Optical planar waveguide for cell counting

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Low cost counting of cells has medical applications in screening, military medicine, disaster medicine, and rural healthcare. In this report, we present a shallow, buried, planar waveguide fabricated by potassium ion exchange in glass that enables low-cost and rapid counting of metal-tagged objects that lie in the evanescent field of the waveguide. Laser light transmitted through the waveguide was attenuated proportionately to the presence of metal-coated microstructures fabricated from photoresist. This technology enables the low-cost enumeration of cells from blood, urine, or other biofluids. © 2012 American Institute of Physics. [doi:10.1063/1.3678339]

Waveguides are optical structures that can guide light by total internal reflection. Waveguides have been in widespread use in the telecommunications industry for over 25 years, and have been employed in recent years as a bio-sensor for detection and diagnostics.^{1–10} In any optical waveguide, the electromagnetic field of the guided light extends beyond the waveguide core, called an evanescent field (Figure 1(a)). The evanescent field has been exploited in many ways as a sensing mechanism.^{11–13} Typically, the sensing principal is a change in effective refractive index of the guided mode in the waveguide because of a change in refractive index of an overlying material.^{14–18} We use the evanescent field to sense objects, such as metal-tagged cells. In our approach, visible light from a low-cost diode laser is sent through the planar waveguide and the intensity of the light can be measured upon output by an inexpensive photodetector. The field is attenuated proportionally to the number of labeled objects lying above the waveguide, such as cells, which serve as efficient loss points along the guide (Figure 1(b)). There are many examples of optical waveguides used for biological and chemical sensing,^{9,19–27} though we believe this report is the first describing using attenuation in a waveguide-based sensor to count cells.

We chose to use ion-exchange waveguides for several reasons. Because the waveguides are diffused into the surface of a glass wafer, we are left with a flat substrate. This makes them an ideal choice on which to build microfluidic devices or other structures to capture the cells to be enumerated. The index changes achievable with ion-exchanged waveguides make them relatively easy to integrate with fibers. Additionally, they have the advantage of needing minimal capital investment to produce low-loss waveguides, enabling the production of a low-cost platform for counting cells. For optical waveguiding to occur, a high index core must be surrounded by a low index “cladding” material. In the ion-exchange process, a glass is typically immersed in a molten alkali salt bath, allowing ions from the bath to exchange with mobile ions in the glass. Often, the glass ion is Na⁺ as it has a high mobility, and is found in inexpensive

soda lime and borosilicate glasses. The first ion-exchanged waveguides were reported in 1972.²⁸ Subsequently, ion exchange waveguides have been formed using a wide variety

FIG. 1. (Color online) (a) Schematic side view showing buried planar waveguide. (b) Attenuation of transmitted signals is expected to be proportional to the number of metal particles above. (c) Laboratory bench-top setup showing fiber-coupled laser, waveguide, and photodetector.

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of ions,^{29,30} and with several variations on the ion exchange process, including thermal exchange from a molten salt, field assisted exchange from a molten salt, field assisted burial, and thermally annealing.³¹ For this work, we have chosen a K^+ - Na^+ ion exchange system, known to produce low loss waveguides with an index change Δn comparable to optical fibers.

The fabrication process for our planar waveguides started with Schott BK7 glass wafers that were cleaned with piranha solution (3:1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide). We deposited a 500 nm thick aluminum film on the wafers by magnetron sputtering. The glass wafers were then covered with Shipley 1822 photoresist by spin coating at 3000 rpm with HMDS spun on as an adhesion promoter prior to the application of the photoresist. After soft baking (100 °C for 30 min), the wafers were exposed to UV in a mask aligner using a chrome mask to define a linear pattern where the waveguides will lie. After post-exposure baking and developing, we etched the exposed aluminum using Transcene Type A Aluminum Etchant (50 °C until visual stop). Residual photoresist was removed with acetone and oxygen plasma. The glass wafers with the aluminum masking layer were submerged in a molten salt bath composed of 0.75 wt% KNO_3 + 99.25 wt% $NaNO_3$ at 350 °C for 1 h. The glass was then cleaned and stripped of the aluminum film. We diced the waveguides using a diamond saw (Disco DAD321), then polished the waveguide ends with diamond paper of sequentially smaller size grit, on an Allied Multi-Prep polishing tool, ending with a 0.5 μm diamond grit.

To analyze the doped layer of potassium, we used Energy-Dispersive X-ray spectroscopy (EDX) on the surface and on a cut edge of the glass. We found that potassium was enhanced in the waveguide regions by 2.4 fold when scanning across the surface (Figure 2(a)), and similarly at the cut edge, as compared to bulk glass (Figure 2(b)). Elemental analysis also showed that potassium was enriched to a depth of $\sim 6.5 \mu m$, as compared to bulk glass (Figure 2(c)). The potassium signal was noted to begin at ~ 350 nm depth (Figure 2(c)), confirming that the enriched potassium was buried at a shallow depth.

To verify the concept of our detector, we conducted a 2-D simulation using Beamprop software (RSoft Design Group). The waveguide was modeled as 6 micron layer with an index change of 0.009 on top of glass with an index of $n_g = 1.515$. In the absence of any features on the surface of the waveguide, the power in the waveguide stays constant after launching a Gaussian wave (Figure 3(a)). To simulate the capture of circulating lymphocytes such as T cells, we placed $4 \mu m \times 4 \mu m$ squares with an index of $n_c = 1.4$ onto the waveguide. To simulate these cells coated with metal nanoparticles, our squares were surrounded on three sides with a metal film. We used the complex index of refraction for aluminum at a wavelength of 0.8 μm , $n = 2.767 + 8.354i$ for the metal film. The cladding index was set at $n = 1.35$ to represent the aqueous solution that we would expect in the microfluidic channel. The simulation was set so that the 100 scattering squares occurred after the first 400 μm of

FIG. 2. (Color online) (a) Elemental analysis looking at potassium concentration across the surface of the glass (green scan line) shows enrichment in the waveguide stripes (highlighted with white dashed lines). Gray line, measured potassium concentration in arbitrary units. Blue line, smoothed fit of gray line. (b) Elemental analysis showed that potassium was enriched comparing the shallow buried waveguide and bulk glass. Perspective view shows the cut face and the waveguide face (facing downwards), and the zoomed view shows the two rectangular regions interrogated along the cut face. The histogram shows the elemental analysis, revealing only a difference in potassium. (c) Scanning along the cut edge from the surface into the bulk glass, potassium enrichment extended $\sim 6.5 \mu m$ and was noted to be buried at a shallow depth (~ 300 nm) as compared with the surface. Gray line, measured potassium concentration in arbitrary units. Blue line, smoothed fit of gray line.

FIG. 3. (Color online) (a) Simulation of waveguide power in the absence of simulated captured "cells." (b) Waveguide geometry with 100 simulated captured "cells." (left) is the geometry with the simulated metalized $4\text{ }\mu\text{m} \times 4\text{ }\mu\text{m}$ captured "cells." (middle) is the contour map of the field and (right) shows the steadily decreasing power in the waveguide in the region of the interaction with the "cells."

waveguide, and were dispersed over the next $2300\text{ }\mu\text{m}$. The modeled geometry is shown in Figure 3(b) (left). We analyzed the field in the waveguide and the total power in the waveguide in this simulation (Figure 3(b), middle and right). The power starts out constant, decreases in the region of the scattering sites, and then remains constant in the absence of the scattering sites. This simulation showed that our system could feasibly detect metal-coated, cell-sized objects that lie the evanescent field of an ion-exchange waveguide of our geometry.

To quantitate the effect of the loss per scatterer, we analyzed the above simulations, which showed that modeling cells as round metal shells on the surface of the waveguide yielded a 0.1% optical power reduction per metal shell. That analysis was performed for an idealized geometry in which every metal shell interacts with the entire light field. When we further factored in the planar geometry of the region of interaction, the power reduction per metal shell was reduced by another factor of 20, yielding a 0.005% optical power reduction per metal shell. Given our plan to use an inexpensive laser source on the order of 5-10 mW, and adding in optimistic values for combined propagation loss and coupling loss at 3 dB, the expected optical power reduction would be

$\sim 0.25\text{ }\mu\text{W}$ per metal shell. This attenuation is far above the noise-equivalent power for ordinary silicon photodetectors, so detector noise will not be a limiting factor. Our preferred architecture includes an inline optical tap of the source light, so power fluctuations of the source would not likely be a limiting factor either. Theoretically, we should be able to detect single events. However there are other sources of optical power variations that would limit sensitivity including variations in thickness of the metal, variations in refractive index of the surrounding fluid, which could influence the evanescent wave profile, and coupling between modes in the guide. Nonetheless, our simulations support that, given solutions to practical engineering issues, our system will allow for at least semi-quantitative detection of labeled objects.

To begin to experimentally test the capacity of the waveguides to detect metal-tagged cells, we prepared cell-sized metal-coated objects out of reflowed photoresist (Figure 4(a)). These objects were designed to be sized approximately as a typical lymphocyte from human blood ($\sim 5\text{ }\mu\text{m}$) and were called "faux cells." To test a broad range of faux cells, we developed a pattern of coverage whereby 0%, 1%, 2%, 5%, 10% or 20% of the surface area of the waveguide is covered by faux T cells (Figure 4(a)). Various photoresists

FIG. 4. (Color online) (a) Faux cells fabricated from reflowed photoresist and coated with aluminum, SEM image and close up. (b) Faux cells were fabricated in a grid pattern over the optical waveguide where surface area coverage was varied by the density of the cells. (c) Laser light was measured after transmission through each waveguide underneath the patterned faux cells. Transmitted power is shown in relation to the coverage of faux cells lying above the waveguide.

were tried for their ability to reflow into cell-shaped objects, and AZ1518 was chosen because of its suitable material properties including ability to reflow into cell-shaped structures. AZ1518 was patterned on the waveguides as faux cells, and they were metalized with aluminum. The faux T cells were imaged with scanning electron microscopy, confirming that they form a regular and complete array (Figure 4(b)) in a variety of coverages.

To test whether the waveguides could quantitatively measure the faux cells, we introduced 561 nm laser light into the waveguides using a low-cost green laser pointer and a benchtop optical setup comprising a 10× objective to focus light into the waveguide, a 20× objective to focus light out of the waveguide, and an inexpensive silicon photodiode as the detector. We found the transmitted light power through the waveguides diminished by the presence of the metalized faux cells, proportional to the number of faux cells above (Figure 4(c)). The relationship was fit by a single exponential: $\text{power } (\mu\text{W}) = 15.9 \times \exp(-0.202 \text{ coverage}\%)$.

In summary, we have developed a method of using planar buried waveguides to enumerate metal-tagged objects lying in the evanescent field of the waveguide. This technology can be used for counting metal-tagged cells or other molecules that can scatter energy from the waveguide.

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