MICROFABRICATED FUSED SILICA FLOW CHAMBERS FOR FLOW CYTOMETRY

Daniel Sobek Stephen. D. Senturia Martha L. Gray

Microsystems Technology Laboratories and Laboratory for Electromagnetic and Electronic Systems
Department of Electrical Engineering and Computer Science
Massachusetts Institute of Technology
Cambridge, MA 02139

ABSTRACT

The long-term goal of our research effort is to develop a low–cost microfabricated flow cell with integrated optics and electronics for applications in flow cytometry, a method for high–throughput measurements of physical and chemical characteristics of microscopic biological particles. The goal of the work reported here was to fabricate a low-cost flow cell ($10/cell) which supports hydrodynamic focusing and integration of optical fibers. A key feature of the device described here is that it is fabricated entirely from fused silica (quartz) wafers using two wafers, a single mask, standard etchants, and wafer–level alignment and bonding. Tests showed that the resulting devices easily allow hydrodynamic focusing and have flat, optically transparent surfaces. Integrated optical fibers provided illumination of the sample stream adequate for the detection of fluorescent particles. The use of quartz —which does not autofluoresce and is transparent over a broad range of wavelengths including the UV— suggests these flow cells will be useful for high sensitivity optical sensing applications.

INTRODUCTION

Flow cytometers are instruments widely used for high throughput optically–based measurements of cells and other microscopic biological particles [1]. These devices have proved to be particularly useful in studies that require classification of subpopulations of biological particles or detection and investigation of rare subpopulations. Flow cytometry is used extensively in clinical hematology and oncology and is applied in a number of diverse research settings ranging from immunology to ecology.

The basis of flow cytometric analysis is that specific optical characteristics (e.g. light scatter, fluorescence) can provide a measure of specific physical or chemical properties of the particle (e.g. size, DNA content, membrane receptors). The optical measurements are made as particles flow through a "sensing region" delimited by the intersection of the illumination and collection regions provided by light source and optical detector assemblies. Typically particles are constrained to the centermost flow stream of two concentric fluid flows and are thereby made to pass single-file through the sensing region. The technique for achieving the concentric flows, referred to as hydrodynamic focusing, imposes an absolute requirement for laminar flow in all positions within the flow cell.

Existing commercial flow cytometers are relatively complicated instruments requiring frequent adjustment of optical components used to align the optics to the sample flow. In order to simplify the operation of such instruments, Shapiro et al. [2] introduced the use of optical fibers as an alternative to lenses for sample illumination and light collection. In addition, they proposed combining a flow channel, integrated optical elements, laser diodes, detectors, and signal conditioning electronics to form a "flow cytometer on a chip." Simplified integrated flow cytometers could potentially be more accessible for routine clinical and research applications and require less expertise for their operation.

Several microfabricated sheath flow cells have been reported previously including a five–layer stainless steel/glass laminate described by Miyake et al. [3] and a four-wafer silicon micromachined flow chamber built by our group [4]. These reports were important in demonstrating the feasibility of fabricating devices capable of supporting hydrodynamic focusing; however, the fabrication schemes were relatively complex. The long-term goal of our research effort is to develop a low–cost device, possibly using a hybrid chip approach, which includes a flow cell with integrated optics, fluid handling, and electronics for applications including flow cytometry. This paper reports on the fabrication and testing of the first steps toward such a "flow cytometer on a chip": a quartz micromachined flow cell with provisions for hydrodynamic focusing and fiber optic illumination.

FLOW CHAMBER DESCRIPTION

The flow cell (Fig. 1) has a sample injector used to introduce a dye or particle suspension into a flowing sheath liquid and a two–dimensional nozzle to focus the sample into a narrow stream. The sample and sheath flows pass through the capillary, and exit through ports in the outlet region. In some devices optical fibers are positioned perpendicular to the capillary axis to allow for illumination and (ultimately) scatter and fluorescence detection.

Our initial mask designs are conservative, allowing only 8 devices per 4 inch wafer. The final "chip" dimensions are roughly those of a microscope slide (30 mm × 15 mm × 1 mm) with a 1 cm-long capillary of oval cross-section (height 250 μm and width 500 μm) — dimensions which are comparable to the flow cells on commercially available flow cytometers. The sample injector has a nearly circular cross-section with a height of 250 μm and width of 280 μm. The sample fluid is introduced using a #32 stainless steel hypodermic needle. External connections to the sheath inlet and outlet ports are achieved by securing the chip in a custom-machined package with an intervening O-ring seal. The grooves for the optical fibers are slightly larger than the cladding diameter of the multimode optical fibers. The fibers used were selected for 442 nm excitation, have a 0.3 numerical aperture, and have core and cladding diameters of 100 and 140 μm, respectively.

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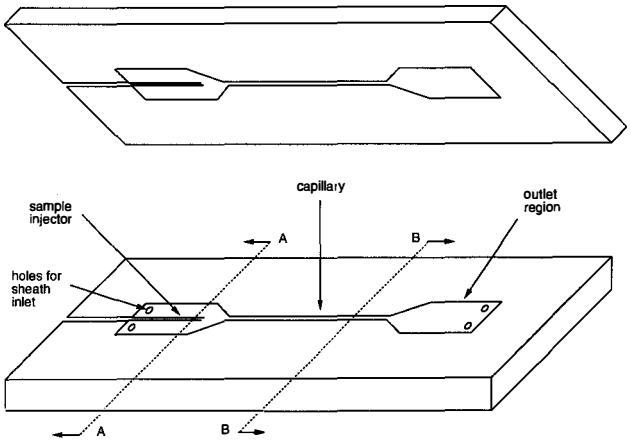

Figure 1: Exploded view of the fused silica flow chamber. The top and bottom plates have matching injection and outlet areas with a capillary joining them. Through–holes in the bottom plate are for sheath inflow and outflow. The sample is introduced through the injector into the center of the sheath flow.

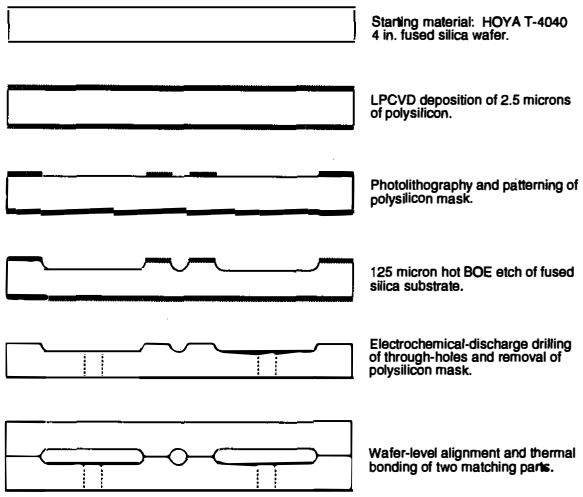

Figure 2: Fabrication sequence (shown along cross-section A–A of Fig. 1).

FLOW CELL FABRICATION

Fabrication of the flow cells involves using a single mask to create two fused silica wafers with matching isotropically–etched flow features and openings for optical fibers. After through-holes for fluid inlet and outlet are drilled in the bottom wafer, the two wafers are aligned and thermally bonded. Finally, the needle and optical fibers are secured in place.

As illustrated in Fig.2, the specific fabrication process starts with a LPCVD deposition of 2.5 μm of polysilicon onto HOYA T-4040 synthetic quartz (i.e., fused silica) wafers. This substrate material has low impurity levels making it compatible with CMOS production environments. Process characterization studies showed that such thick masking layers were necessary to ensure negligible pin hole formation during the wet etching. This use of poysilicon as a mask for etching of quartz was independently reported by Kaplan et al. at MEMS 94 [5]. The use of fine grained polysilicon masking layers may allow lower poly thicknesess. The openings for the optical fibers and the flow features are defined photolithographically, and established by plasma etching of the polysilicon masking film followed by an isotropic hot (50°C) 7:1 BOE etch of the fused silica substrate. Fused silica etch rates of 23.0 μm/hr were measured under these conditions. The inlet/outlet holes are drilled using electrochemical discharge machining [6]. (The precise position of the holes is not critical.) Finally, the polysilicon film is removed using 20 wt % KOH at 60°C and the wafers are cleaned, aligned using a customized attachment for a standard contact aligner[7], and bonded at 1000 °C. Immediately prior to testing, the needle and fibers are secured into place using UV curable glue. After the fibers or needles are positioned in their corresponding grooves under microscopic observation, the glue is introduced by capillary action and its flow stopped with the exposure to UV illumination. This method assured precise filling and sealing of the fiber and needle grooves.

RESULTS

The technical feasibility of the fabrication process has been validated with the fabrication of several prototypes. The circular and ovoid cross–sections of the injector and capillary are apparent from the SEM micrographs (Figs. 3 & 4). The smooth, flat surface of the capillary is consistent with the good optical properties observed in the tests described below. There was no obvious asymmetry between the top and bottom wafers; the slight offset observed in the capillary and injector resulted from a slight misalignment of roughly 1 μm. Preliminary data suggest that the fabrication sequence, including the etch characterization is reliable.

The hydrodynamic tests were performed after mounting the package on a microscope with either a video camera or photomultiplier tube connected to the video port. Sample and sheath flows were driven by a syringe pump and pressure vessel, respectively. A differential pressure sensor mounted between the inlet and outlet ports allowed us to relate flow rates to pressure drops. Hydrodynamic focusing in the horizontal plane was verified by microscopic visualization of a device operating with a water sheath and dye-containing sample (Fig. 5). The width of the sample stream changed in accordance with changes in the relative sample and sheath flow rates (Fig. 6). Focusing was maintained for mean velocities of up to at least 10 m/s (requiring an inlet to outlet pressure drop of 28 psi). Hydrodynamic focusing in the vertical plane was verified in two ways. First, for sample stream diameters smaller than 10 μm, conservative estimates based on measured sample and sheath flow rates indicate that the height of the sample stream must be less than 160 μm, far less than the 250 μm height of the capillary. Second, by adjusting the focal point along the vertical axis the dye did not appear to contact the capillary wall. It was not possible, however, to use this approach to reliably identify the vertical position of the interface between sheath and dye. Although the precise dimensions of the sample stream are not known, these results are significant in demonstrating that this comparatively simple device design provides hydrodynamic focusing in both the horizontal and vertical dimensions.

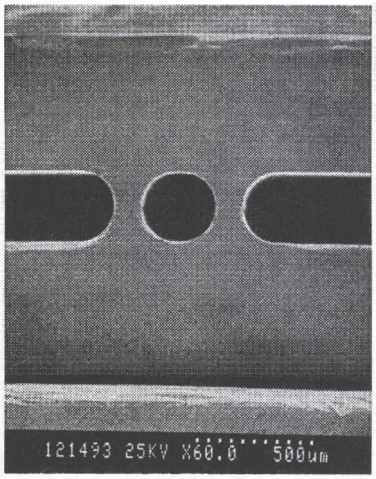

Figure 3: SEM micrograph showing a cross-section (section A–A of Fig. 1) of the sample injector and part of the passages for the surrounding sheath flow.

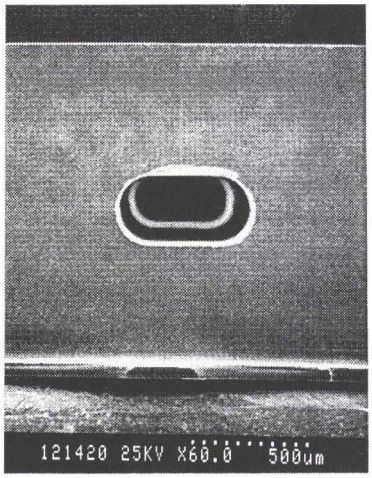

Figure 4: SEM micrograph showing a cross-section (section B–B of Fig. 1) of the capillary duct. The bottom and top surfaces are optically flat.

The good optical characteristics of the device suggested by the optical clarity of the hydrodynamically-focused dye stream were further illustrated by fiber optic illumination of a sample stream containing fluorescein isothiocyanate (FITC) (Fig. 7). These studies were done using a 60mW He-Cd laser beam (tuned to 442 nm), coupled to the multimode optical fiber using a lens and precision positioner. The adequacy of the coupling of the laser beam to the optical fiber was determined by measuring the power output at its end. This measurement was made using an optometer with a silicon photodiode head (calibrated at 442 nm) before placing the fiber in the flow cell. At optimal alignment, we measured a 30 mW power output.

The presence of the fiber *per se* did not appear to perturb the fluid flow (as long as the fiber did not protrude into the channel). In some cases the groove containing the fiber was not sealed so that through a venturi effect, air bubbles were periodically released into the flow stream, perturbing the flow. The normal flow patterns were subsequently reestablished. Fluorescence of the sample stream could be quantified using a PMT mounted in the video port of the microscope. The distribution of fluorescent intensity resulting from 2 μm fluorescent particles illuminated with the optical fiber was determined by connecting the PMT output to standard flow cytometry amplifier and data acquisition system. This analysis resulted in distributions having coefficients of variation (CV; standard deviation/mean) on the order of 8% (Fig. 8) which compares well the the CV's obtained on commercially available cytometers. Improvements in the CV would presumably result from the use of a single mode rather than multimode optical fiber (both because of the gaussian intensity distribution and the smaller illumination volume) and from more substantial sample stream focusing in the vertical plane.

CONCLUSIONS

In summary, the work described in this paper demonstrates the feasibility of microfabricating flow chambers from fused silica wafers using a comparatively simple two wafer, single mask process. Although the structure geometry directly imposes focusing in the horizontal plane, the resulting flows clearly show focusing in both the horizontal and vertical planes. Improved vertical focusing can probably be achieved by decreasing the vertical dimensions of the injector, a change which could be implemented with a second mask. Initial tests using the precision-positioned optical fibers are encouraging in that illumination of the sample stream and detection of fluorescence were demonstrated. Additional studies are necessary to determine the flow conditions for which the illumination volume includes the entire sample stream and whether integrated optical fibers can be used for detection of fluorescence or scatter.

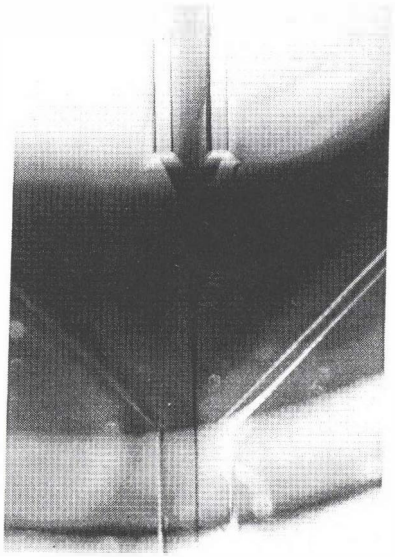

Figure 5: Demonstration of hydrodynamic focusing. The photograph shows dye being introduced through the injector into the nozzle. In this picture the dye stream at the entrance of the capillary is focused to a 10 micron width.

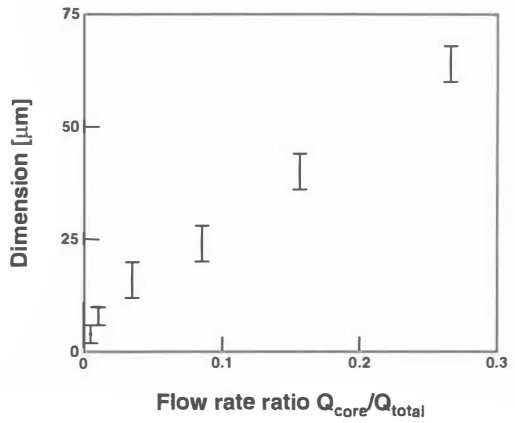

Figure 6: Width of the dye stream as a function of ratio between core flow rate (Q_{core}) and total flow rate (Q_{total}).

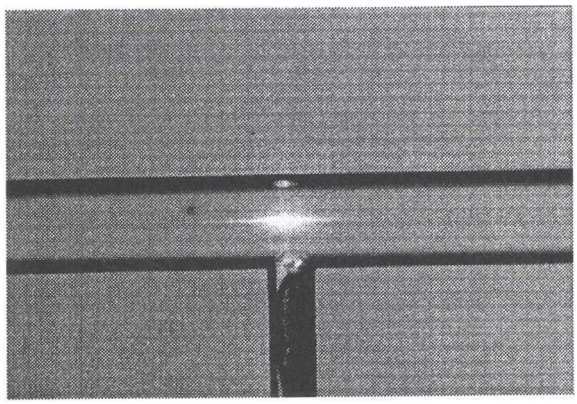

Figure 7: Fiberoptic illumination of the flow channel. Sample stream contains FITC.

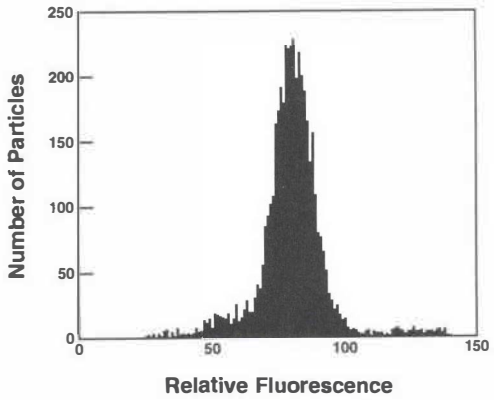

Figure 8: Fluorescence intensity distribution for 2 micron diameter fluorescent particles, illuminated at 442 nm through a multimode optical fiber, and detected using a PMT attached to the microscope.

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