Micro flow cytometers with buried SU-8/SOG optical waveguides

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Abstract

This paper reports an innovative micromachine-based flow cytometer integrated with buried optical waveguides on soda-lime glass substrates. A novel optical waveguide using SU-8/spin-on-glass (SOG) double-layer structure is demonstrated, which increases light guiding efficiency due to smoother channel surface and larger difference of refractive index between SU-8 and organic-based SOG. Instead of using complex optical alignment system, detection light source is coupled with the waveguide with direct insertion of an etched optical fiber. A very high coupling efficiency can be achieved using this approach. In this study, the performance of the waveguides and insertion losses are measured. Experimental results show that the optical loss is less than 15 dB for a 40 mm long waveguide. With the integrated optical waveguides, a micro flow cytometer capable of particle counting has been realized. Data show that microparticles can be hydrodynamically focused and counted successfully without fluorescent labeling using the miniaturized flow cytometer with the integrated optical detection system.

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1. Introduction

Flow cytometry is a general method for analyzing microparticles such as cells, bacteria and even euglena with high efficiency. It has been a popular diagnostic equipment for clinical and environmental applications [1,2]. Typically, fluorescence-labeled microparticles are hydrodynamically focused in a flow chamber and pass through a region where scattered light, fluorescence emission or absorption light is then collected by several sophisticated optical detection instruments. The complicated optical alignment procedures make the equipment not portable and expensive. Several micromachined flow cytometers have been reported in the literature [3,4]. Nonetheless, delicate optical alignment methods and apparatus are still required in their designs.

Recently, integration of buried optical waveguides with microfluidic devices for optical detection was wildly investigated. Several integrated waveguide structures fabricated by surface micromachining techniques have been reported, which used nitride/oxide or GeO2/SiO2 structures for planar waveguide [5,6]. However, the size of core region was limited by thin-film deposition process and may not meet the requirement for larger flow channel applications. In addition, Grewe and co-workers reported an optical leaky waveguide device in fused silica using wet chemical etching and bonding techniques [7,8]. Nevertheless, surface roughness of the etched channel [9,10] and a small refraction index difference at the core/wall interface affected the performance of the device. Conversely, polymeric channel waveguides are very attractive since core materials can be properly chosen to improve desired properties. However, previous study reported that high optical attenuation in the core (more than 50 dB/cm) could be a serious problem for practical applications [11].

In this study, a polymer (SU-8 negative photoresist) was used as a core material of a buried optical waveguide. SU-8 has been popular for MEMS applications recently. It has several excellent characteristics for optical applications, including high transmittance for light from visible to near-IR range (Fig. 1), a high refractive index of 1.8 after hard-baking [12] and a low polymer shrinkage rate of 7.5% after curing [13]. These properties make SU-8 become an excellent candidate for the core material of buried
optical waveguides. In this paper, the organic-based SOG layer was also used to increase the difference of refraction index between core/wall interfaces, resulting in better light guiding efficiency. Meanwhile, it also improves the surface smoothness of the etched channel on soda-lime glass substrates.

Coupling of a light source to a microfluidic chip could be cumbersome in practical applications. In this study, a simple light coupling method was demonstrated to couple the detection light into the polymeric waveguide. An asymmetrical top/bottom substrate forms an “insertion guide”, which could be used for insertion of an etched optical fiber. The efficiency of light coupling can be very high using this coupling technique because there is no gap between optical fiber and SU-8 core materials, resulting in a very low dispersion loss. In this study, the waveguide performance and insertion loss of the cytometer chips were measured. Microparticles were hydrodynamically focused and counted successfully by the innovative micro flow cytometers proposed in this paper.

2. Principle and design

The working principle of the microfluidic device is shown in Fig. 2. We integrate a micro flow cytometer with the buried optical waveguides for on-line cell counting. Two neighboring sheath flows are used to squeeze the center cell flows into a narrow stream. Cells in the sample flow can be hydrodynamically focused into a narrow stream prior to counting and then detected by the light emitted from the buried optical waveguides [14]. The signal is collected by another waveguide buried at the opposite side of the sample flow channel (Fig. 2A). Fig. 2B shows a schematic of the buried optical waveguide structure. The large difference of reflective index between SU-8/SOG double layers can form a waveguide structure. The detection light can propagate in the waveguide structure by total internal reflection. Light intensity changes when cells pass through the integrated waveguide structures and is then collected by the other waveguide buried on the other side of the channel. Therefore, numbers of cells can be counted without any microscope and delicate optical alignment.

3. Fabrication

Low-cost soda-lime glass was used in this study. Usually, a layer of polysilicon or metal is used for glass etching, which implies a more-expensive process. Instead, a layer of positive photoresist (AZ 4620) was used as an etching mask for etching of glass substrates in BOE (buffered oxide etch) solution in this study [15]. A simplified fabrication process is shown in Fig. 3. Three major steps were involved for fabrication of the device, including: (1) glass etching and bonding; (2) SOG filling; and (3) SU-8 filling. The detailed fabrication process is shown in Fig. 3. Three major steps were involved for fabrication of the device, including: (1) glass etching and bonding; (2) SOG filling; and (3) SU-8 filling. The detailed fabrication process is shown in Fig. 3.
The process for formation of microchannels and bonding was described in our previous work [15]. Note that an asymmetrical top/bottom substrate forms an insertion guide after bonding (Fig. 4). The microchannels designed for waveguides were filled with SOG (200F, Filmtronics, USA), which was driven only by capillary forces. A vacuum pump was then used to suck out extra SOG. The SOG film was then cured at 425 °C in N2 atmosphere for 1 h. The cross-linked SOG film has a refractive index of 1.36 and acts as a cladding layer of the waveguide. In order to couple a optical fiber with the buried optical waveguides, commercially available single-mode optical fibers were first stripped of the plastic buffer layer and then immersed into a 6:1 BOE solution for etching of the cladding layers. Optical fibers with 40 μm in diameter could be obtained after 4 h of etching. The insertion guide on bottom glass substrate can easily direct the etched optical fiber into the waveguide channel (Fig. 4). The optical fiber was then fixed using UV-sensitive glues.

The last step of the fabrication process is the filling of SU-8 core material. Prior to filling, SU-8 photoresist was first baked at 120 °C for 12 h in order to remove solvent contents. Vacuum suction was then used to increase the filling efficiency. Note that we filled the SU-8 at 100 °C in order to reduce the viscosity of the material since the glass transition temperature ($T_g$) of the unexposed SU-8 was 55 °C. The chip was then exposed and hard-baked at 180 °C for 20 min.

4. Results and discussion

Fig. 5A shows a cytometer chip fabricated using the developing process. The dimension of the cytometer chip is 3.5 cm × 2.5 cm. The channels are filled with red dye in order to get a better image. Note that there is no leakage observed in the cytometer chip after bonding. One pair of waveguide channels was placed orthogonally at the downstream of the flow channel. The dimension of the waveguide channel is 50 μm in depth and 80 μm in width. Fig. 5B and C shows the SEM images of close-up views of the etched waveguide channel and the cytometer nozzle. The images indicate that the shapes are well defined and the etched surface is smooth. The cross-section views of the channels are shown in Fig. 6. Successful bonding was observed since the interface between the two glass substrates was no longer visible after the bonding (Fig. 6A). It is important to form a solid SU-8 core inside the channel to assure excellent light guiding efficiency. In some cases, bubbles or a hollow SU-8 structure could be formed in the waveguide channel after hard-baking (Fig. 6B), which reduced the waveguide efficiency drastically. The possible solution is to eliminate the solvent contents of the photoresist before filling. Then the filling percentage of SU-8 photoresist could be significantly improved (Fig. 6C).

Fig. 5. (A) Microfabricated flow cytometers with buried optical waveguides. (B) A close-up view of the waveguide channel and the sample flow channel. (C) Micronozzles of a flow cytometer. The width of the nozzle is 140 μm and the depth is 25 μm.
Fig. 7A shows images of an unetched (125 µm in diameter) and an etched (40 µm in diameter) optical fiber. The surface of the etched fiber is as smooth as the unetched one. The high flexibility of the etched fiber could sustain the mechanical forces during insertion process. Furthermore, the etching will not affect the performance of the optical fiber because the core diameter is 9 µm for a single-mode optical fiber. The residual cladding layer is thick enough for reflection of the detection light. An assembled waveguide is shown in Fig. 7B. Since the etched optical fiber is surrounded by the SU-8 photoresist, there is no gap between optical fiber output and the SU-8 waveguide. As a result, no dispersion loss will occur during coupling. The image also shows a very high intensity light comes out from the outlet of a 20 mm long SU-8/SOG waveguide buried inside the glass chip. The waveguide efficiency was measured by a photomultiplier tube (PMT) photodetection system (R928, Hamamatsu, Japan). The system was placed in a dark environment in order to reduce background noises. Fig. 8 shows the measured optical loss of the buried SU-8/SOG waveguide. The overall loss is less than 15 dB for a 40 mm long waveguide, which is caused mainly due to nature attenuation of the light propagating in the SU-8 core material.

The light guiding effect of the developed SU-8/SOG optical waveguide is shown in Fig. 9. Experimental data indicated that SU-8/SOG optical waveguide could guide light through sample channel successfully. A high intensity
of light passes through the sample flow channel and reaches
the inlet of the other waveguide. The measured insertion loss
of six different chips is 30.1 ± 5.8 dB while the channel was
filled with de-ionized water. The expected loss was mainly
caused by the dispersion of light propagating form the input
waveguides to the output waveguide at different sides of the
channel. In spite of this issue, the loss shows little affection
to the applications of differential measurement in cell
counting.

Fig. 10 shows the experimental setup for the micro flow
cytometers. Two syringe pumps provided the hydrodynamic
forces for sample and sheath flows. One end of the optic fiber
was connected to a fiber coupler and the other end was
connected to an APD optical sensor (C5460-01, Hamamatsu, Japan). The detection light (633 nm, He–Ne laser) was focused by a lens and then coupled into the input optic fiber. The optical signals were then detected and amplified by the APD module. The signals were monitored by an oscilloscope and collected using a DAQ card on a personal computer.

Fig. 11 shows the hydrodynamic focusing effect inside a
micro flow cytometer. The flow rate for the sheath flows and
sample flow is 0.2 and 0.05 μl/min, respectively. Experimental results showed that dye was hydrodynamically focused into a narrow stream successfully. The width of the stream is about 10 μm and remains unchanged while reaching the optical sensing area, which is about 7.5 mm downstream away from the cytometer nozzle.

Microparticles were injected into the flow cytometer to
investigate the performance of the microfluidic device. Fig. 12 shows results of a real particle counting test. The particles (POROS®, Selfpack poros 20MC, Framingham, USA) used in this test are hydrophilic metal chelates with a size of 20 μm. Each peak in the figure indicates that one particle passes through the optical sensing area. Results show the developed device can sense particles without fluorescent labeling.
optical waveguides have been integrated with buried optical waveguides on soda-lime glass substrates has been demonstrated. The SU-8 photosensitive epoxy resin was used as a core material for the optical waveguide. Another SOG cladding layer was utilized to increase the difference of refractive index between the core and the cladding layer. The rough surface commonly occurred for wet chemical etching of glass substrates could be alleviated with this approach. The SU-8/SOG double layers could form a high-performance solid-core buried waveguide. The optical loss of the waveguide was measured to be less than 4 dB/cm, which was suitable for most microchip detection applications. A simple and high efficient method to couple the detection light into the microchip device was also developed in the study. No complicated optical alignment system was required for the coupling using an insertion guide formed by an asymmetrical top/bottom substrate. Etched optical fiber could be inserted into the waveguide channel with ease. The buried optical waveguides have been integrated with a micro flow cytometer successfully. Hydrodynamic focusing effect inside a two-dimensional flow cytometer using two neighboring sheath flows has been demonstrated. At last, the functionality of the developed flow cytometer has been verified using microparticles of 20 μm hydrophilic metal chelates.

5. Conclusions

An innovative method to fabricate a flow cytometer integrated with buried optical waveguides on soda-lime glass substrates has been demonstrated. The SU-8 photosensitive epoxy resin was used as a core material for the optical waveguide. Another SOG cladding layer was utilized to increase the difference of refractive index between the core and the cladding layer. The rough surface commonly occurred for wet chemical etching of glass substrates could be alleviated with this approach. The SU-8/SOG double layers could form a high-performance solid-core buried waveguide. The optical loss of the waveguide was measured to be less than 4 dB/cm, which was suitable for most microchip detection applications. A simple and high efficient method to couple the detection light into the microchip device was also developed in the study. No complicated optical alignment system was required for the coupling using an insertion guide formed by an asymmetrical top/bottom substrate. Etched optical fiber could be inserted into the waveguide channel with ease. The buried optical waveguides have been integrated with a micro flow cytometer successfully. Hydrodynamic focusing effect inside a two-dimensional flow cytometer using two neighboring sheath flows has been demonstrated. At last, the functionality of the developed flow cytometer has been verified using microparticles of 20 μm hydrophilic metal chelates.

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References


Fig. 12. Optical signals of a real article counting test. The bottom line is the signal without particles in the sample solution. The upper line shows the particle counting signal.