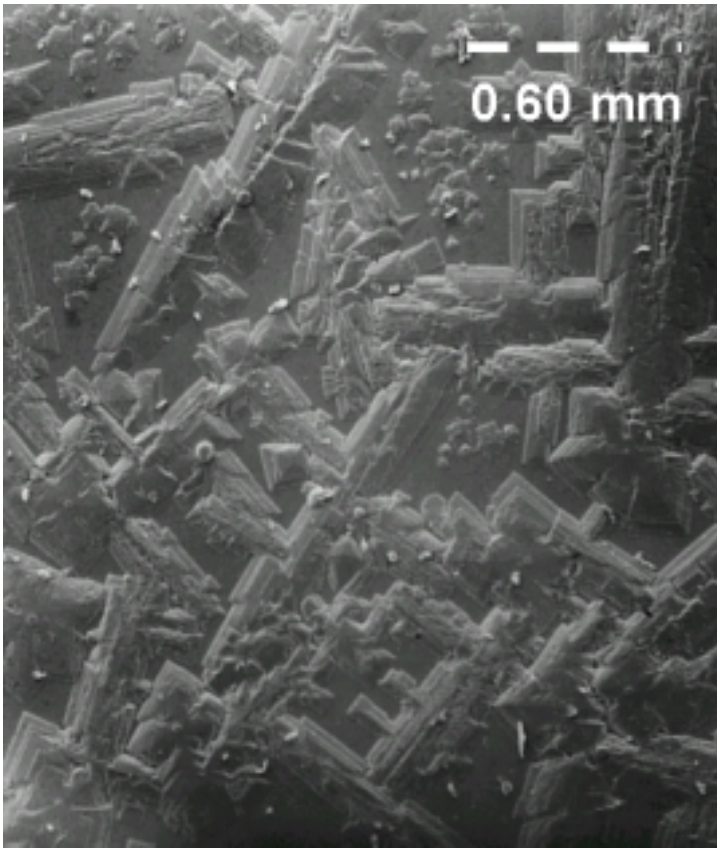

Fig 5

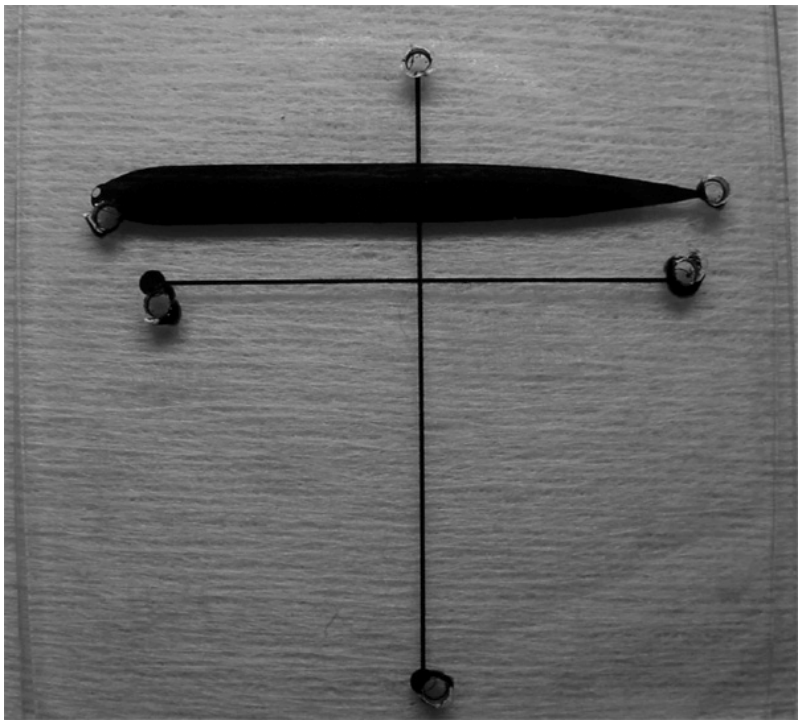

Fig 6

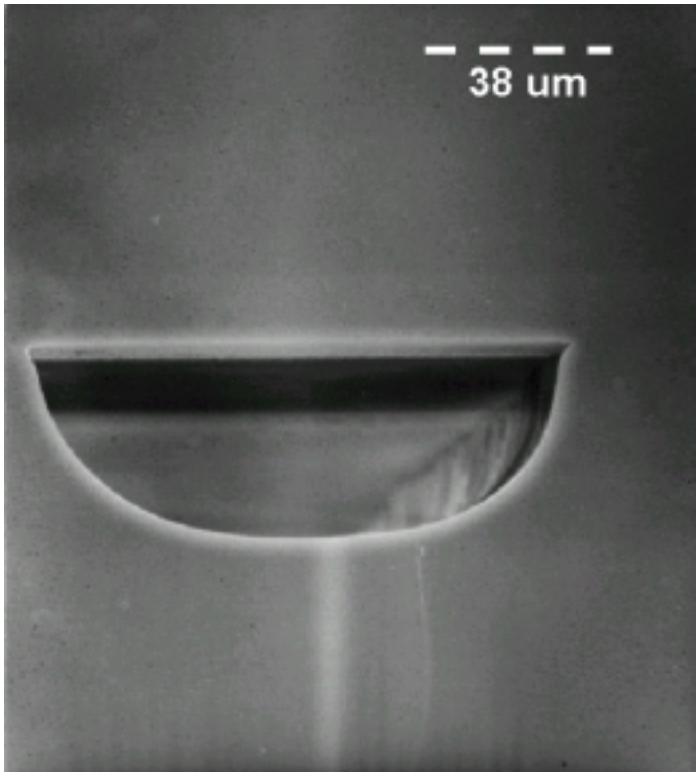

Fig 7

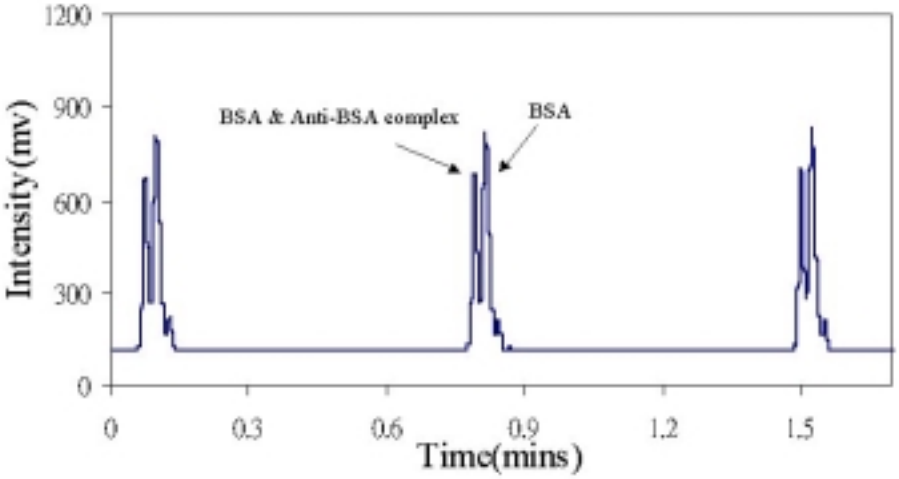
1200
900
600
300
0
Intensity(mv)
BSA & Anti-BSA complex
BSA
0
0.3
0.6
0.9
1.2
1.5
Time(mins)

Fig 8