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An Optical Biosensor for Rapid and Label-Free Detection of Cells

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Rapid and sensitive detection and identification of pathogenic bacteria, such as *Escherichia coli*, *Salmonella*, and *Bacillus anthracis* (anthrax), is extremely important in biological research and medical diagnosis.[1] The number and variety of new detection systems that are continually being developed reflect the increasing demand for greater sensitivity, speed, and ease of use.[2,3] Several protocols involving microarrays,[4−7] quartz crystal microbalance resonators (QCM),[8] polymerase chain reaction (PCR),[9] fluorescent-bioconjugated silica nanoparticles,[10] monoclonal antibody-coupled ferromagnetic nanoparticles,[11] carbohydrate-mediated cell recognition using gold glyconanoparticles,[12] light-addressable potentiometric detection,[13] amperometric detection of enzymatic reaction products,[14] diffraction-based cell detection,[15] and nanowire-based detection[16] are presently available. Recently, multiplexed flow cytometry methods[17] and pathogen sensors that can achieve an optimal combination of speed and sensitivity through the use of B lymphocytes[18] have emerged as laboratory-based assays. Among some of the recently reported methods, a microarray protocol can detect a minimum of 6000 cells from a suspension of 5×10^6 cells/mL,[5] a fluorescence-based sensor system can detect 480 *Pseudomonas aeruginosa* cells present in a suspension of 2.4×10^5cells/mL,[19] and a silicon chip-based light-addressable potentiometric biosensor can detect 119 *Salmonella* cells from a suspension of 10^6cells/mL.[13]

Many of the currently available methods for detecting trace amounts of pathogenic bacteria require signal amplification or enrichment of the target bacteria in the sample or expression of fluorescent protein markers and antibodies in the cells. As a consequence, these methods tend to include additional steps and time-consuming assay procedures. A simple detection system that can achieve high sensitivity without the need for target amplification and labeling is highly desirable.

In this communication, we present a simple, rapid, and label-free optical method that allows the detection of as few as 45 bacterial cells specifically captured (from a suspension of 5×10^4-cells/mL) on a transparent surface. Cells are detected via the amount of laser light they block/transmit. The method we describe here does not involve relatively laborious labeling and purification steps in sample preparation procedures and offers the flexibility to repeat the measurements multiple times without deterioration in signal intensity which occasionally occurs in fluorescence-based detection systems due to issues, such as photobleaching and quenching. The biosensor described here provides a simple and commercially inexpensive screening of immobilized cell samples.

In the method presented here, a laser beam is passed through a small diameter well whose glass surface contains cells specifically captured by antibodies (Figure 1), and the transmitted laser intensity is measured using a photodiode. The transmission intensity for an adjacent well functionalized only with antibodies is considered as reference. The laser transmission intensity value for the cell-containing well is less than that of the reference due to the additional absorption caused by cells. The difference between readings from the two wells is the decrease in transmission intensity ΔI, which is proportional to the number of cells bound to the well surface. ΔI represents a differential signal that inherently suppresses disturbances and nonspecific effects that are expected to influence both wells similarly.

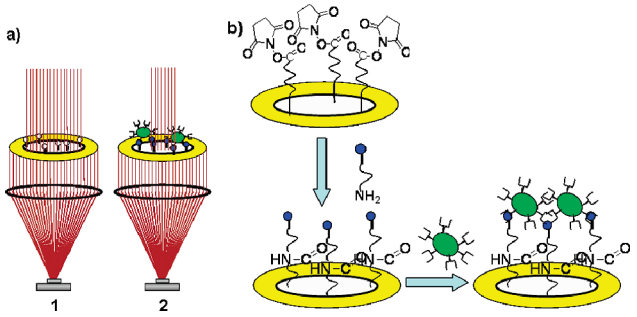

Figure 1. (a) Schematic of the detection method (1) reference well with only immobilized antibodies transmitting the laser beam; (2) well containing both antibodies and *E. coli* cells blocking a part of the laser beam. (b) Schematic of the coupling of antibodies to the activated glass slide followed by binding of *E. coli* cells to the antibodies.

In the experimental setup, a laser diode (Sanyo DL3148−025, 635 nm, 5 mW max output power) and a silicon photodiode with 12V reverse-bias (Thorlabs DET 110) in conjunction with a band-pass filter (Thorlabs FL635−10) were arranged on an optical bench to ensure consistent alignment of the light path from the laser source to the photodiode. The photodiode was connected to a variable resistor (1 kΩ) to convert the current output of the diode to voltage. The resulting signal was connected to a low-pass filter/amplifier (Stanford Research 640) and readout by a computer with a National Instruments LabView interface. The sample slide containing isolated wells was placed between the laser source and the photodiode and aligned to the path of the laser beam using a translation stage (Thorlabs LT3) and by finely adjusting the position of the sample glass slide until a maximum transmittance was achieved. At this point, the reading from the computer display was noted. For each well, the transmission intensity was measured three times, and average values and standard deviations were calculated off-line. An activated glass slide (CodeLink) attached to a silicone gasket with holes (250 μm diameter and 500 μm depth) arranged in two columns was used to form the activated wells of the sample.

Into each individual well, 2.5 μL of the *E. coli* antibody solution (1 mg/mL in PBS buffer, pH 7.4) was spotted and incubated for 30 min. At the end of this period, the sample slide was gently rinsed with Nanopure water to wash away the unbound antibodies. The first column of wells on the glass slide was not exposed to *E. coli* cells (contained only antibodies), and the transmission intensity recorded for these wells was considered as the reference. The second column of wells, already functionalized with *E. coli* antibodies, was spotted with 2.5 μL of *E. coli* (O7:K1) cells in Nanopure water

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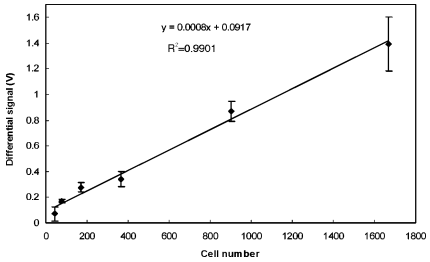

Figure 2. Variation in differential signal intensity ΔI with cell number (error bars indicate 95% confidence interval).

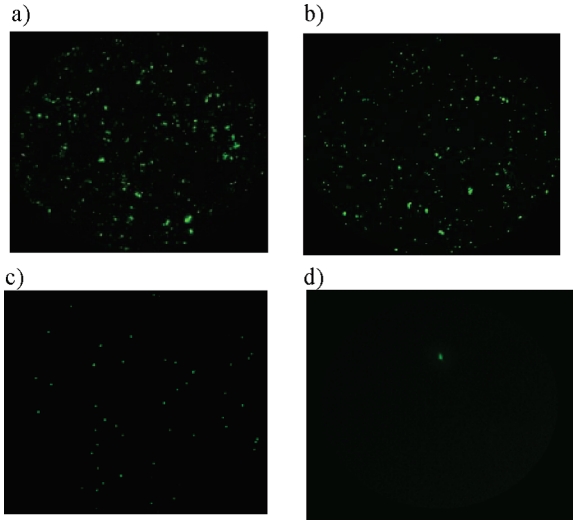

Figure 3. Fluorescence micrographs of (a) 903, (b) 364, and (c) 45 *E. coli* cells immobilized on a series of well surfaces. (d) Fluorescence image showing only 1 bound Pseudomonas cell on the well functionalized with *E. coli* antibodies.

of varying concentrations (50−10 000 cells/μL) and incubated at room temperature for 30 min (to ensure capture of sufficient number of cells). At the end of this period, the glass slide was rinsed three times with Nanopure water so as to wash away the unbound *E. coli* cells and dried under a stream of nitrogen. The sample slide thus prepared was subjected to laser transmission intensity measurement studies.

The differential intensity ΔI increased proportionally with the number of cells bound to the well surface. Figure 2 shows ΔI versus the number of cells bound (verified by fluorescence). The fairly linear relationship between ΔI and the number of cells indicates the method's strong potential as a quantitative biosensor. At very high cell numbers, the response deviated from linearity and began to saturate above 32 000 cells (data included in the Supporting Information). With this experimental setup, we could measure as few as 45 cells bound to a well surface. To see the cells clearly and count their number after measuring the transmission intensities, the wells that contained *E. coli* cells were incubated with a 10 μM green fluorescent FITC dye solution (Microprobes) for 15 min followed by rinsing three times with Nanopure water to wash away the excess dye. The stained sample was examined under a fluorescence microscope. As can be seen from the fluorescence micrographs (Figure 3a−c; additional figures are included in the Supporting Information) and verified by optical microscopy, the cells were well distributed in a monolayer arrangement. In addition to individual cells, a few aggregates of cells were also observed. As few as 45 cells distributed sparsely on the well surface can be detected by the relatively fast and simple system presented here. The specificity of the *E. coli* antibodies was tested by incubating the antibody-functionalized glass slide in a suspension of *Pseudomonas* bacteria cells (5000 cells/μL), which resulted in negligible cell binding, thus demonstrating the specificity of the antibodies (Figure 3d).

The present work demonstrates a rapid, simple, inexpensive, and completely label-free method for detection of cells. The approach described here may readily be expanded to encompass the detection of a wide variety of pathogenic, infectious, and malignant cells without the need for a fluorescent or a radio-labeling process and other optical microscopic methods. On the basis of the preliminary results, the method is promising for developing a portable biosensor for high-throughput detection of pathogenic bacteria and bioterrorism agents present in low quantities.

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Supporting Information Available: Experimental procedures and schematic of the experimental setup are presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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