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Portable 24-analyte surface plasmon resonance instruments for rapid, versatile biodetection

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Abstract

Field use of surface plasmon resonance (SPR) biosensors for environmental and defense applications such as detection and identification of biological warfare agents has been hampered by lack of rugged, portable, high-performance instrumentation. To meet this need, we have developed compact multi-analyte SPR instruments based on Texas Instruments' Spreeta sensing chips. The instruments weigh 3 kg and are built into clamshell enclosures measuring 28 cm × 22 cm × 13 cm. Functions are divided between an electronics unit in the base of the box and a fluidics assembly in the lid. Automated valves and pumps implement an injection loop flow system that allows sensors to be exposed to sample, rinsed, and treated with additional reagents (such as secondary antibodies) under computer control. Injected samples flow over the surfaces of eight sensor chips fastened into a temperature-controlled silicone flowcell. Each chip has 3 sensing regions, for a total detection of 24 areas that can be simultaneously monitored by SPR. Coating these areas with appropriate antibodies or other receptors allows a sample to be screened for up to 24 different substances simultaneously. The instruments report refractive index (RI) values every second, with a typical noise level of 1–3 × 10^{-6} RI units. The design of the device is described, and performance is illustrated with detection of six distinct analytes ranging from small molecules to whole microbes during the course of a single experiment.

Keywords: Surface plasmon resonance biosensing; Detection of chemical and biological warfare agents; Portable biosensors

1. Introduction

Tools enabling the rapid detection and identification of chemical and biological substances are needed for applications including detection of chemical and biological warfare (CBW) agents, biomedicine, environmental monitoring, food monitoring, drug screening, and process control. Biosensors capable of directly detecting target analytes (proteins, small organics, microbes, viruses, or toxins) by means of specific recognition elements (e.g. antibodies) immobilized on the sensor surfaces have attracted much attention. Optical methods are of great interest for biosensing because they allow continuous non-destructive measurement. Considerable research has been carried out on surface plasmon resonance (SPR) based optical biosensors that can detect an analyte's presence directly, without the use of labeled molecules (Liedberg et al., 1983; Lundström, 1994; Lawrence et al., 1996; Homola et al., 2005; Singh and Hiller, 2006). The SPR sensing principle has been exploited by Biacore Life Sciences, who manufacture and sell "point of reference" SPR-based systems tailored to research, high throughput testing, rapid food sample analysis, and quality control. These instruments require a large financial investment and extensive user training to learn device operation and proper use of associated software. Leica Microsystems, GWC Technologies, IBIS Technologies, and Toyobo Co., Ltd. have also developed SPR systems for laboratory use, which are too large and delicate for field applications.

Miniaturization and ruggedization is of vital importance for the development of portable SPR-based biosensors for field applications such as detection of pollutants in natural water supplies (Mauriz et al., 2006), sensing TNT for landmine detection (Shankaran et al., 2005), and detection of mycotoxins in food supplies (van der Gaag et al., 2003). Various approaches to the miniaturization of SPR biosensors have included optical fibers

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(Jorgenson and Yee, 1993, 1994), integrated optical waveguides (Harris and Wilkinson, 1995; Homola et al., 1997), and miniaturized versions of the attenuated total reflection (ATR) method (Karlsen et al., 1996). The β-SPR system (Sensia, Spain) and Spreeta (Texas Instruments, USA) are examples of miniaturized SPR biosensors that have been commercialized (Mauriz et al., 2006; Suzuki et al., 2002; Jiang et al., 2005; Melendez et al., 1996). The Spreeta SPR sensing components developed by Texas Instruments are unique in that the all of the optoelectronic components needed to perform an SPR measurement are incorporated into a rugged molded plastic chip the size of a fingertip. Spreeta 2000 sensors exhibit very low noise when used in conjunction with optimized readout hardware and data analysis techniques (Chinowsky et al., 2003). Because the Spreeta 2000 has resolution comparable to other available SPR sensing platforms yet is extremely compact, robust, and commercially available in large quantities for low cost, the Spreeta sensors provide an ideal technology for development of portable SPR systems. For several years, our team has been working with the Spreeta components to develop handheld sensors to be used in the field for detection and analysis of chemical and biological warfare agents. This research has resulted in the development of several versions of SPR-based instruments capable of real-time detection of chemical and biological substances, including whole bacteria, viruses, protein toxins and small molecules. We recently reported the development of a six-channel sensor system (Naimushin et al., 2002, 2003, 2005). Here, we describe a semi-automated 24-channel Spreeta-based SPR biosensor system. This lunchbox-size instrument, dubbed Surface Plasmon Instrumentation for the Rapid Identification of Toxins ("SPIRIT"), is completely self-contained and easily portable (Fig. 1).

1.1. The surface plasmon resonance sensing principle

SPR is a well established method for detection of biological binding events. As implemented here, one surface of a prism is coated with a thin (50 nm) layer of gold. Monochromatic light

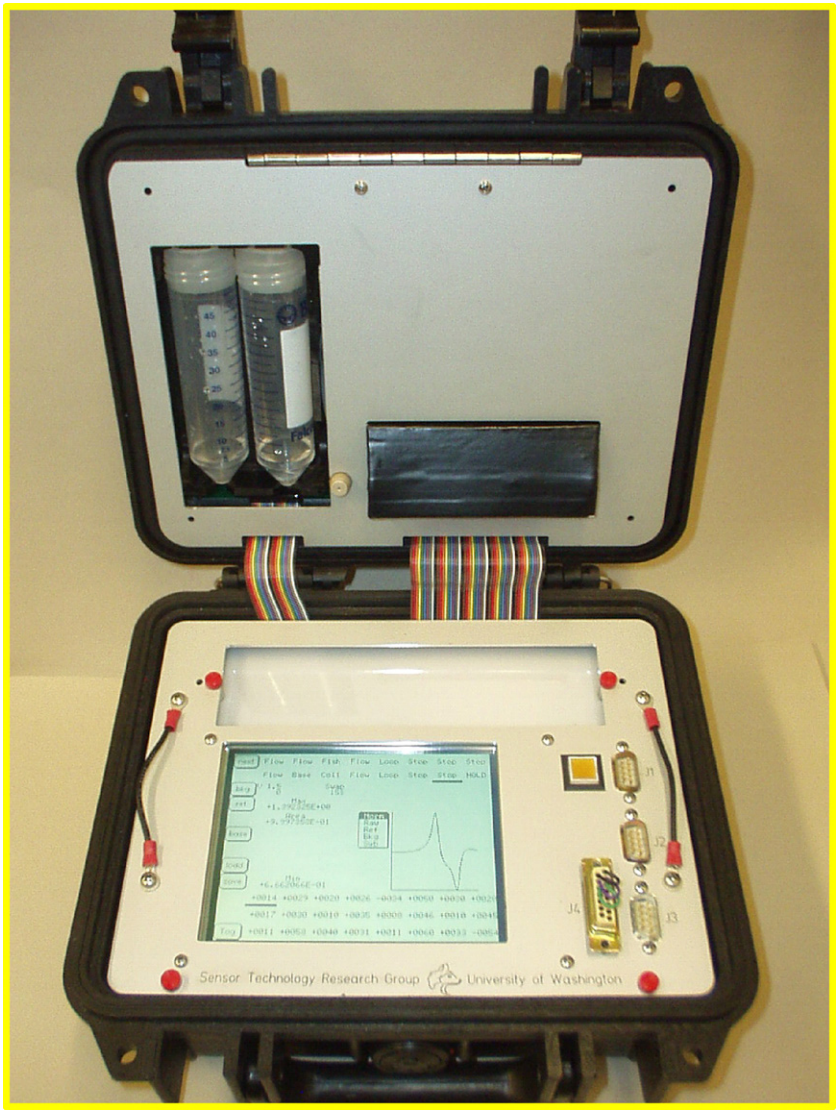

Fig. 1. Photograph of SPIRIT biosensor unit.

($\lambda = 830$ nm) travels through the prism and strikes the back side of the gold-coated surface at a range of angles greater than the critical angle. For a certain angle of incidence, much of the illumination energy will be absorbed in a surface plasmon wave traveling along the gold surface. This resonance angle strongly depends upon the refractive index (RI) of the material immediately adjacent to the gold surface. Determination of this angle provides a sensitive measurement of refractive index near the surface. Because biological substances have higher RI than water, the presence of tiny quantities of such substances on the surface can be detected and quantified in real time. To make the sensor specific for particular biological analytes, the surface is treated with chemistries such that the specific sensing areas bind the selected analyte and resist binding of other substances. In the SPIRIT system, SPR is observed using Texas Instruments' Spreeta 2000 sensing chips. These components consist of a plastic prism molded onto a small printed circuit board. Light emitted by the sensor's light emitting diodes (LEDs) strikes the gold-coated SPR surface, reflects from a mirror on top of the sensor, and strikes a linear diode array. Because the incident angle varies with position along the diode array, reading the array gives a measurement of reflectivity as a function of angle. This reflectivity measurement may then be analyzed to obtain measurements of biomolecular binding in real time.

2. Instrument design

2.1. Instrument packaging

Our group has developed several versions of miniature multichannel SPR instruments based on Spreeta devices. The present version is packaged in a Pelican 1200 clamshell case, weighs approximately 3 kg, and measures 28 cm × 22 cm × 13 cm with the lid closed. Instrument functions are divided between an electronics unit in the base of the Pelican case and a fluidics assembly located in the lid.

2.2. Fluidics

2.2.1. Flowcell

The SPIRIT system incorporates fluidics fabricated from cast silicone. Silicone can be readily cast with good feature resolution, seals well with pressure, can serve as the active element of pumps and valves, and is readily available in tubing form as well as uncast resin. Pre-existing silicone components such as tubing can be joined together or integrated into a custom mold with additional resin, with perfect bonding between components. The fine features available with silicone cast in precision machined molds allow construction of highly detailed flowcell structures.

The fluidics assembly is built around 8 three-channel Spreeta 2000 devices fastened into a cast silicone flowcell. The flowcell design allows easy removal and replacement of individual sensor elements (Fig. 2). Individual Spreeta sensors slide into aluminum mounting brackets that snap into Delrin clips on the top and bottom of the flowcell, sealing the Spreeta sensor surface

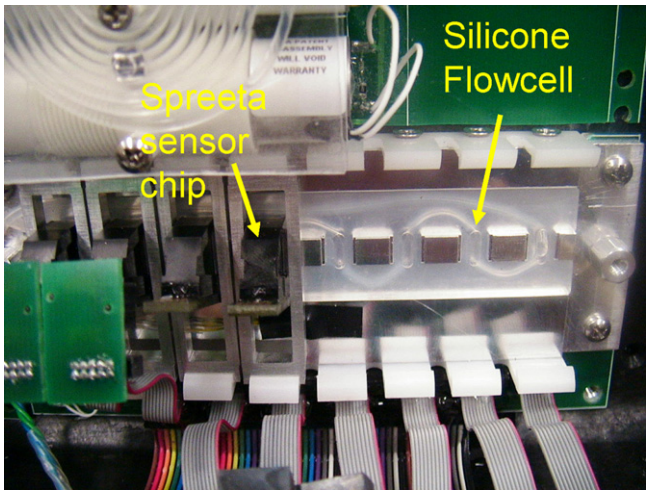

Fig. 2. SPIRIT flowcell, showing clip-in sensor mounts, molded chambers, and interconnects.

onto the silicone flowcell. The flowcell is held by an aluminum backing plate that serves to equilibrate the sensors at a constant temperature, equilibrate the fluid stream to constant temperature and mate with a thermoelectric heater/cooler attached to a heat sink extending from the lid of the instrument.

Inside the flowcell, liquid sample flows over each of the sensor chips in series. Each chip's surface has three sensing areas that are coated with receptors for a particular CBW agent or simulant.

2.2.2. Sample handling and injection loop

Sensors are exposed to samples using a flow injection system coupled to the sensor flowcell. A sample injection port leads through a three-way valve into a sample loop backed with a pressurized buffer reservoir. The injection valve switches between the injection port and the flowcell input to control flow of sample over the sensors' path. Additional valves allow for automated purging of the injection loop and flow of auxiliary reagents across the sensors.

The main purpose of the injection system is to produce a controlled flow of the samples to be analyzed across the sensor surfaces. In addition, the fluidics valving can switch to flow amplifying reagent (e.g. secondary antibodies) over the sensors or to purge the sample loop (to wash the sensor surfaces and prevent cross-contamination between samples). Fluidics were constructed using commercial pumps and three-way solenoid valves, and were designed for ease of use, simple construction and ease of maintenance. The valves and pumps are actuated by the sensor's microprocessor in the sequence necessary to perform a sample analysis. The basic sequence is to collect a sample, flow the sample over the sensors, determine the rate of binding of analyte to the receptors on the sensor surface, and calculate the concentration of analyte from a standard curve. The fluidic schematic shown in Fig. 3 illustrates how valves are switched to perform the collection, detection, and flush steps. In the collection step, sample is injected into the sensor's sample port and through the injection loop, with excess flowing into the waste reservoir (W). When the injection loop has filled with

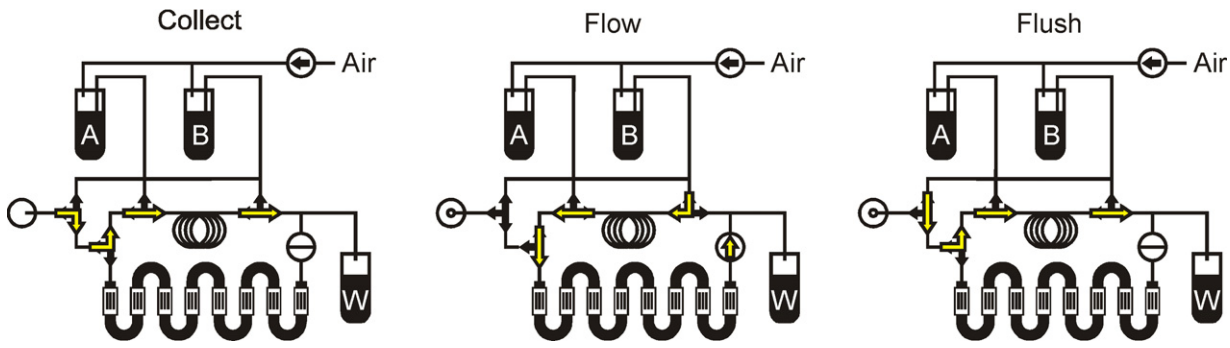

Fig. 3. Schematic of SPIRIT fluidics during sample collection, flow, and flushing of the sample loop. Three-way valves (arrows) and a peristaltic pump switch to control fluid flow. During collection, fluid flows from the sample port into the injection loop. During analysis, the injected sample flows from the loop into the serpentine sensor flowcell, exposing the surface of eight sensor chips to the sample. In the flush step, buffer (B) is automatically injected into the sample loop, clearing. Amplification solution was added by manual injection through the injection port, however the system allows for automatic injection of amplifiers (A). All waste was collected in a closed system waste collection tube (W).

sample (and the previous contents of the injection loop have been emptied to waste), the valves are switched such that fluid flow reverses and contents of the injection loop flow across the sensor surface at a rate metered by a peristaltic pump. The flush step is identical to the injection step, except that the sample flow is directed from the sensor's buffer reservoir instead of the sample port.

A key innovation that led to greatly improved fluidics performance was the development of pressurized (∼10 psi) fluidics. Compared to unpressurized systems, we find pressurized systems wet out more easily, exhibit smaller and more quickly resolved interference from injected bubbles, and show no tendency to bubble evolution over time. With a pressurized system, the gas-permeability of the silicone fluidic components becomes a desirable feature—gasses in the liquid stream tend to be pushed out through the walls of the silicone material. As a result, degassing of solutions is generally not necessary.

2.3. Electronics

The instrument electronics are built around eight replaceable three-channel Spreeta 2000 SPR sensors, each of which contains a diode array detector and three LEDs (one for each sensing channel). The sensor base unit contains components necessary to perform RI measurements with the Spreeta chips, control the sensor fluidics, and drive the sensor's user interface. A 1 MHz analog-to-digital converter and a digital signal processing (DSP) microcomputer are used to digitize the Spreeta outputs, process sensor data, and control the instrument. Custom electronics boards drive the Spreeta LEDs, provide control of fluidics pumps and valves, and control the temperature of the sensor elements by driving the thermoelectric elements mated to the flowcell.

System electronics also contain switching power supplies for efficient generation of instrument voltages and drive of thermoelectric elements, a touch-screen liquid crystal display (LCD) user interface, flash memory for non-volatile storage of sensing results, and a USB interface for connection to PC. The instrument measures and reports RI values once per second. Instrument temperatures, current consumption, and fluidic state are also reported.

2.4. Software and user interface

2.4.1. DSP software

The SPIRIT sensor is controlled via a touch screen built into the unit. The user interface functions are divided between several interface screens, which the user may cycle between. These screens display numerical values of baselined refractive index for all 24-sensor channels, with values updated once per second. If the user touches on the value for a particular channel, the current SPR curve for that channel is displayed. Touching on the SPR curve display causes the graph to cycle between display of the normalized SPR curve, the raw SPR curve, the reference SPR curve (measured in a high RI reference solution), and the background curve (measured with the LED off). Soft keys on the left of the screen allow the user to record background and reference spectra, establish a baseline for the displayed RI values, and store and recall reference spectra in flash memory. A scrolling "strip chart" displays sensor temperature, temperature set point, and instrument current consumption. The upper two rows of the screen show the currently programmed fluidic steps. The user may jump to a given step by touching the label for that step. To program the function of these steps, the user may touch the soft key in the upper left corner of the screen to change to the fluidics programming screen.

On the fluidics programming screen, the user can enter and store up to four programmed sequences of fluidic operations to be automatically performed by the sensor. Each sequence can contain up to 15 steps, listed along the top two rows of the screen. Each of these steps has an associated type, time, flow rate, air/vacuum pump state, and temperature. Touching one of the 15 step labels along the top of the screen causes that step to be selected, with its current values displayed on the on-screen ring controls. The user may change a step by pausing the program (by touching the "HOLD" soft key) and entering new values on the ring controls.

2.4.2. PC software

For long-term monitoring and more convenient display of full 24-channel sensor data, we have written Windows software that allows a PC to connect to the SPIRIT unit over its RS-232 data

port. The software continuously records RI values for all sensing channels, as well as instrument temperatures, current consumption, and fluidic state. The user may select which channels to display, and zoom and pan within the sensor data.

3. Biosensing with SPIRIT: 24-channel, six-analyte experiment demonstrating sensing of small molecules, proteins, viruses, and whole microbes

3.1. Target analytes

In the experiments presented here, a 24-channel SPIRIT sensor unit was used to detect multiple analytes sequentially, and then each of the analytes tested were verified by testing them separately at the low detection threshold for each analyte. The analytes chosen were all simulants of or inactivated forms of potential CBW agents, with the exception of *Staphylococcus* enterotoxin B (SEB) which was an active toxin molecule. Easily detectable concentrations were chosen for the sequential experiment, and an additional individual experiment (three replicates with three reference channel replicates) was performed at a lower concentration to confirm detection as well as to establish the lower limits of detection. Data from both the sequential experiment and individual experiments are summarized in Table 1. The classes of analytes tested were as follows:

Small molecules: 2,4-Dinitrophenol-L-lysine (DNP-lysine; MW 348.74, Sigma, #259934) was chosen as a representative small molecule. DNP is used because it is similar in structure and size to the explosive molecule trinitrotoluene (TNT) and both antigen and antibody are readily available. Monoclonal antibodies to DNP (US Biologicals #3G136) were used for these experiments.

Proteins: The bacterial toxin SEB (MW 28,000) and ricin A chain (MW 32,000) were selected as representative proteins. The active form of ricin contains both the A and B chains (MW 66,000). The B chain is required for transport into the cell, and the A chain is the active toxin molecule; therefore A chain alone is not toxic. SEB and antibody to SEB were obtained from Toxin Technology (SEB #BT202, Goat anti-SEB #LBI202). Anti-ricin antibodies were obtained from Dr. Robert Bull at the Naval Medical Research Center (NMRC), Silver Spring, MD (monoclonal), and the ricin A chain and polyclonal anti-ricin antibodies were obtained from the Department of Defense Critical Reagents Program (DOD CRP) in Frederick, MD. A monoclonal anti-ricin antibody (RIC-07-A-G1) was used for the surface antibody, and a mix of a monoclonal (RIC-03-A-G1) and goat polyclonal (ABE 129) was used for secondary amplification.

Virus: Norwalk virus is a small (27 nm) highly communicable virus that causes gastrointestinal illness. Norwalk virus virus-like particles (VLP's) are non-infectious because they are hollow viral capsules and lack nucleic acid (Hutson et al., 2003). Monoclonal anti-Norwalk virus (MAb 3901) (Hardy et al., 1996) was used to coat the sensor surface, while a polyclonal (rabbit) anti-Norwalk virus was used to amplify the signal from direct binding.

Bacteria: Francisella tularensis LVS, Irradiated. A killed vaccine strain of the tularensis bacteria was selected to demonstrate detection of the organism that causes tularemia. *F. tularensis* LVS was provided along with death certificates from irradiation treatment by Dr. Robert Bull at the Naval Medical Research Center (NMRC). Affinity purified goat anti-*F.t.* (Lot #270504-01) was also obtained from Dr. Bull and was used to coat the sensor as well as for the amplification step.

Spores: *Bacillus subtilis* (*Bg*) spores. As a simulant for *B. anthracis* spores, *B. subtilis* (*Bg*), var. Niger, spores were employed. Spores and antibody (rabbit anti-*Bg* spores. Lot #240699-01) were provided by Dr. Bull at NMRC. The rabbit anti-*Bg* spore polyclonal antibody was used to coat the sensors as well as to amplify the signal.

Table 1
Summary of surface functionalization, antigen selection, and detection results for the 24-channel, six-analyte experiment

Sensor	Surface molecule	Antigen	Sequential experiment			Individual experiments at lower concentration		
			Concentration	Signal (RU)	Control (RU)	Concentration	Signal (RU)	Control (RU)
1	PC (goat) anti-SEB	SEB	1 nM	72 ± 18	0 ± 2	NA	NA	NA
2	MC (mouse) anti-Norwalk virus	Norwalk VLP's	6×10^9 VLPs/ml	52 ± 3	8 ± 1	1×10^9 VLP's/ml	29 ± 4	11 ± 2
3	PC (goat) anti-*F. tularensis*	*F. tularensis*	1×10^5 CFU/ml	195 ± 20	43 ± 16	1×10^4 CFU/ml	40 ± 4	4 ± 1
4	MC Reference	–	–	–	–	–	–	–
5	BSA-Dinitrophenol	DNP-Lysine	5 μM	−36 ± 5	−1 ± 2	1 μM	−21 ± 6	−5 ± 3
6	MC anti-ricin	Ricin A chain	100 ng/ml (3.1 nM)	44 ± 8	4 ± 1	10 ng/ml (0.31 nanomolar)	28 ± 11	−8 ± 5
7	PC reference	–	–	–	–	–	–	–
8	PC (rabbit) anti-*Bg* spores	*Bg* spores	1×10^6 CFU/ml	102 ± 11	3 ± 6	9×10^4 CFU/ml	31 ± 2	−6 ± 7

Each sensor contained three channels functionalized with the surface molecule specific to that sensor. The three-channel average and standard deviation of the detection signal for each sensor at the end of the amplification step is shown adjacent to the response of the corresponding reference sensor which provides a control. *Abbreviations*: SEB, *Staphylococcus* enterotoxin B; MC, monoclonal; PC, polyclonal; CFU, colony forming units; VLP, virus-like particle; RU, resonance units. The MC reference surface was normal goat serum (sensor #4). The PC reference surface was anti-*Bacillus anthracis* (sensor #7). Sensor curves for the sequential experiment are shown in Fig. 4. In the individual experiments, each analyte was detected separately at a lower concentration, in order to estimate the detection limit of the assay. Each sensor contained three channels functionalized with the surface molecule specific to that sensor and each experiment was run with a reference sensor similar to that used in the six-analyte experiment. SEB detection limit data were reported previously (Naimushin et al., 2002) and so are not shown.

3.2. Sensor and antigen preparation

Seven different antibodies were used to coat the surface of seven separate three-channel Spreeta sensor chips. The eighth sensor, used for detection of DNP via displacement assay, was coated with BSA conjugated to DNP. The antibody coating procedure was as follows: each sensor chip was first cleaned with nitric acid and ethanol. The gold surface of each sensor was immersed in 10% nitric acid for 10 min. The sensors were then rinsed with 95% ethanol and wiped off with lens paper soaked in ethanol. Immediately after cleaning the sensor surface, antibodies were attached. Each antibody was diluted into Dulbecco's phosphate buffered saline (PBS, pH 7.3) to a final concentration of 500 μg/ml. A volume of 40 μl of this solution was pipetted onto the sensor surface, covering the entire gold surface for each sensor (20 μg antibody total). Non-specific adsorption (physisorption) of proteins (including antibodies and BSA) onto a bare gold surface is a fast and easy way to immobilize to the sensor surface (Storri et al., 1997; Casreo et al., 2002). Antibodies were allowed to adsorb to the gold for 15 min at 37 °C followed by 1 h at room temperature, then rinsed with 1 ml PBS. Sensors were then treated with blocking solutions of 1 mg/ml casein in PBS, followed by neat fetal bovine serum (FBS) (Sigma, #F-6178). A volume of 40 μl of each blocking solution was pipetted onto each sensor, incubated for 1 h at room temperature, and then rinsed with 1 ml PBS.

DNP-BSA coating was performed as follows. Following cleaning, 40 μl of BSA solution (1 mg/ml in PBS) were placed on the sensor surface for 1 h at room temperature. Following a rinse with 1 ml PBS, 40 μl of 2 mg/ml dinitrobenzene-sulfonate (DNBS, Aldrich, #259934) in carbonate buffer (0.1 M potassium carbonate with 0.1 M NaCl, pH 9.2) were applied. The reaction was allowed to proceed for 1 h, and was terminated with a PBS rinse. FBS (neat) was used as described above to further block the sensor surface.

Each antigen to be tested was diluted into a final volume of 2 ml PBS–Tween (Dulbecco's phosphate buffered saline; 0.1% Tween 20). The surface treatment of each sensor, together with the corresponding analyte with which the sensors were tested, is summarized in Table 1.

3.3. Experimental procedure

The sensors were used immediately following coating. Each sensor was placed into the sensor cartridge and a flow of PBS–Tween was initiated to wet the sensors. The sensors were then initialized by injecting a 20% sucrose solution. This sucrose solution has a high refractive index relative to the buffer solution and causes the SPR signal to shift well outside the range for monitoring protein binding events in buffer solutions, thus allowing a background initialization to be performed without drying the sensor. Following initialization the sucrose solution was flushed out with PBS–Tween and the sensor signals were set to zero values before beginning the experiment.

The sequential analyte experiment consisted of six separate injections of 2 ml samples. The injected samples were allowed to flow over the eight-sensor bank for a total of 10 min. Each injection, except for the small molecule test (DNP), was followed by a 10-min antibody amplification step at a concentration of 25 μg amplifying antibody/ml. Pump flow was 40 μl/min. The sensors were set to zero prior to each injection, including the amplification injection, and the injection loop was automatically flushed to complete each 10-min cycle.

At the beginning of the experiment, anti-DNP antibodies at a concentration of 10 μg/ml were loaded onto the surface of the DNP-BSA sensor. Displacement of these antibodies by DNP-lysine resulted in a negative detection signal that was proportional to analyte concentration.

3.4. Results and discussion

The sensor readings for each of the eight sensors are shown overlaid in Fig. 4. The three channels of each sensor were averaged to give a single trace for each sensor. When the sensor

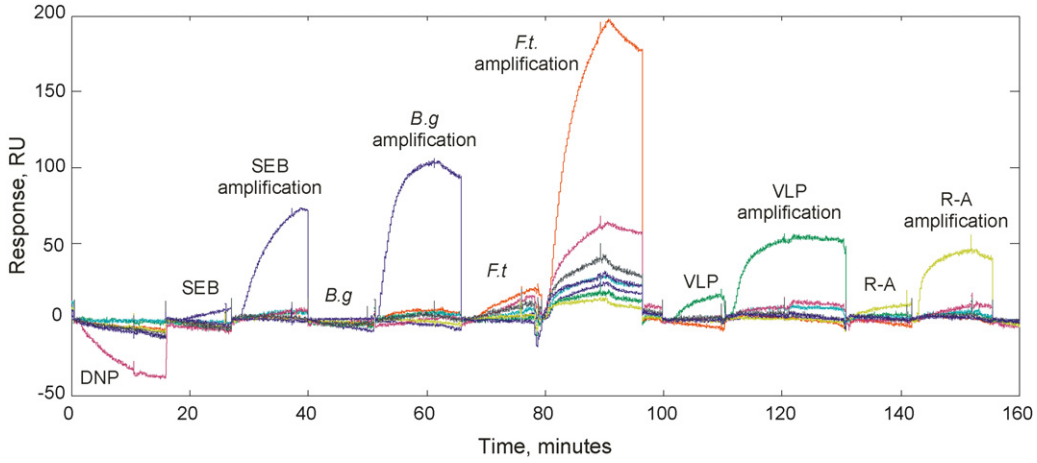

Fig. 4. Six-analyte detection-all sensors. Overlaid plots of refractive index traces from each of the 8 three-channel sensors used during the experiment. The sensor specific for each analyte responded as that analyte was introduced. Flow of secondary antibodies then amplified the signal. *Abbreviations*: DNP, dinitrophenol; SEB, *Staphylococcus* enterotoxin B; *Bg*, *Bacillus subtilis* spores; *F.t.*, *F. tularensis*; VLP, Norwalk virus-like particles; R-A, ricin A chain.

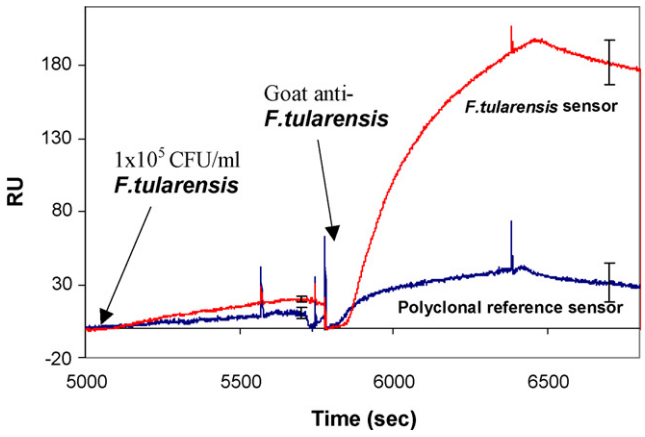

Fig. 5. Detection of 1×10^5 CFU/ml *F. tularensis*. The average response of three sensor with goat anti-*F. tularensis* surfaces is compared to the average of two channels with normal goat serum surfaces. Error bars indicating the deviation between channels are shown every 900 s. Arrows indicate when sample was injected and when the amplifier was injected into the SPIRIT system.

bank was exposed to each analyte and then to amplifying antibody specific to that analyte, the sensor specific for that analyte exhibited a large response relative to the other sensors.

In Fig. 5, the response of the *F. tularensis* sensor to 10^5 CFU/ml *F. tularensis* is shown in isolation. At the end of the initial direct detection step ($t = 5700$ s) the *F. tularensis*-specific sensor gave a signal of approximately 20 RU, compared with 10 RU from the PC reference sensor. At the end of the amplification step ($t = 6400$ s), the binding of secondary antibodies greatly increased the signal, to 195 RU on the *F. tularensis*-specific sensor, compared to 43 RU from the PC reference sensor. The responses of the other sensors were also specific for each target analyte, and are tabulated in Table 1. For each target, the response of a three-channel sensor decorated with the specific antibody is listed next to the response of a three-channel reference sensor. The reference displayed was either the response from the normal goat serum sensor surface (when the detection surface is a polyclonal antibody) or from the monoclonal anti-anthrax antibody surface (when the detection surface was a monoclonal antibody). For the small molecule detection (DNP), the normal goat serum reference was used.

Detection events were demonstrated for each of the six analytes. Of the 24 sensor channels tested in the multi-analyte experiment, only the middle channel of sensor #7 (normal goat reference sensor) did not give a functional SPR signal, most likely due to a scratch on the gold surface from the cleaning process necessary for reuse.

4. Conclusions

We describe here a portable and versatile SPR-based biosensor system potentially capable of detecting as many as two-dozen analytes in a semi-automated fashion. Analyte detection varied from small organics to viruses and microbes, depending on their surface binding characteristics. *F. tularensis* exhibited appreciable non-specific binding, while SEB displayed virtually none. Analyte detection also differed with respect to the efficacy and necessity of secondary amplification. For example, Norwalk virus VLPs displayed a clear direct signal that could be amplified upon exposure to secondary antibodies, while *Bg* spores provided an appreciable signal only when amplified with a second antibody. Detection time was dependent on analyte accumulation on sensor surfaces, occurring in seconds to several minutes. While experiments presented here were qualitative, we have previously demonstrated analyte quantification using a similar system (Naimushin et al., 2002).

In addition to detection of microorganisms and their products for defense purposes, this technology has potential for use in other arenas, such as homeland security, environmental monitoring, and food and drug research and monitoring. Although portable, the system is also useful for the general laboratory study of many different inter-molecular binding events, including DNA/transcription factor interactions and mRNA detections using DNA probes. Further multi-analyte assay performance optimization will require individual enhancement of direct detection and/or amplification efficiency, and control of non-specific binding for each analyte. Further enhancement of the system user interface, in-line sample concentration, automation of sample collection and amplification, and flow cell volume reduction may also contribute to refinement of the instrument.

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