Chapter 22

Integrating Waveguide Biosensor

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Summary

The Integrating Waveguide Biosensor was developed for rapid and sensitive detection of bacterial cells, spores, and toxins. A sandwich format of immunoassay was employed using *Salmonella* as model. The analyte was immunocaptured on the inner surface of the waveguide and then detected by the antibody conjugated with fluorescent dye. The waveguide was illuminated by an excitation light at a 90° angle. The emitted light from fluorescent labels on the surface of the waveguide was efficiently collected and channeled to a detector at the end of the waveguide, while minimizing interference from the excitation light. Utilizing fluorescent dye Cy5, a 635-nm diode laser for excitation, and a photomultiplier tube detector, the Integrating Waveguide Sensor System was able to detect approximately ten captured cells of *Salmonella*.

Key words: Biosensor, Integrating waveguide sensor, Integrating waveguide biosensor, Fluorescence detection, *Salmonella*.

1. Introduction

When fluorescent labels emit light, the emission is typically in all directions such that only a small fraction of the light is collected by the detector as signal. Simultaneously, light from the excitation source, auto fluorescence from the sample and the sample container, Raman emission from water, and other light collected by the detector contribute to background noise. The ability to maximize the signal while minimizing the background noise, i.e., high signal-to-noise ratio results in a lower limit of detection and improved instrument sensitivity.

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A platform to detect analyte on the surface of a waveguide is shown in **Fig. 1**, demonstrating a captured analyte on the inside surface of the capillary waveguide binding a fluorescent detection antibody. The signal is obtained by illuminating the capillary tube at a 90° angle relative to its length and subsequent collection of the emitted fluorescence from one end of the waveguide. The emitted fluorescent light is efficiently gathered and guided to the end of the capillary tube waveguide and goes through a set of lenses and optical filters to the optical detector. The signal is maximized by integrating the emitted light from all of the fluorescent labels on the capillary tube surface. At the same time, the background noise is reduced as a consequence of the excitation light being directed at a 90° angle to the waveguide surface. The Integrating Waveguide Biosensor (IWB) has an inherently high signal-to-noise ratio, because it gathers a high percentage of the fluorescence signal, and because the noise contributed by the excitation is reduced.

The IWB was originally developed by Ligler et al. at the Naval Research Laboratory (NRL) in Washington, DC *(1, 2)*. Ligler et al. *(2)* reported a detection limit of 40 pg/mL for mouse IgG and 30 pg/mL for staphylococcal enterotoxin B (SEB) in a sandwich assay format, which is about 100-fold more sensitive than the evanescent-wave fiber-optic and array biosensor technologies previously developed *(3–8)*.

We have constructed a compact and portable instrument using a low-cost capillary tube as the waveguide. When the IWB was applied to the detection of *Salmonella*, the limit of detection was ten captured *Salmonella* cells.

The IWB utilizing solid phase assay of analyte on the waveguide surface belongs to a family of integrating waveguide sensors detection principles. The implementation of the liquid-phase

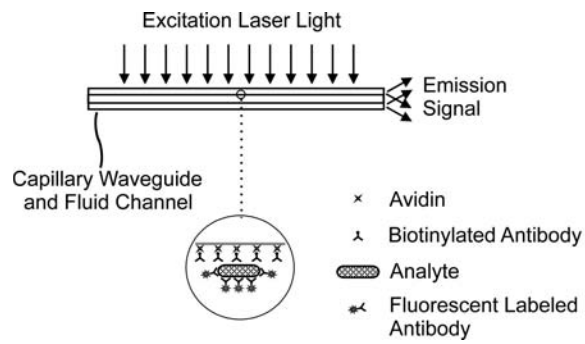


Fig. 1. Basic principle of the integrating waveguide sensor where a capillary is used as the waveguide. The analyte is captured on the inner surface of the capillary waveguide.
Fig. 2. Schematic of Creatv's Integrating Waveguide Sensor.

detection of the Integrating Waveguide Sensor is described in Chap. 24 *(9)*.

2. Materials

2.1. Instrument Components

1. *Illumination light source.* Diode laser of 635 nm, 30 mW (LaserMax, Rochester, NY).
2. *Signal cleanup filters.* A RG665 color glass filter (Edmund Industrial Optics, Barrington, NJ) and an interference band-pass 670-nm filter (Omega Optical, Brattleboro, VT).
3. *Detector.* Photomultiplier tube (PMT) H7827-11 (Hamamatsu, Bridgewater, NJ).
4. *Signal digitalizing.* DT9802 Data acquisition card (Data Translation, Inc., Marlboro, MA).
5. *Laser line generator.* A rod lens from LaserMax, Rochester, NY, combined with a cylinder lens from Edmund Industrial Optics, Barrington, NJ.
6. *Signal collimating lens.* An aspheric lens combined with a double convex lens.
7. *Signal focusing lens.* Double convex lens from Edmund Industrial Optics, Barrington, NJ.
8. *User interface.* LabVIEW™ software (National Instruments Corp., Austin, TX).

2.2. Simulation and Analysis

1. *Optical ray tracing.* TracePro software (Lambda Research Corp., Littleton, MA).

2.3. Capillary Waveguide

1. *Capillary waveguides.* 50 mm long borosilicate glass capillary tube with 1.66 mm outer diameter and 1.23 mm inner diameter (Drummond Scientific Company, Broomall, PA).

2.4. Chemicals and Reagents for Assay

1. NeutrAvidin™ (Pierce Biotechnology, Rockford, IL).
2. *GMBS.* 4-maleimidobutyric acid *N*-hydroxysuccinimide ester (Sigma-Aldrich, St. Louis, MO).
3. *Buffers.* PBST (10 µg/mL in PBS containing 0.05% Tween) and PBSTB (PBST containing 2% BSA).
4. *Analyte. Salmonella typhimurium* (ATCC 53648) (ATCC, Manassas, VA).
5. *Capture antibody.* Biotinylated rabbit polyclonal antibody (Biodesign International, Saco, Maine).
6. *Detector antibody.* Cy5-conjugated goat polyclonal antibody (KPL, Gaithersburg, MD).

3. Methods

The IWB consists of an illumination optical system, capillary tube waveguide, detection optical system, and data acquisition system (Fig. 2); the details of each component part will be discussed in the following sections. One of the design changes from NRL's experimental instrument was the elimination of the laser chopper and lock-in amplifier, which were necessary to minimize ambient background light. In contrast, the light-tight construction of Creatv's instrument eliminates interference from ambient light, obviating the chopper and lock-in amplifier, thereby reducing both the size and the cost. Other design changes are automation of data acquisition and packaging into a portable bench-top format.

Laser beam is expanded and collimated to fit the dimension of the capillary tube, so that the capillary tube can be evenly illuminated. Light exiting from the end of the capillary tube is collimated before passing through optical filters, in order to block excitation light and pass emission light. After passing the optical filters, the signal is focused down to the detection area of the PMT.

3.1. Illumination Optical System

The illumination system provides a collimated beam to the analyte-sensing surface. The system consists of a laser, a cylinder lens to generate a line pattern, and a collimating cylinder lens, as shown in Fig. 2. The features of the system include:

- Stable laser illumination
- Collimated beam
- Low noise in the fluorescence emission range
- Illumination of the whole length of capillary waveguide

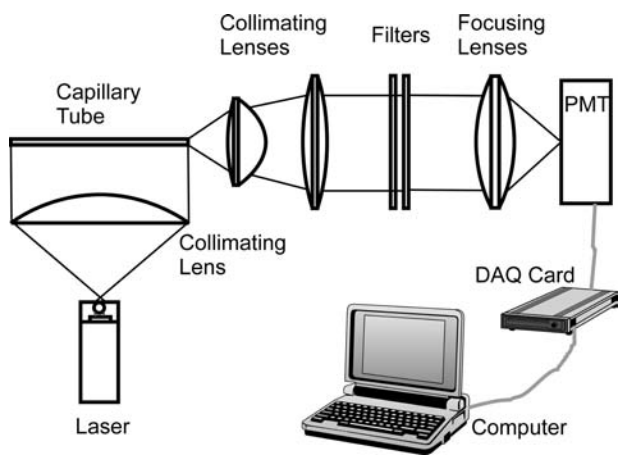


Fig. 3. Results of tracepro simulation of NRL's fused-silica capillary tube (0.70 mm inner

The optical system was optimized using Cy5 as the fluorescent dye. The peak absorption wavelength of Cy5 is 647 nm, while the peak emission wavelength is 670 nm, with a range of 650–750 nm. A diode laser (LaserMax, Rochester, NY) at 635 nm was chosen to provide maximum separation of the excitation and emission wavelengths.

Capillary tube is 1.66 mm in diameter and 50 mm long. In order to illuminate the whole tube, the laser beam needs to be about 2 mm × 50 mm in size.

The dimensions of the laser beam was expanded to 2 mm × 50 mm to cover the capillary tube waveguide and collimated to provide illumination at a 90° angle to the capillary surface.

3.2. Waveguide

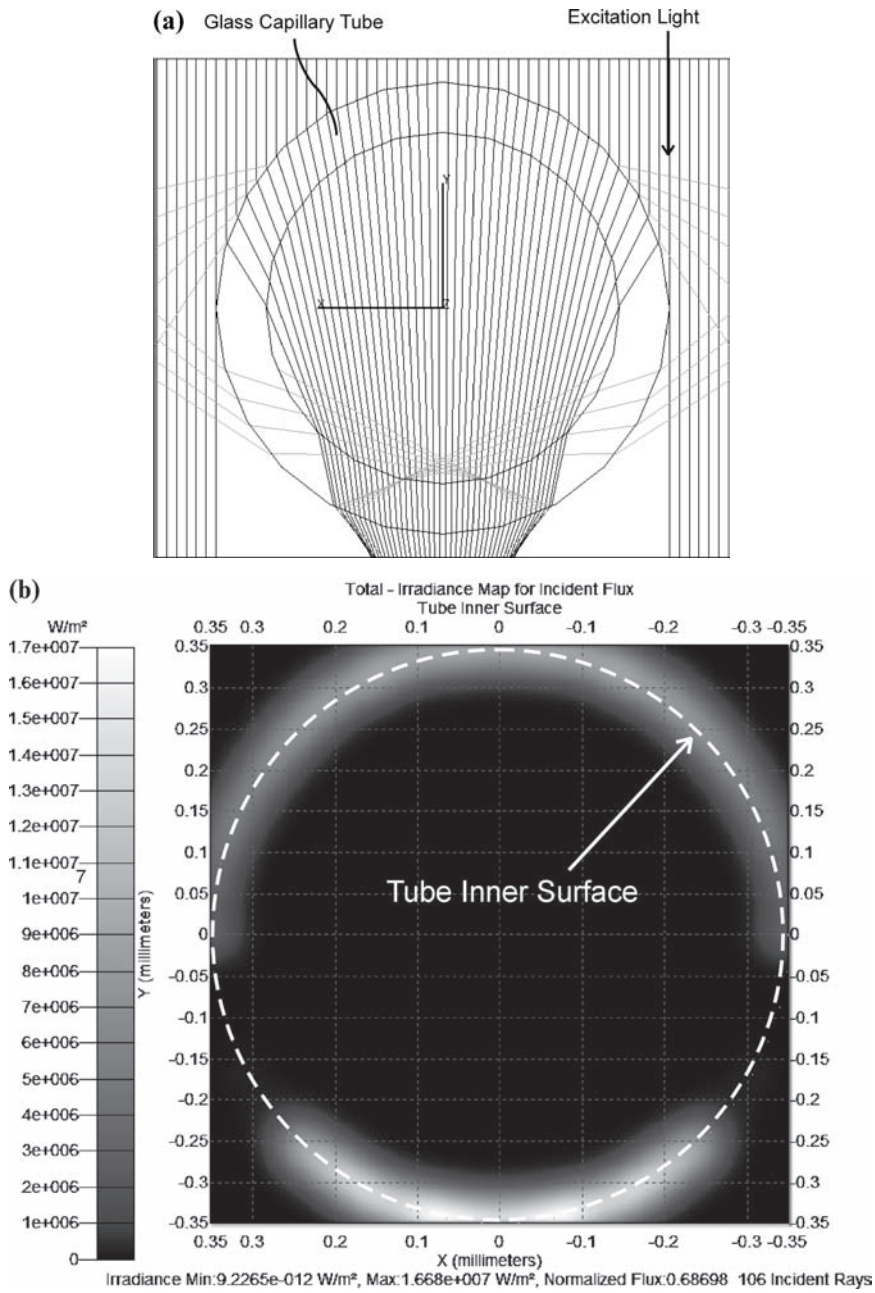
Borosilicate capillary tube is chosen as waveguide and they have the following properties:

- Good transmission for both laser excitation and fluorescent emission wavelengths
- Low autofluorescence
- Convenient and inexpensive
- Compatible with bioassays

In NRL's initial development, the capillary tubes they used is fused-silica capillary pieces 38 mm long (0.70 mm inner diameter and 0.85 mm outer diameter) with optically polished ends and coated on the outer surface with PTFE were obtained from Polymicro Technologies (Phoenix, AZ). These capillary tubes provided a low limit of detection, but their high cost and fragile nature made them unsuitable for routine use.

In order to select the optimum capillary tube waveguide geometry, simulations of the optical properties of various geometries and materials were conducted using the optical ray tracing software TracePro (Lambda Research Corp., Littleton, MA) to perform analysis of illumination of the fluorescent labels on the surface of the capillaries. TracePro uses Monte Carlo simulations to set up the rays for ray tracing and to compute optical flux. TracePro accounts for absorption, reflection, refraction, and scattering of light according to the material and surface properties of the capillary tube. Simulations were performed using incident rays of collimated 635-nm laser light.

Initial simulations of the NRL capillary tube waveguide indicated that the NRL's capillary geometry did not provide uniform illumination of the analyte sensing surface on the inner surface of the capillary. TracePro ray tracing results for the NRL capillaries is shown in **Fig. 3a.** The trajectories of the incident rays from the excitation laser light (entering from the top of the figure), are bent in the silica capillary (index of refraction = 1.46), and are reflected at the capillary tube surfaces due to change of the refraction index. The capillary is filled with buffer, because



diameter and 0.85 mm outer diameter) with liquid inside ray trajectories (**a**) and intensity plot (**b**).

NRL's experiments obtained better results with fluid inside the capillary. The rays shown in **Fig. 3a** are displayed in shades of gray: black rays denote the rays with flux equal or greater than 67% of the incident laser light; medium gray rays denote the rays with flux between 33 and 67% of the incident laser light; while

light rays denote the rays with flux less than 33% of the incident laser light. **Figure 3a** shows that bottom portions of the capillary surface received concentrated excitation light, while some areas to either sides of the capillary received no excitation light.

TracePro can also display the results of the excitation laser intensity for a cross section of the capillary tube, where the grayscale from black to white indicates no laser light to brightest laser light, respectively. The dotted white circle in **Fig. 3b** denotes the location of the inner surface of the capillary tube used by NRL and shows that this capillary geometry does not provide uniform illumination of the analyte sensing surface.

We subsequently analyzed several borosilicate glass capillary tubes of various sizes (index of refraction $n = 1.52$) commercially available from Drummond Scientific. Representative results are shown for the capillary tube with outer diameter of 1.661 mm and inner diameter of 1.226 mm, which was easy to handle and had superior performance (**Fig. 4a**, **b**). The TracePro simulation was performed with empty capillaries. Ray tracing trajectories (**Fig. 4a**) and grayscale cross-sectional view of laser excitation intensity (**Fig. 4b**) show nearly uniform illumination of the analyte sensing surface, indicated by the dotted white circle. Based on these simulations, this capillary geometry was chosen for the experiments.

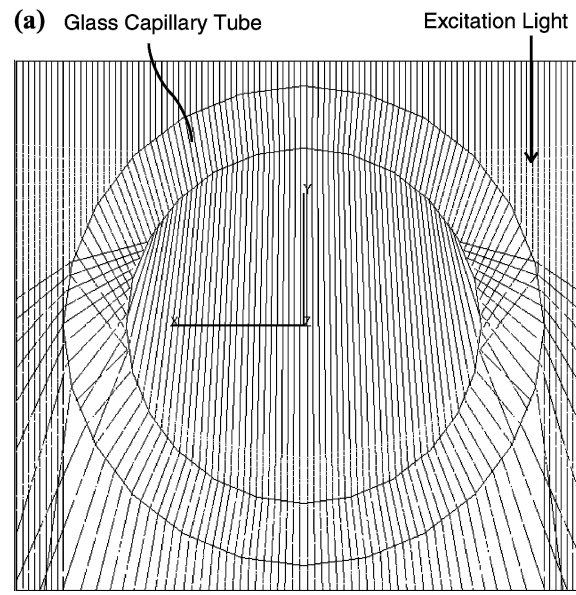


Fig. 4. Results of tracepro simulation of Drummond borosilicate capillary tube (1.226 mm inner diameter and 1.661 mm outer diameter) with air inside ray trajectories (**a**) and intensity plot (**b**).

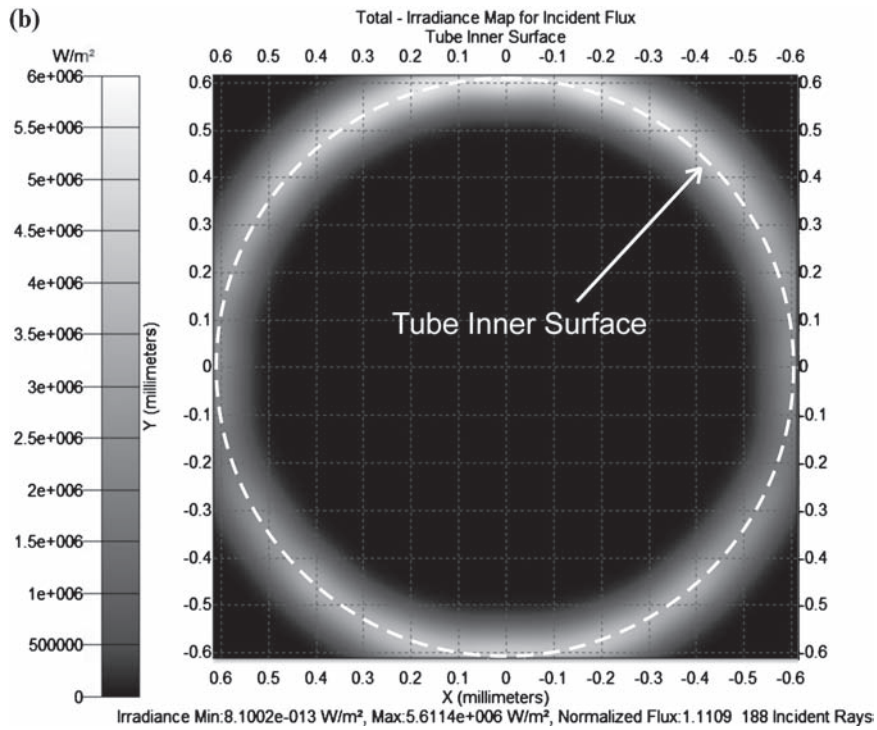


Fig. 4. (continued)

In order to design the detector optical system, we needed to estimate the properties of the signal that exits from the end of the capillary tube. A different TracePro simulation of the propagation of fluorescent emission signal from Cy5 dyes on the inner surface of the capillary was gathered and guided to the end of tube. The result is presented in **Fig. 5** as a candela plot of luminous intensity plot or emission flux distribution vs. angle at the exit of the capillary tube from one dye with 10,000 emission rays. Darker gray indicates more flux. Simulation with large number of dyes would provide uniform angular distribution. The software TracePro, however, can only simulate one dye at a time. **Figure 5** indicates that the emission signal exits the tube end in a cone shape with wide angles ranging from 30 to 60°. Thus, it is very important for the detecting optics to collect all the signals efficiently from the large emission angles from the end of the capillary tube.

3.3. Detection Optical System

The purpose of the detector optical system is to:

- Efficiently collect the emission light from the end of the waveguide
- Filter out any laser light

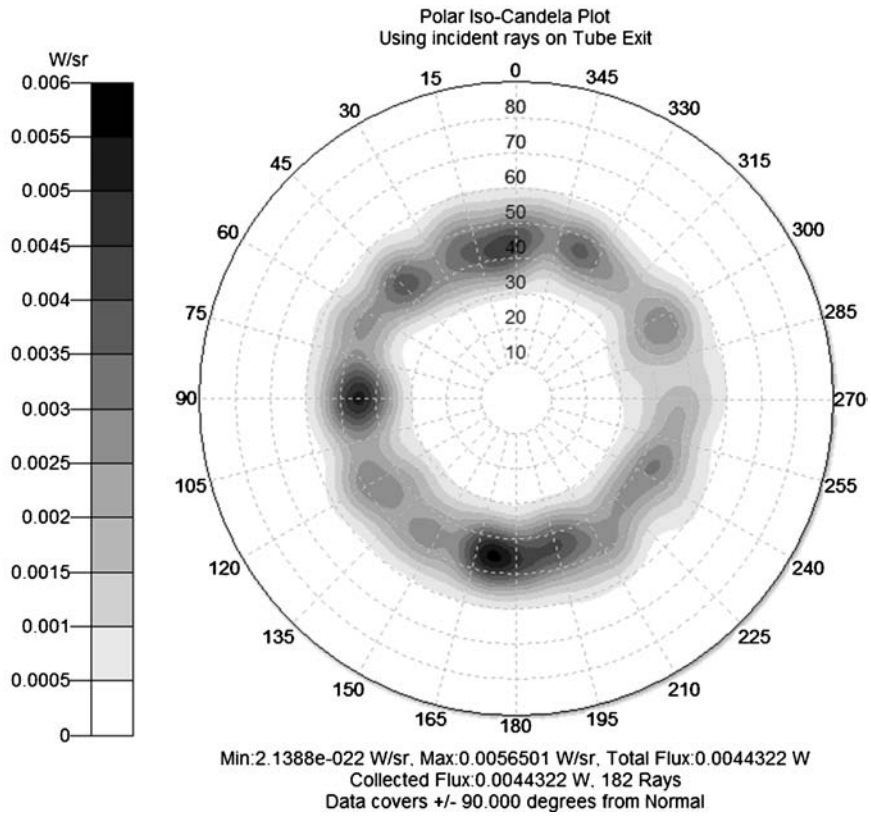


Fig. 5. Candela plot of emission flux distribution from Drummond capillary tube vs. angle at the exit of the capillary tube. *Darker gray* indicates more flux.

- Select the relevant portion of the emission light for detection
- Focus the transmitted signal to the detector

The light from the end of the waveguide includes both excitation and emission light. The excitation light has to be separated out before testing the signal. A 670-nm band-pass filter combined with a 665-nm long-pass filter are used to block excitation light. In order for band-pass filter to work properly, the light has to be collimated. The light from the end of the capillary has large angles (about 60° half angle). An aspheric lens plus a double convex lens are incorporated to collimate the light, as shown in **Fig. 2**. After passing through the collimating lenses and filters, the excitation light is removed, and only the emission light passes through. Finally, a focusing lens focuses the signal onto the PMT.

It is important that the capillary tube should be aligned with the illumination light and the detection system. The alignment of optics is guaranteed by mounting the illumination optics, signal detection optics, and optics holder in a precision machined chamber.

3.4. Data Acquisition and User Interface

The output form PMT is voltage signal ranging from 0 to 10 V. A data acquisition card is used to digitalize the voltage signal and send it to a computer. The gain of PMT is adjustable. If the signal is too low, the PMT gain can be raised; and if the signal is too high and saturates the PMT, the gain can be lowered. The signal has to be normalized accordingly.

LabVIEW software is used to control the IWB and collect the data. The user interface, shown in **Fig. 6**, consists of three panels: SET-UP PANEL, TEST PANEL, and RESULT PANEL. User fills in the information on the SET-UP PANEL. User starts the test by clicking the TEST button. For each testing, the chart at the right side of the front panel will show all the readings collected by PMT as a function of time. An average value will be calculated and displayed on top of the chart. The test data can be saved in EXCEL format. The file name and path will be shown on the RESULT PANEL.

Figure 7 shows a schematic of the interior of the instrument. This model uses a H7827-11 Hamamatsu PMT, a DT9802 data acquisition card (Data Translation, Inc.), and a computer-controlled user interface using LabVIEW™ software (National Instruments Corp.).

3.5. Testing Results

IWB was applied to the detection of *Salmonella* using sandwich immunoassay. Glass capillary tubes were prepared as previously

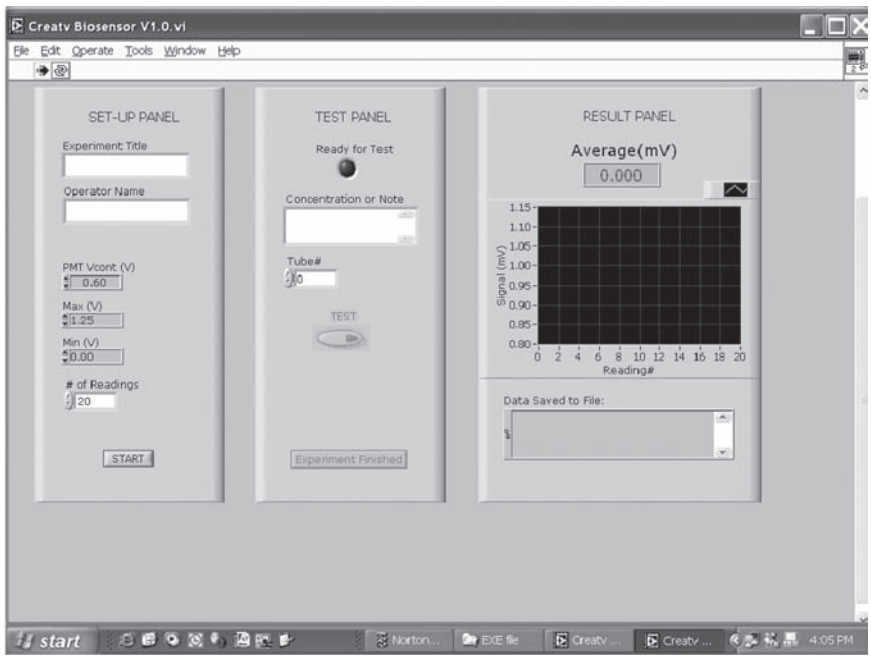


Fig. 6. Computer interface of Creatv's prototype Integrating Waveguide Biosensor.

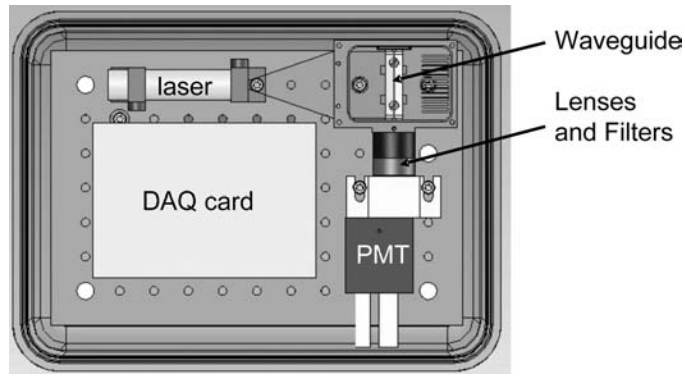


Fig. 7. Schematic of Creatv's portable prototype IWB using capillary tubes as waveguides for Cy5 fluorescent dye.

described *(2)*. Briefly, capillary tubes were cleaned with methanol/HCl and sulfuric acid, dried with nitrogen, silanized with 3-mercaptopropyl trimethoxysilane in anhydrous toluene under nitrogen atmosphere, incubated with 4-maleimidobutyric acid *N*-hydroxysuccinimide ester (GMBS) (Sigma-Aldrich, St. Louis, MO), and then treated with NeutrAvidin (Pierce Biotechnology, Rockford, IL). After conjugation of NeutrAvidin inside the capillary tubes, they were washed with PBST, and stored at 4°C until use.

The capillary tubes were incubated with PBSTB containing 2% BSA, and then a biotinylated capture antibody in PBSTB was immobilized inside the capillary. After rinsing with PBST, sample was introduced into the capillary tubes and incubated for 1 h at room temperature. The capillary tubes were subsequently washed with PBSTB, and then filled with Cy5 conjugated detector antibody in PBSTB for 1 h. After removal of the unbound detector antibody and thorough washing with PBST, the capillary tubes were tested using the IWB instrument.

A serially diluted culture of *S. typhimurium* (ATCC strain 53648) in PBST buffer (phosphate buffered saline + Tween + Triton) was tested using the Creatv's IWB. Biotinylated rabbit polyclonal antibody from Biodesign International (Saco, Maine) was used for capture and Cy5 conjugated goat polyclonal antibody from KPL (Gaithersburg, MD) was used for detection. The fluorescence signals obtained using 40–4 × 10^6 *Salmonella* cells per capillary are shown in **Fig. 8a**. The detection threshold (Mean + 3 SD (Standard Deviation) of zero concentration) was 23.4 mV. These data indicate that as few as 40 *Salmonella* cells per capillary resulted in a detectable signal of 26.9 mV. Based on an estimated capture efficiency of 18–29% (data not shown), the absolute detection sensitivity of the IWB is approximately ten cells. This is consistent with our previous assay to detect *E.*

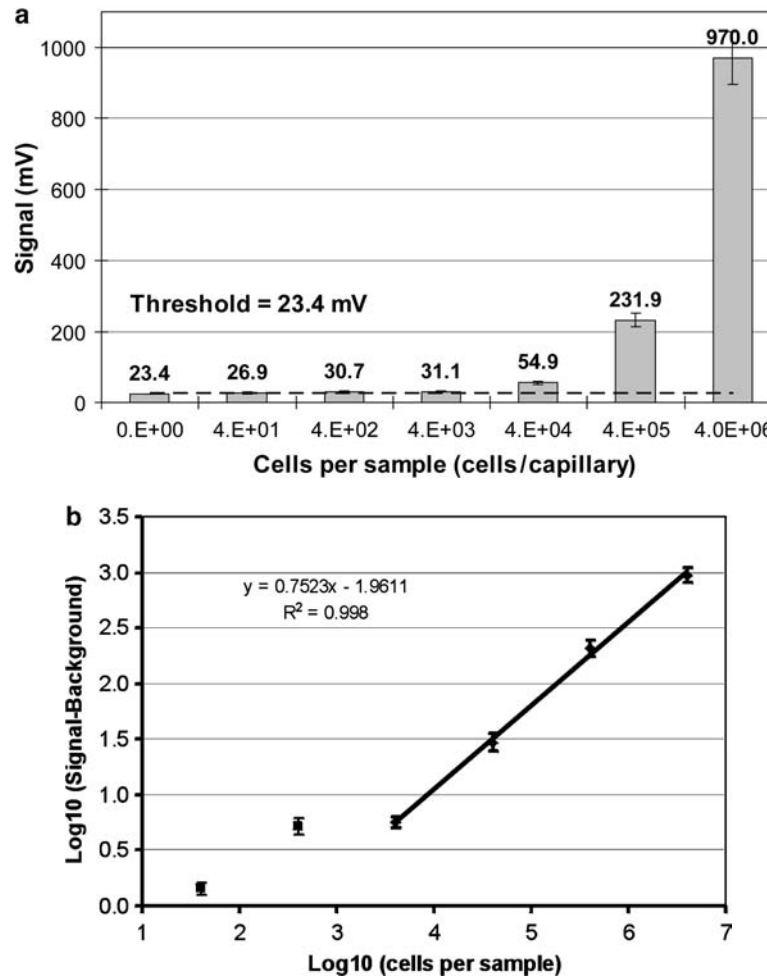


Fig. 8. Sandwich immunoassay results for detection of *Salmonella* using the IWB using Cy5 fluorescent label. **a** Fluorescence signal response to total cell number of *Salmonella* in a capillary. Threshold detection limit (mean value plus three times of standard deviation) is 23.4 mV, as indicated by the *dashed line*. **b** Linear correlation between log10 of net fluorescence signal and log10 of total cells per capillary.

coli (10). For high cell concentrations, the R^2 value is 0.998 (**Fig. 8b**). When the cells per sample are low, the signal readings are accurate, but cells per sample and percentage of cells captured by the waveguide are susceptible to sampling error. We do not have means to provide accurate count of the number of cells captured on the capillary waveguide. The linear relationship between log10 of fluorescence signal and log10 of input cell concentration (**Fig. 8b**) allows for quantitative detection for high concentrations and only semiquantitative detection for lower concentrations.

4. Notes

1. Collimating the laser light or signal through the interference filter is important to reduce background noise.
2. In capillary tube preparation, all the steps from cleaning until up to the immobilization of NeutrAvidin should be done on the same day. After conjugation of NeutrAvidin inside capillary tubes, the capillary tubes were washed with PBST, and may be stored at 4°C until use.
3. The instrument requires a warm up time of about 20 min. This is for the PMT to stabilize.

Acknowledgments

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