Counting cells with a low-cost integrated microfluidics-waveguide sensor

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The capability to count cells from biofluids at low cost has important diagnostic implications in resource-poor settings. Many approaches have been developed to address this important need, and while most envision a low per-test cost, the detector instrument can be quite expensive. In this report, we present a novel device that enables low-cost and rapid counting of cells from a drop of blood. We demonstrate a shallow, buried, planar waveguide fabricated by ion exchange in glass that underlies a microfluidic structure for capturing cells. Laser light transmitted through the waveguide was attenuated by the number of metal nanoparticles tagged to the cells because of the interaction of the metal particles with the evanescent field of the waveguide. Calibration of the sensor using bead-tagged lymphocytes captured from human blood showed that the sensor could semi-quantitatively count as few as 100 cells/μL of blood. This technology enables the enumeration of specifically captured cells, allowing for a point-of-care, hand-held device for fast and affordable cell counting in screening, remote, or resource-poor settings. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.3689857]

Microfluidics is a powerful platform for biomedical diagnostic tests because of portability, low sample requirement, low costs of fabrication, minimal power consumption, and ease of disposal.[1] Many commercially available, FDA-approved diagnostic tests employ microfluidics, including products from Fluidigm, Caliper, Gyros, Agilent, Abbott (I-Stat), and others. Microfluidic devices are fabricated using a technique called soft lithography to pattern polymers such as poly-dimethylsiloxane (PDMS). The microfluidic aspects of our design facilitate the capture and labeling of cells of a particular type (e.g., lymphocytes) and overlie our waveguide sensor.[2]

Waveguides are optical structures that guide light by total internal reflection. Waveguides have been in extensive use in the telecommunications industry, and have made inroads in the field of biological and chemical sensing,[3–15] but have made only a limited entry into actual clinical diagnostics.[16] Typically, the evanescent field of the waveguide has been exploited, sensing a change in the refractive index of an overlying material as a change in the effective index of the waveguide. In our case, we use the evanescent field to interact with captured cells (Figure 1(a)). The field is attenuated proportionally to the number of captured cells, i.e., labeled cells in the channel serve as efficient points of scattering out of the waveguide[2] (Figure 1(c)). An optical setup (Figure 1(b)) was constructed using three-axis optical stages (Newport, USA). One end of an ion-exchange waveguide was aligned and coupled to a 635 nm solid-state laser using a single mode fiber patch cord and fiber holder. Light emerging from the waveguide was focused onto a silicon photodiode (Thor Labs, USA) using a microscope objective lens (20×, 0.4 NA, Plan N, Olympus, USA) and an iris diaphragm and directly measured in μA with a sourcemeter (Keithley, USA).

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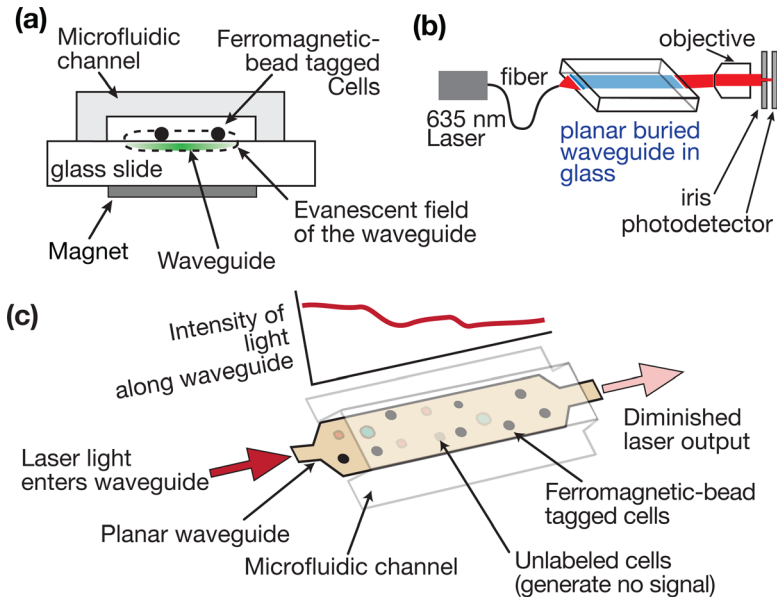

FIG. 1. (a) Schematic side view showing buried planar waveguide and the overlying microfluidic channel. Cells tagged with ferromagnetic beads are captured in the channel by the magnet. (b) Schematic of the optical setup. (c) Attenuation of transmitted light due to the number of metal-tagged cells above.

K^+ ion-exchange waveguides were prepared as detailed previously.[2] To test the capability of the waveguide to detect changes, we added incremental amounts of dilute Black India ink (Higgins, USA) to a PDMS-based microfluidic well positioned above the waveguide. India ink is colloidal carbon in water and was diluted serially in isopropanol with aliquots of the dilutions sequentially added to the PDMS well. The solvent was allowed to completely evaporate before the signal was measured and the next dilution was added. Laser light transmitted through the blank (no ink) waveguide elicited a photocurrent of $2.71 \pm 0.29\,\mu$A (95% confidence interval (CI), $n = 4$ independent experiments). After each addition of diluted ink, the photocurrent was measured. Increments of ink reduced the photocurrent (Figure 2). After the equivalent of neat ink was added, the measured photocurrent was $0.75 \pm 0.13\,\mu$A. Most of the recorded change in waveguide signal occurred between 1/10 and 1/100 diluted ink and was largely insensitive to ink more diluted than that. These results demonstrate that the waveguide could sensitively measure the presence of dilute colloidal ink in a microfluidic chamber positioned just above the waveguide.

We next tested the capability of the waveguide to count cells. We sought to count peripheral blood lymphocytes, which express the cell-surface molecule CD45, from whole blood. A PDMS microfluidic channel was positioned so that the lumen lay above the waveguide. To tag the cells for cell-type specific capture, we coated goat anti-mouse IgG (Fc) ferromagnetic particles (4.4 μm diameter, Spherotech, USA) with biotinylated mouse anti-human CD45 antibody (Biolegend, USA) (4 °C for 30 min with rocking). A magnet was glued beneath the waveguide glass to facilitate capture of the ferromagnetically tagged cells within the channel (Figure 1(a)). Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood of healthy adult donors after informed consent using density-gradient centrifugation (Ficoll-Paque, GE Healthcare, USA), were incubated with the antibody-coated ferromagnetic particles (4 °C for 30 min), and then were counted by hemocytometer in duplicate. Following the incubation, the cells were washed in phosphate-buffered saline (PBS) and diluted serially to produce various concentrations. Suspensions containing increasing concentrations of bead-tagged PBMCs were added to the microfluidic channel over the waveguide chip, resulting in progressively higher numbers of bead-tagged cells covering the waveguide (Figure 3(a)). Light microscopy and analysis with IMAGEJ were employed to count the number of cells that covered the waveguide area.

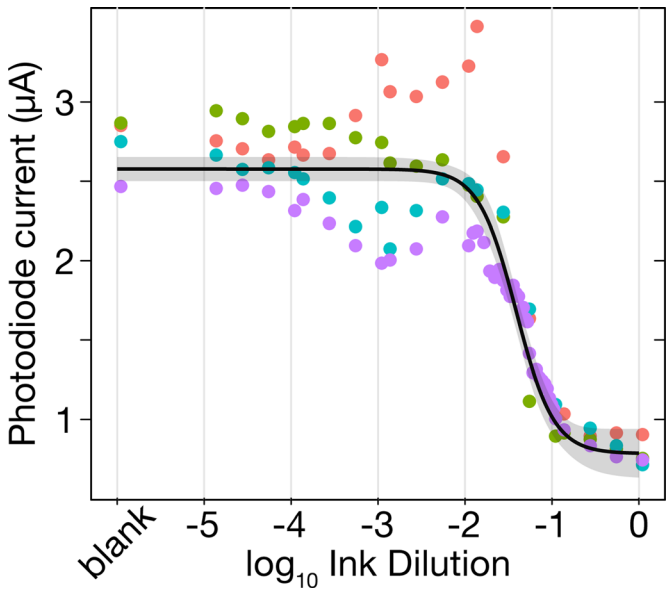

FIG. 2. Photocurrent shows decrease in signal upon serial addition of dilute ink. Four independent experiments are shown (each color represents an independent experiment) with a sigmoidal fit and the 95% confidence interval of the fit.

Light through the blank waveguides elicited a photocurrent of 2.53 ± 0.27 μA (95% CI, n = 3 independent experiments). Cells were introduced gradually and allowed to settle on the waveguide surface for 10 min before measurement of the photocurrent. Adding cells reduced the photocurrent (Figure 3(b)). A saturating number of cells introduced into the channel ($\sim 1 \times 10^6$) reduced the photocurrent to 0.59 ± 0.18 μA. We found that a 50% reduction in photocurrent was seen when ~950 lymphocytes had been added to the channel. These results show that the waveguide-based sensor could detect a very small number of cells from blood and could reasonably count the expected numbers of most blood cells. Notably, low numbers of tagged cells generate more photocurrent, which makes this methodology ideal for screening for numerical deficiencies of cells, e.g., screening T cell counts in newborns to diagnose severe combined immune deficiency (SCID).[17] This kind of screening test requires only a binary test result, indicating the presence or absence of T cells.

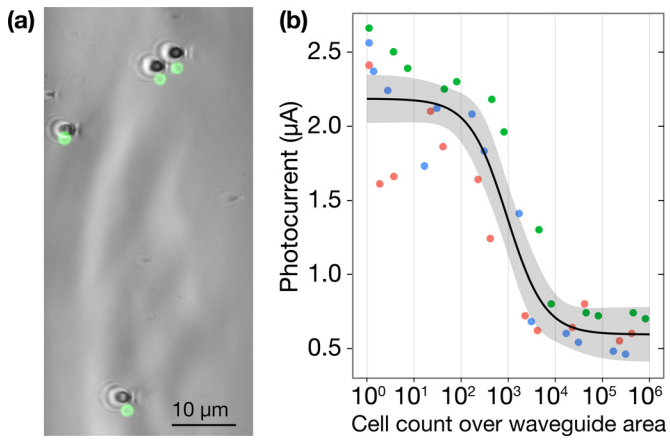

FIG. 3. (a) Composite micrograph of bead-tagged cells. Lymphocytes were labeled with CD45-AlexaFluor488 and tagged to ferromagnetic beads bearing anti-CD45 mAbs. Generally, each cell was tagged with one or more beads. (b) Photocurrent shows decrease in signal upon serial addition of dilute cells. Three experiments are shown (each color represents an independent experiment: red, green, blue) with a sigmoidal fit and the 95% confidence interval of the fit.

We have demonstrated enumeration of primary cells from whole blood by attenuation of waveguide-transmitted light. Our approach works in ambient light by avoiding the use of fluorescently tagged reagents, which may have a short half-life due to sensitivity to light. Waveguides are fabricated side by side, which enables easy multiplexing. The simple methodology lends itself to resource-poor settings where trained technicians may not be available. A detector was built using a low-cost photodiode and $<$ \$50 of circuitry. Because our technique can work with virtually any cell type, we expect this technology to impact on cell counting applications in resource-poor settings, in military medicine, in disaster settings, and in rural healthcare.

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