

Photonic wire biosensor microarray chip and instrumentation with application to serotyping of *Escherichia coli* isolates

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Abstract: A complete photonic wire molecular biosensor microarray chip architecture and supporting instrumentation is described. Chip layouts with 16 and 128 independent sensors have been fabricated and tested, where each sensor can provide an independent molecular binding curve. Each sensor is 50 μm in diameter, and consists of a millimeter long silicon photonic wire waveguide folded into a spiral ring resonator. An array of 128 sensors occupies a $2 \times 2 \text{ mm}^2$ area on a $6 \times 9 \text{ mm}^2$ chip. Microfluidic sample delivery channels are fabricated monolithically on the chip. The size and layout of the sensor array is fully compatible with commercial spotting tools designed to independently functionalize fluorescence based biochips. The sensor chips are interrogated using an instrument that delivers sample fluid to the chip and is capable of acquiring up to 128 optical sensor outputs simultaneously and in real time. Coupling light from the sensor chip is accomplished through arrays of sub-wavelength surface grating couplers, and the signals are collected by a fixed two-dimensional detector array. The chip and instrument are designed so that connection of the fluid delivery system and optical alignment are automated, and can be completed in a few seconds with no active user input. This microarray system is used to demonstrate a multiplexed assay for serotyping *E. coli* bacteria using serospecific polyclonal antibody probe molecules.

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References and links

1. A. Densmore, D.-X. Xu, P. Waldron, S. Janz, P. Cheben, J. Lapointe, A. Del  ge, B. Lamontagne, J. H. Schmid, and E. Post, "A silicon-on-insulator photonic wire based evanescent field sensor," *IEEE Photon. Technol. Lett.* **18**(23), 2520–2522 (2006).
2. S. Janz, A. Densmore, D.-X. Xu, P. Waldron, J. Lapointe, J. H. Schmid, T. Mischki, G. Lopinski, A. Delage, R. McKinnon, P. Cheben, and B. Lamontagne, "Silicon photonic wire waveguide sensors," in *Advanced Photonic Structures for Photonic and Chemical Detection*, X. Fan, ed. (Springer, 2009), pp. 229–264.
3. A. Densmore, D.-X. Xu, S. Janz, P. Waldron, T. Mischki, G. Lopinski, A. Del  ge, J. Lapointe, P. Cheben, B. Lamontagne, and J. H. Schmid, "Spiral-path high-sensitivity silicon photonic wire molecular sensor with temperature-independent response," *Opt. Lett.* **33**(6), 596–598 (2008).
4. D.-X. Xu, A. Densmore, A. Del  ge, P. Waldron, R. McKinnon, S. Janz, J. Lapointe, G. Lopinski, T. Mischki, E. Post, P. Cheben, and J. H. Schmid, "Folded cavity SOI microring sensors for high sensitivity and real time measurement of biomolecular binding," *Opt. Express* **16**(19), 15137–15148 (2008).
5. R. Halir, P. Cheben, J. H. Schmid, R. Ma, D. Bedard, S. Janz, D.-X. Xu, A. Densmore, J. Lapointe, and I. Molina-Fern  ndez, "Continuously apodized fiber-to-chip surface grating coupler with refractive index engineered subwavelength structure," *Opt. Lett.* **35**(19), 3243–3245 (2010).

6. D.-X. Xu, M. Vachon, A. Densmore, R. Ma, A. Del  ge, S. Janz, J. Lapointe, Y. Li, G. Lopinski, D. Zhang, Q. Y. Liu, P. Cheben, and J. H. Schmid, "Label-free biosensor array based on silicon-on-insulator ring resonators addressed using a WDM approach," *Opt. Lett.* **35**(16), 2771–2773 (2010).
7. R. Halir, P. Cheben, S. Janz, D. X. Xu, I. Molina-Fern  ndez, and J. G. Wang  mert-P  rez, "Waveguide grating coupler with subwavelength microstructures," *Opt. Lett.* **34**(9), 1408–1410 (2009).
8. R. Halir, L. Zavargo-Peche, D.-X. Xu, P. Cheben, R. Ma, J. H. Schmid, S. Janz, A. Densmore, A. Ortega-Mo  ux,   . Molina-Fern  ndez, M. Fournier, and J.-M. F  d  li, "Single etch grating couplers for mass fabrication with DUV lithography," *Opt. Quantum Electron.* **44**(12-13), 521–526 (2012), doi:10.1007/s11082-012-9563-2.
9. J. Homola, ed., *Surface Plasmon Resonance Based Sensors* (Springer-Verlag, 2006).
10. K. Tiefenthaler and W. Lukosz, "Sensitivity of grating couplers as integrated-optical chemical sensors," *J. Opt. Soc. Am. B* **6**(2), 209–220 (1989).
11. W. Lukosz, "Principles and sensitivities of integrated optical and surface plasmon sensors for direct affinity sensing and immunosensing," *Biosens. Bioelectron.* **6**(3), 215–225 (1991).
12. J. H. Schmid, W. Sinclair, J. Garc  a, S. Janz, J. Lapointe, D. Poitras, Y. Li, T. Mischki, G. Lopinski, P. Cheben, A. Del  ge, A. Densmore, P. Waldron, and D.-X. Xu, "Silicon-on-insulator guided mode resonant grating for evanescent field molecular sensing," *Opt. Express* **17**(20), 18371–18380 (2009).
13. B. J. Luff, J. S. Wilkinson, J. Piehler, U. Hollenbach, J. Ingenhoff, and N. Fabricius, "Integrated optical Mach-Zehnder biosensor," *J. Lightwave Technol.* **16**(4), 583–592 (1998).
14. H. Sohlstr  m and M.   berg, "Refractive index measurement using integrated ring resonators," *Proceedings of the Eighth European Conference on Integrated Optics*, 322–325 (1997).
15. C.-Y. Chao and L. J. Guo, "Biochemical sensors based on polymer microrings with sharp asymmetrical resonance," *Appl. Phys. Lett.* **83**(8), 1527–1529 (2003).
16. K. De Vos, I. Bartolozzi, E. Schacht, P. Bienstman, and R. Baets, "Silicon-on-Insulator microring resonator for sensitive and label-free biosensing," *Opt. Express* **15**(12), 7610–7615 (2007).
17. A. Yariv, "Universal relations for coupling of optical power between microresonators and dielectric waveguides," *Electron. Lett.* **36**(4), 321–323 (2000).
18. A. Del  ge, D.-X. Xu, R. W. McKinnon, E. Post, P. Waldron, J. Lapointe, C. Storey, A. Densmore, S. Janz, B. Lamontagne, P. Cheben, and J. H. Schmid, "Wavelength-Dependent Model of a Ring Resonator Sensor Excited by a Directional Coupler," *J. Lightwave Technol.* **27**(9), 1172–1180 (2009).
19. D.-X. Xu, A. Del  ge, R. McKinnon, M. Vachon, R. Ma, J. Lapointe, A. Densmore, P. Cheben, S. Janz, and J. H. Schmid, "Archimedean spiral cavity ring resonators in silicon as ultra-compact optical comb filters," *Opt. Express* **18**(3), 1937–1945 (2010).
20. P. Cheben, S. Janz, B. Lamontagne, and D.-X. Xu, "A method of optical off-chip interconnects in multichannel planar waveguide devices," *US Patent 7,376,308 B2* (2008).
21. D.-X. Xu, M. Vachon, A. Densmore, R. Ma, S. Janz, A. Del  ge, J. Lapointe, P. Cheben, J. H. Schmid, E. Post, S. Messaoud  ne, and J.-M. F  d  li, "Real-time cancellation of temperature induced resonance shifts in SOI wire waveguide ring resonator label-free biosensor arrays," *Opt. Express* **18**(22), 22867–22879 (2010).
22. M. S. Luchansky, A. L. Washburn, T. A. Martin, M. Iqbal, L. C. Gunn, and R. C. Bailey, "Characterization of the evanescent field profile and bound mass sensitivity of a label-free silicon photonic microring resonator biosensing platform," *Biosens. Bioelectron.* **26**(4), 1283–1291 (2010).
23. K. De Vos, J. Girones, T. Caes, Y. De Koninck, S. Popelka, E. Schacht, R. Baets, and P. Bienstman, "Multiplexed antibody detection with an array of silicon-on-insulator microring resonators," *IEEE Photon J.* **1**(4), 224–235 (2009).
24. S. R. Wasserman, Y.-T. Tao, and G. M. Whitesides, "Structure and reactivity of alkylsiloxane monolayers formed by reaction of alkyltrichlorosilanes on silicon substrates," *Langmuir* **5**(4), 1074–1087 (1989).
25. A. D. Taylor, Q. Yua, S. Chena, J. Homola, and S. Jiang, "Comparison of E. coli O157:H7 preparation methods used for detection with surface plasmon resonance sensor," *Sens. Actuators B Chem.* **107**(1), 202–208 (2005).
26. M. Zourob, J. J. Hawkes, W. T. Coakley, B. J. Treves Brown, P. R. Fielden, M. B. McDonnell, and N. J. Goddard, "Optical Leaky Waveguide Sensor for Detection of Bacteria with Ultrasound Attractor Force," *Anal. Chem.* **77**(19), 6163–6168 (2005).
27. M. Zourob, S. Mohr, B. J. Brown, P. R. Fielden, M. B. McDonnell, and N. J. Goddard, "An integrated optical leaky waveguide sensor with electrically induced concentration system for the detection of bacteria," *Lab Chip* **5**(12), 1360–1365 (2005).

1. Introduction

Silicon photonic wire waveguides are one of the most sensitive optical transducers for label-free molecular sensing. The extremely small size and high refractive index of these waveguides result in a strong interaction of the guided light with molecules bound to the waveguide surface [1,2]. Molecular affinity binding sensors can provide critical information on molecular interactions in genomics, proteomics, and drug screening. They can be used as transduction element for chemical and biological sensors to monitor contaminants in water or to detect and identify pathogenic micro-organisms. The silicon photonic wire evanescent field

(PWEF) sensor platform offers the advantages of extremely small sensor size, high levels of function integration, and low cost manufacturing that comes with the use of established semiconductor fabrication processes. Since many applications require simultaneous monitoring of many different binding reactions, the possibility of integrating tens or even hundreds of independent molecular sensors on a single disposable sensor chip is very compelling. However, several design and manufacturing issues must be addressed to arrive at a practical multiplexed molecular sensor chip technology. To be deployable in field testing and in laboratories devoted to biological or medical research, the sensor chip and associated instrumentation must be self-contained and amenable to operation by users with no specialized expertise in photonics. The system should require a set-up time of only a few minutes per test. The technology should be fully compatible with existing infrastructure in molecular analysis and research. Finally, the manufactured sensor array chip cost should be low enough that the chips can be considered disposable.

This paper presents an original design for a multiplexed photonic wire sensor chip and a reader instrument that fulfills all of these requirements. The silicon sensor microarray chips incorporate several silicon photonic components that have been developed individually [1–8], but are integrated here for the first time on one chip to enable rapid multiplexed binding measurements with rapid turn-around time. The chips can be quickly prepared for multiplexed molecular binding measurements using commercially available automated spotting tools. The reader instrument and chip design allow external microfluidics connections and optical input/output coupling to the sensor chip to be completed automatically in less than one minute. Taken in combination with the reader instrument, the system comprises a practical sensor microarray platform that allows up to 128 independent binding reactions to be monitored simultaneously. The utility of the system is demonstrated by performing a serotyping assay that can distinguish between several closely related *E. coli* bacteria serotypes.

2. Sensor microarray design and fabrication

The binding of molecules to the waveguide surface produces a change in the effective index N_{eff} of the guided optical mode propagating through a waveguide [2]. This effective index change δN_{eff} is a result of the coupling of the mode evanescent field with the molecules near the waveguide surface, which causes N_{eff} to vary linearly with the density of molecules at the surface. For a sensor waveguide of length L , the total induced phase shift in response to the effective index perturbation δN_{eff} is

$$\delta\phi = \delta N_{\text{eff}} L. \quad (1)$$

Perturbation of a guided mode is also the basis of surface plasmon resonance (SPR) sensing [9], in which the phase velocity of light (i.e. the plasmon mode) propagating along a metal surface is altered by molecules binding to the surface. Both SPR and evanescent field waveguide sensors have been established and used for more than two decades [10, 11]. However SPR has been the most widely used molecular binding sensor, due to its relative ease of implementation. In its simplest form, an SPR instrument monitors the change in reflectivity of a thin gold metal film deposited on a glass prism [9]. In the case of waveguide sensors, additional integrated on-chip components are required to measure the molecule induced phase shift. For example, sensor response can be monitored using grating couplers [10,12] a Mach-Zehnder interferometer with the sensor waveguide placed in one arm [1,3,13], or by measuring the resonance wavelength shift of a ring resonator sensor [4,6,14–16].

However, it is now established that the molecular response of silicon photonic wire waveguides with silicon thicknesses in the range from 200 nm to 260 nm can be much higher than that of SPR, due to the concentration of the evanescent field at the sensor surface, particularly for the TM polarized waveguide mode [1,2], and also because of the long optical propagation length possible in a sensor waveguide [1,2,12]. In SPR the achievable sensitivity

is limited by the intrinsic attenuation length of a plasmon at a metal surface, which is few tens of micrometers or less. On the other hand Eq. (1) predicts that the phase response of a waveguide sensor can be increased simply by making the waveguide longer. In a silicon waveguide the propagation length can be several centimeters, being limited only by scattering loss caused by fabrication imperfections. Advances in silicon fabrication, optical coupling methods, and photonic integration now make it possible to produce cost effective waveguide sensor chips with dense arrays of sensors for multiplexed sensing applications.

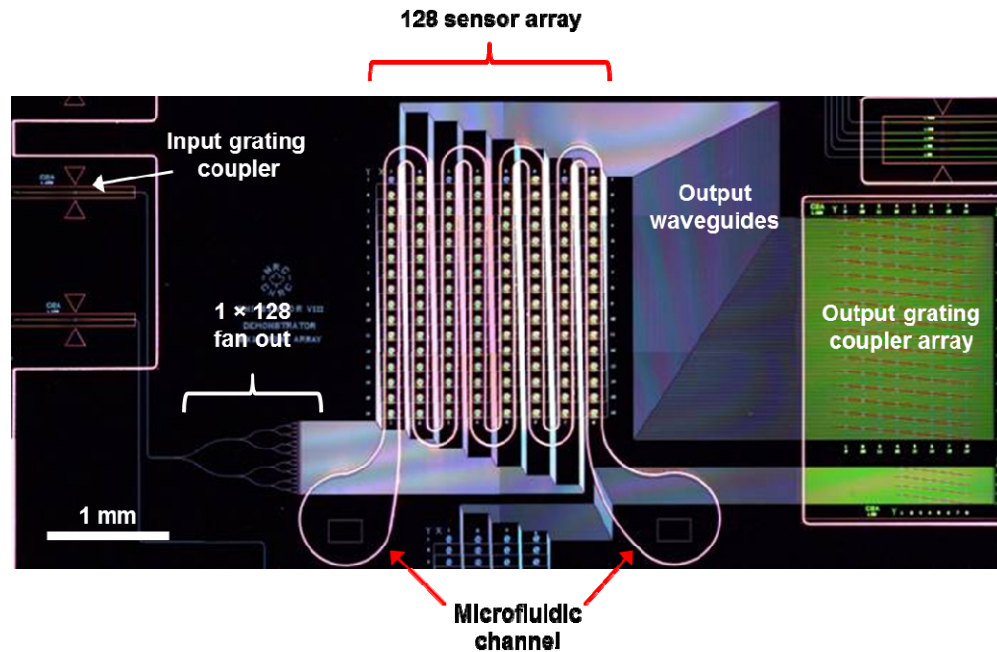


Fig. 1. A Nomarski microscope image of a 128 sensor PWEF microarray chip, showing layout of 128 sensor array, microfluidic channels and input and output couplers.

The sensors are fabricated on a $6 \times 9 \text{ mm}^2$ silicon-on-insulator (SOI) chip. Figure 1 shows a 128 sensor chip, and 16 sensor chips were also fabricated that are identical in chip size and layout apart from the number of sensor and input/output waveguides. The 128 sensor array occupies a $2 \times 2 \text{ mm}^2$ area in the middle of the chip, and the 16 sensor array occupies about 1 mm^2 . All waveguides and the sensor elements are formed using 450 nm wide silicon photonic wire waveguides etched in a 260 nm thick silicon layer. Each independent sensor element is a ring resonator with a $L = 940 \mu\text{m}$ cavity length formed in a closed spiral configuration shown in Fig. 2. Light is coupled from the through waveguide to the ring across a $4 \mu\text{m}$ long directional coupler with a 400 nm gap between the through and ring waveguides. The transmission spectrum of the through waveguide exhibits a comb of minima at wavelengths λ satisfying the ring resonance condition $m\lambda = L/N_{\text{eff}}$, where m is an integer and N_{eff} is the waveguide effective index [17,18]. The molecules binding to the surface cause N_{eff} to change, and as a result the surface density of the captured molecules is directly proportional to a shift in ring resonance wavelength [4,15,16,18]. The width and depth of the minima are determined by the ring waveguide loss and the coupling coefficient to the through waveguide [17,18]. In our sensors, the ring free spectral range (i.e. the comb spacing) is 0.6 nm with a resonance width of approximately 50 pm (FWHM). The observed contrast ratios of resonance minima are between -10 dB and -15 dB.

The spiral resonator geometry of Fig. 2(a) is adopted to increase the propagation length of light within the resonator [3,4]. Longer resonator waveguides can increase the quality factor

Q and produce narrower resonance features [18,19], leading to better resolution in measuring wavelength shift. At the same time the densely wound spiral waveguide configuration results in a greater fraction of the exposed chip surface area contributing to the sensor response, while enabling the sensor element to be contained within a circular area less than 100 μm in diameter. Spiral sensors are therefore compatible with automated spotting tools used to deposit appropriate probe molecules to biochip arrays. In the experiments described later in this work the sensor arrays are functionalized with probe antibodies using the GeSiM Nano-Plotter 2.1 arraying tool, which can deposit picoliter drops of liquid at precise locations on a chip.

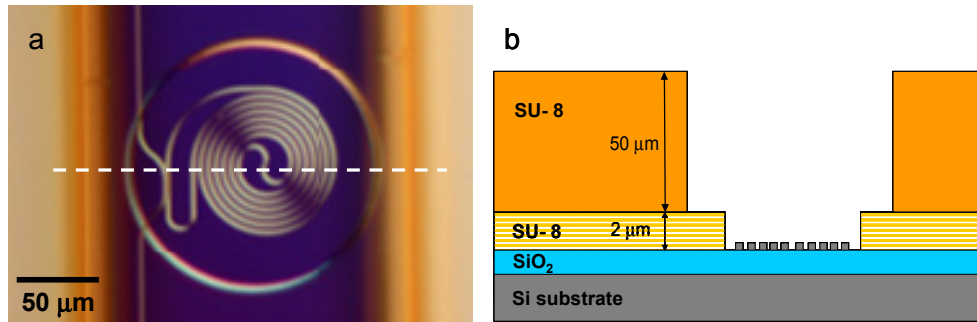


Fig. 2. (a) An optical microscope image of a single sensor element showing the circular window in the 2 μm -thick SU-8 protective layer, and the SU-8 microfluidic channel. (b) A schematic cross-section of the sensor and microfluidic channel corresponding to the plane cut across the sensor indicated by the dashed line in (a).

Light is coupled to and from the chip waveguides using the sub-wavelength patterned grating couplers [5,7,8] shown in Fig. 3. Unlike conventional grooved gratings, these couplers are formed by rows of holes that are etched completely through the silicon layer. Along the row (i.e. perpendicular to the direction of light propagation) the nominal hole width and pitch are 150 nm and 300 nm respectively. The hole width parallel to the direction of light propagation is 250 nm. Since the hole pitch along a row is less than the wavelength of light in the silicon, there is no diffraction in the lateral direction and the row behaves like a homogeneous grating line. The grating line contrast is controlled by the volume ratio of silicon to hole along a row, rather than groove depth as in a conventional grating structure. These rows are arranged with an 840 nm periodicity along the propagation direction to form a $15 \times 15 \mu\text{m}^2$ diffraction grating that couples p-polarized light incident at 12° into the TM mode of the Si waveguide. The grating directs light to the 450 nm wide single mode photonic wire waveguides through an adiabatic taper section. Numerical simulations indicate that the taper length can be as short as 120 μm long with negligible additional loss. Every grating coupler is bracketed by identical tapers on both sides as shown in Fig. 3(c). The second taper leads back to a photonic wire waveguide that captures and carries residual and backscattered light from the grating to a beam dump at the edge of the chip. After the input light has been distributed to all the sensor elements, an array of identical gratings arranged as in Fig. 3(c) is used to couple light from each sensor into an array of free space beams propagating upwards from the sensor chip [20]. The measured coupling loss of the grating couplers is -4.5 ± 0.5 dB for TM polarized light emitted from a SMF 128 optical fiber, with a 3dB coupling bandwidth of approximately 60 nm. The advantage of the sub-wavelength patterning approach for grating couplers is that all waveguides, sensor elements and the grating couplers are fabricated in the same lithography and etch step, in which the etch goes through the silicon layer and terminates at the buried oxide layer. Thus manufacturing is simpler than for gratings requiring a second calibrated etch part way through the silicon waveguide layer.

The light in the single input waveguide is broadcast to the through waveguide of every ring sensor element in the array by a fan-out section (see Fig. 1) containing a series of

cascaded 1×2 Y-junction splitters. Four stages of 1×2 splitters can distribute the light to a 16 sensor array, and seven splitter stages will address 128 sensors. Light is coupled to each ring sensor element, as depicted in Fig. 2(a), by a directional coupler formed by the through

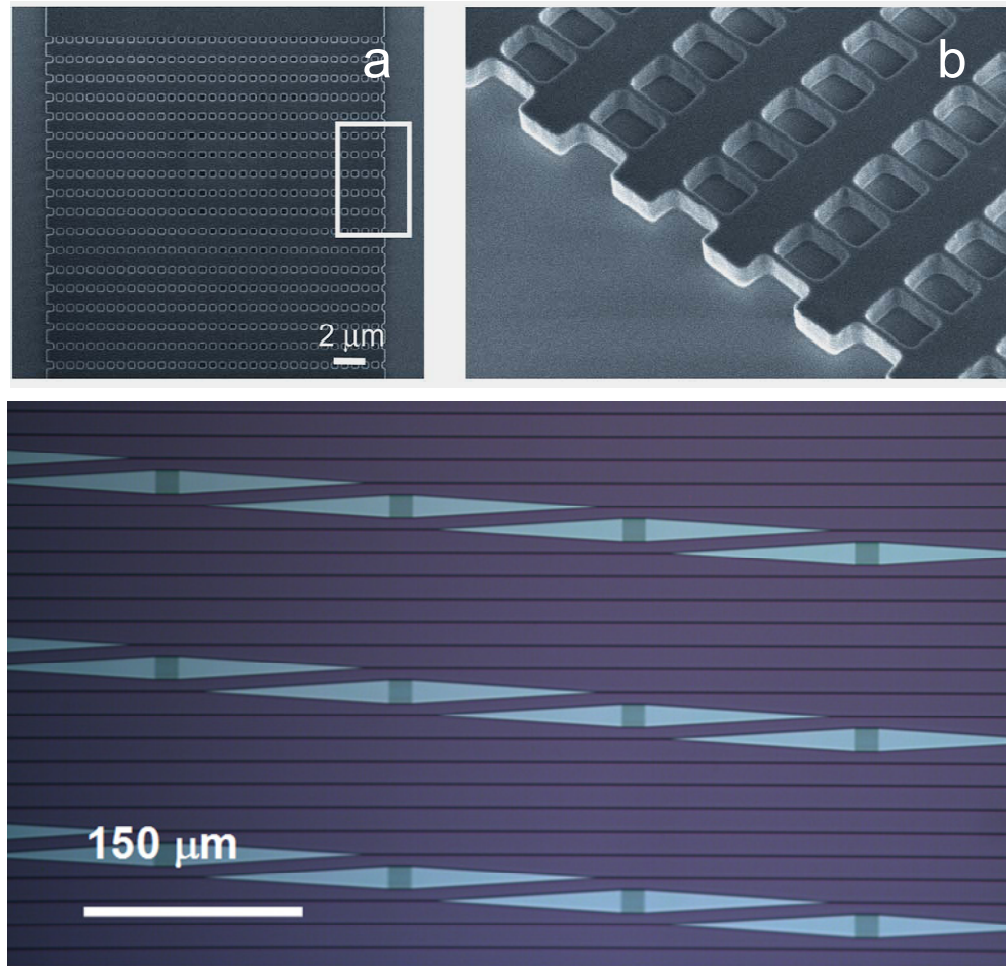


Fig. 3. (a) Scanning electron microscope image of a subwavelength patterned grating coupler and (b) a magnified view of the area indicated by the rectangle. (c) An optical microscope view of the output coupler array on a 128 sensor chip showing the $15 \times 15 \mu\text{m}^2$ coupling gratings (small squares) bracketed by $120 \mu\text{m}$ long adiabatic tapers that connect the $0.45 \mu\text{m}$ wide photonic wire waveguides to the $15 \mu\text{m}$ square grating couplers. Each waveguide and grating coupler carries light from one element of the sensor array.

waveguide and ring waveguide. The through waveguide for each sensor ring continues on past the sensor element and eventually terminates at one of the surface grating couplers shown in Fig. 3(c), that redirect light from the waveguide to an off-chip beam. The fiber-to-fiber insertion loss of a sensor chip (from input to output of one sensor output) is -28 dB for a 16 sensor array, and -40 dB for a 128 sensor array. Removing -12 dB and -21 dB to account for 16 and 128 sensor fan-out respectively, gives -16 dB and -19 dB losses due to coupling, waveguide loss, and other on chip losses. The extra -3 dB insertion loss on the 128 sensor chip is attributed to the longer overall waveguide propagation length and the three extra stages of 1×2 splitters. As will be discussed in section 3, the final output light intensity is more than sufficient due to the high sensitivity of InGaAs photodetector array. Note that the

total fiber-to-detector insertion loss is different in the chip reader described below, since light is coupled to the chip through free space beams rather than cleaved optical fibers.

The sensor chips have been fabricated successfully using both electron beam lithography and deep-UV lithography. The latter method was used to produce sensor chips on eight inch SOI wafers, to provide large numbers of chips needed for biological assay development. In either case, only one lithography step was used to define the waveguide structures and sub-wavelength grating couplers [8] in the resist layer. Subsequently all silicon features were formed in a single etch step using reactive ion etching. After the waveguides were formed, a 2 μm -thick SU-8 polymer layer was put on the chip to isolate the waveguides from the sample liquids. Circular windows shown in Fig. 2(a) were left open in this SU-8 layer to expose only the sensor elements to the overlying sample liquid. On every chip one sensor element is used as a temperature reference [21], and for this sensor the SU-8 layer was left in place over the resonator waveguide. The area over the coupler gratings was also left free of SU-8 to avoid degrading of the grating performance by incomplete hole filling. A second 50 μm thick SU-8 layer is then patterned onto the chip to define the microfluidic channels. These 130 μm wide serpentine channels weave over all the sensors on the chip, and are terminated at both ends by an 800 μm wide reservoir that aligns with the inlet and outlet apertures on the reader instrument fluidic manifold. As before, windows in the second SU-8 layer are also left open over the grating coupler areas. The channel and widened reservoirs are visible in Fig. 1, while Fig. 2(a) shows a channel at higher magnification. The top of the fluid channels are left open, since they will be sealed by the fluidic manifold when the chip is placed into the reader instrument. For ease of handling, chips are fixed to a small ceramic submount for use.

3. Reader instrument design

The chip reader instrument shown in Fig. 4 is designed to allow automated optical alignment of the silicon sensor chip with the input beam and output acquisition optics. During measurement, the chip is connected with the sample fluid delivery system so that sample liquids can be delivered to the chip and flowed over the sensor elements, while the optical signals from each sensor in the array are continuously monitored.

Laser light is delivered to the chip as a free space p-polarized beam at a 12° incident angle onto the input grating coupler described in the previous section. The light source is a fiber coupled swept wavelength laser with 10 mW power. To enable interrogation of the sensor resonance wavelength, the laser wavelength is continuously scanned over a 5 nm range near $\lambda = 1560$ nm at sweep speed of 1.25 nm per second. The light emitted from the laser output fiber is first collimated and then focused onto the input grating coupler using a pair of achromatic lenses with focal lengths $f = 75$ and $f = 100$ mm. The spot size on the chip surface can be controlled by adjusting the lens position, and is typically set to several hundred micrometers in diameter. This is much larger than the $15 \times 15 \mu\text{m}^2$ grating coupler size, so system performance is insensitive to chip misalignments up to about $\pm 50 \mu\text{m}$. The InGaAs detector array used to acquire the intensity data is sensitive enough that a full 12 bit (35 dB)

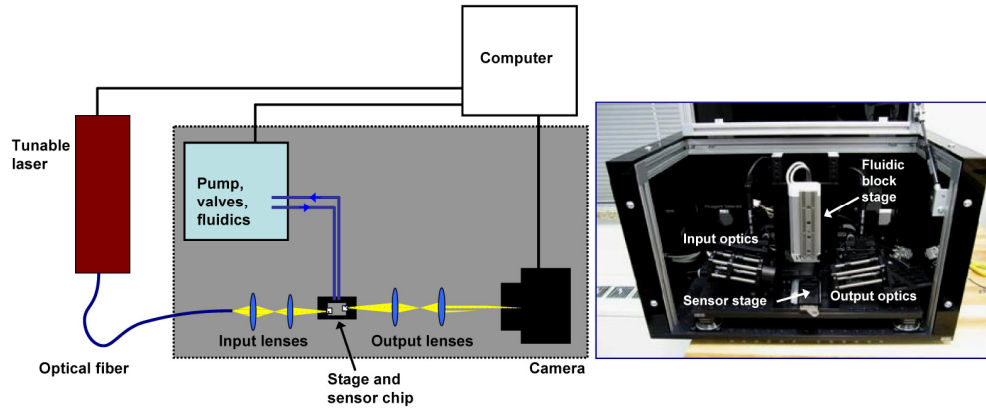


Fig. 4. A schematic diagram of the PWEF reader instrument, and a photograph of the instrument with the cover removed. The instrument enclosure occupies a bench top area of approximately 60 cm by 45 cm, and contains fluid pumps, valves, optics and automated translation stages that deliver light and sample liquids to the PWEF chip.

dynamic range can still be achieved, even with the large mismatch in coupler size and incident beam spot. Another pair of achromatic lenses is used to capture the array of beams emitted from the sensor output coupler array and project them onto the InGaAs detector array to form an image of the output coupler array. For ease of on screen visualization, the imaging system can be configured with a magnification of $3.3 \times$ for the 128 sensor chips (using $f = 30$ mm and $f = 100$ mm lenses), and $10 \times$ for the 16 sensor arrays (using $f = 5$ mm and $f = 30$ mm lenses). The detector array is a 320×256 pixel commercially available InGaAs CCD array, with a $25 \mu\text{m}$ square pixel size.

When the sensor chip is placed onto the instrument stage it is automatically positioned and aligned by a computer controlled translation stages that pushes the chip against a fixed rectangular corner edge stop. In this way the input and output couplers are aligned with the coupling optics, and the reservoirs are aligned with fluid delivery channels in the fluid delivery manifold. The accuracy of chip alignment is therefore ultimately set by the position accuracy of the chip edge relative to the on-chip grating couplers and fluid reservoirs. Using a commercial dicing saw, an edge position accuracy of better than $\pm 50 \mu\text{m}$ can be achieved on

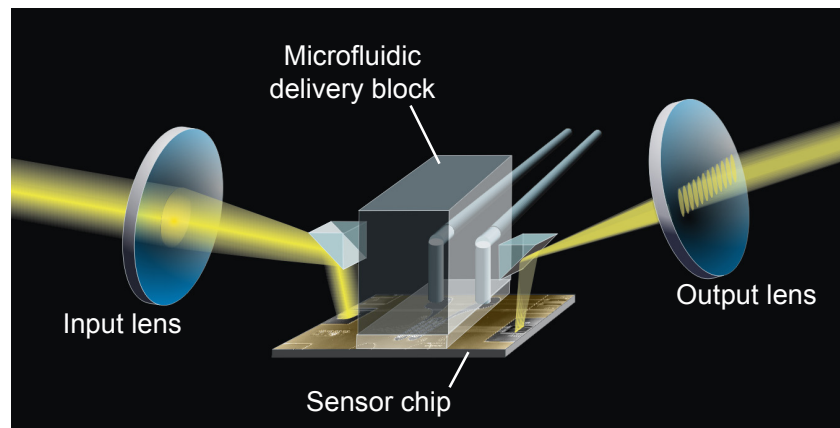


Fig. 5. A schematic diagram showing the sensor chip, microfluidic block, and input and output lenses within the reader instrument. Dimensions shown are not to scale. The output beams are focused onto an InGaAs detector array to the right (not shown).

a routine basis. This is more than sufficient given the 800 μm diameter fluid reservoirs and approximately 200 μm diameter input beam spot size.

Figure 5 is a schematic view of the sensor chip in final position, showing the arrangement of mirrors, chip and microfluidic block. The plastic fluid delivery manifold block is pressed down on the chip, again by automated translation stages. Independent microfluidic delivery channels are machined into the block so that the channels open at the bottom of the manifold block and align with the input/output fluid reservoirs on the sensor chip. A deformable gasket layer covers the bottom of the block. This gasket cushions the chip-manifold contact area and seals the connection between block channel outlets and chip reservoirs. The other ends of the block channels are connected with flexible microfluidic tubing leading from the sample fluid vials, pumps and valves used to control and direct sample flow. Note in Fig. 5 that the block is sized and positioned so that it does not cover the input and output optical grating couplers on the chip, so that clear optical access is always maintained. Once the fluidic block is in place over the chip, fluid is delivered to the chip through the block and passes over the sensors. The sample fluids are driven by a peristaltic pump, and an electronically controlled valve can select fluids from any one of nine different vials. Thus molecular assay tests can include a sequence of up to nine different samples or rinsing fluids delivered sequentially to the chip. Since the on-chip fluid channels are only $130\ \mu\text{m} \times 50\ \mu\text{m}$ in cross section, a typical molecular assay test consumes only about 2 mL of fluid per hour.

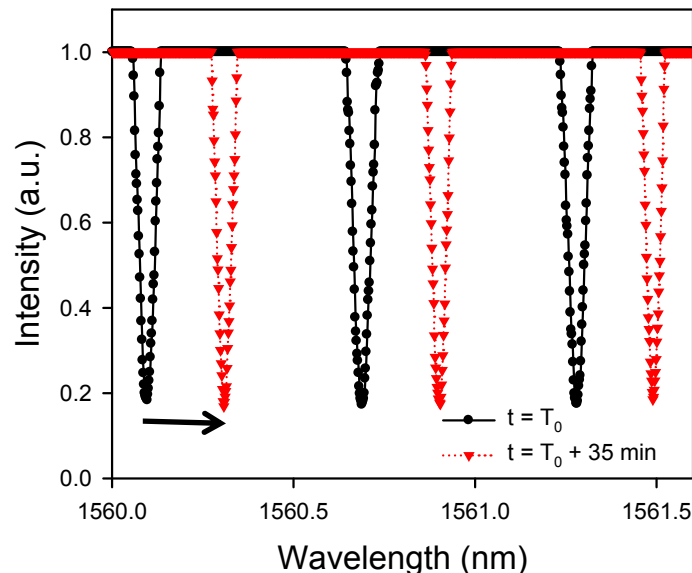


Fig. 6. Two ring resonator sensor spectra as captured by the reader instrument during binding of bacteria antigen isolates to the sensor surface. Each data point corresponds to the intensity captured by one camera exposure. The two spectra were taken 35 minutes apart during the capture of *E. coli* antigen to the sensor surface as described in section 4. Only the resonance minima are visible. To improve accuracy in measuring resonance wavelengths, the coupled optical power is adjusted to improve signal resolution near the resonance minima, while intensity between resonances is allowed to saturate the camera pixels.

All functions of the chip reader instrument are controlled by computer. This includes the swept wavelength laser, microfluidic valves and pumps, and detector array data read-out. The laser tuning speed and detector array frame rate can both be adjusted. In our experiments, the InGaAs detector array captures the output power of each sensor on the chip in real time at frame rates of up to 705 frames per second. Once the sensor chip is aligned within the instrument, image analysis software is used to identify the position of each output beam spot

on the array. During a measurement the output power from each sensor is monitored by following the intensity variation at the pixel in the center of each image spot. The spectra of all the resonators are acquired in parallel by synchronizing the camera frame capture with the wavelength sweep of the laser source. After each wavelength sweep is completed a fitting algorithm locates the ring resonator peaks in each sensor spectrum. The wavelength shifts over time correspond directly to molecular coverage of the sensor surfaces, and rate of molecular capture is inferred from the shift in wavelength of the ring resonances from one sweep to the next. For a typical set of measurement parameters, a 4 nm wide spectrum of every sensor can be acquired in about 4 seconds, with a wavelength resolution of 2 pm. Therefore, with this set of parameters, simultaneous molecular binding curves have a time resolution of four seconds. Figure 6 shows the unprocessed data for a single ring sensor spectrum captured at two different times using this system. In special cases where finer time resolution is required, the system can be reconfigured to monitor a smaller number of sensors or use a coarser wavelength resolution so that spectra may be acquired more quickly. Except for samples with very high target molecule concentrations or extremely fast binding rates, in most applications a time resolution of four seconds is more than adequate.

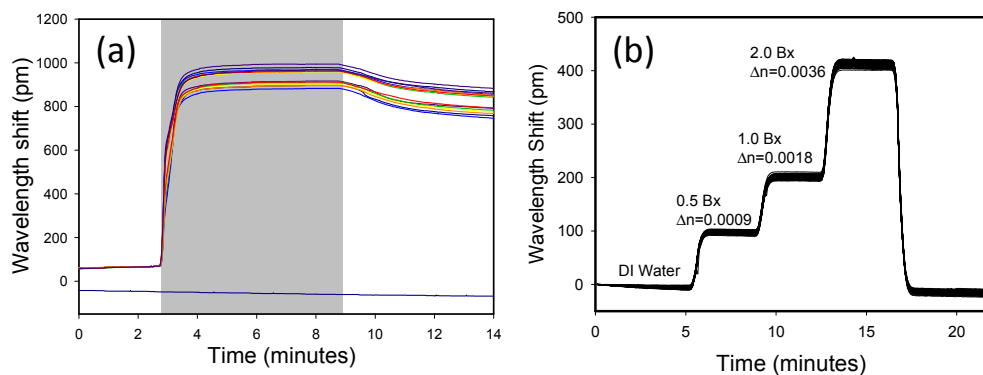


Fig. 7. (a) Streptavidin protein capture binding curves for a 16 sensor PWEF microarray chip as measured in the reader instrument described here. The shaded area indicates the time interval during which the sensors were exposed to streptavidin-containing PBS solution. Before and after streptavidin exposure the liquid was pure PBS solution. The trace at the bottom is the output of the reference sensor that records temperature drifts. (b) A superposition of 128 sensor response curves as the refractive index of the fluid flowing through a 128 sensor array is increased in steps of $\Delta n = 0.0009$ by increasing sucrose concentration in steps of 0.5 Bx.

Molecular binding data obtained in this way are consistently uniform across all sensors on one chip. Figure 7(a) shows the output of 16 sensors on one chip as streptavidin protein molecules (approx. 60 kDa mass) form a monolayer on each of the sensors. In this example streptavidin protein at 100 μM concentration in phosphate buffered saline (PBS) solution was flowed through the on chip microfluidic channels. Each sensor in the array was previously functionalized by covering the surface with a silane layer as described in Section 4, to which the streptavidin protein readily binds. The trace at the bottom is the output of the on-chip reference sensor that records temperature drifts [21]. The molecular binding curves in Fig. 7(a) all saturate at a resonator wavelength shift of approximately 900 pm, corresponding to the formation of a monolayer of streptavidin protein. Uniformity of response for 128 sensor chips has similarly been verified by measuring simultaneous response of all 128 sensors as the refractive index of water flowing over the sensors was increased by varying sucrose concentrations. Figure 7(b) shows 128 overlapped traces. Here again the response (i.e. the induced wavelength shift) of all sensors was identical to within a few percent.

The response of the spiral sensor in terms of induced resonance wavelength shift and bound surface molecular mass density is $2.5 \text{ pg mm}^{-2} \text{ pm}^{-1}$ [4]. For the multiplexed reader instrument reported here, the measurement system limited uncertainty in resonance wavelength is approximately $\pm 0.3 \text{ pm}$, as determined from point to point resonance wavelength scatter in sensor traces acquired by the instrument under static conditions. This corresponds to a minimum level of detection of approximately 1 pg mm^{-2} , in the absence of other sources of signal fluctuation. This is comparable to the minimum levels of detection (MLD) of 1.5 pg mm^{-2} [22] and 3.4 pg mm^{-2} [23] reported for conventional circular ring resonators. This is sufficient to detect the presence of less 0.1% of monolayer of protein (e.g. streptavidin, or IgG) molecules on a surface.

4. *Escherichia coli* serotyping assay

As a practical application of the multiplexed PWEF sensor array, we demonstrate the utility of the system for simultaneous serotyping of *Escherichia coli* bacteria isolates. *E. coli* bacteria occur naturally in many warm blooded organisms including humans. While most strains of *E. coli* are harmless and are common components of the normal gastrointestinal microbiota, several *E. coli* strains are pathogenic and can cause serious illness. Infections can be fatal for children and individuals already in poor health prior to infection. Food contamination and subsequent cases of infection by these pathogenic *E. coli* often trigger wide spread food recalls which have considerable economic consequences. The challenge faced by food safety inspectors and regulators is to detect and identify these pathogenic strains of *E. coli* in food quickly in order to prevent widespread distribution of contaminated products, but avoid unnecessary recalls and plant closures because of a false positive test result. *E. coli* testing is particularly problematic since there are almost two hundred strains, only a few of which represent a hazard. Detection protocols must be able to distinguish the beneficial commensal strains from the pathogenic strains.

One of the most well established methods of identifying *E. coli* is through serotyping – the use of antibodies that selectively bind to molecules (the serotyping antigen) in the bacterial cell wall. These antibodies can be used determine the specific serotype of an *E. coli* isolate. One serotype antigen, the O-antigen, is part of the lipopolysaccharide (LPS) molecule found in the outer membrane of Gram negative bacteria. This is one of the most commonly used antigens for serotyping of *E. coli* strains. Another commonly used serotyping antigen for *E. coli* is the flagellar antigen (H-antigen). Polyclonal antisera (antibody containing blood serum) or monoclonal antibodies specific to these distinct antigens are routinely produced using live animal hosts or cell cultures and these reagents can then be used to characterize strains. For example, a well known particularly virulent form of *E. coli* is denoted as O157:H7 in reference to the two distinct forms of the O and H antigens made by strains of this serotype.

The gold standard protocol for serotyping employs an agglutination test to verify the presence of a target antigen and hence identify the *E. coli* serotype. Agglutination testing relies on an observer's visual assessment of the amount of precipitate falling to the bottom of a tube of cultured bacteria isolate at a fixed time interval after adding a serospecific antibody to the isolate. The results depend on qualitative visual assessment of the agglutination reaction. Furthermore, the traditional agglutination approach consumes relatively large amounts of antiserum. The antibodies themselves vary widely in their specificity and binding affinity, and high quality serospecific antibodies are expensive to produce and purify. Hence the cost of producing antisera in sufficient quantities for agglutination testing is one of the major factors that prevent the routine use of serotyping worldwide. Overall, this process is costly, time consuming, and requires skilled technicians, and is usually only performed by major microbiology reference laboratories. Consequently, it would be of great value to develop a more automated microarray method that provides a highly multiplexed test that

would take only a few minutes, provides quantitative binding data, and consumes extremely small volumes of antibody, is clearly an attractive replacement for existing protocols.

In this experiment we prepared a 16 sensor PWEF array to distinguish between five different *E. coli* O-serotypes. Polyclonal antibodies (PAb) to serotypes O121, O145, O55, O103 and O157 were provided by the National Microbiology Laboratory, Public Health Agency Canada. The assay strategy is to pass the sample liquid under test over the chip and detect whether antigen molecules or cellular fragments containing the antigen bind to the sensor. A positive capture signal is an indication that the specific O-serotype antigen is present.

The sensor chips were prepared by first forming a self-assembled monolayer of $\text{c10-undecenyltrichlorosilane}$, a long chain silane molecule, by immersing the chip in a solution of silane in water-free toluene and then rinsing in dichloromethane. The chip was then immersed in an oxidation solution that converts the terminal silane vinyl group to a carboxylic acid group [24]. Surfaces prepared in this way are able to rapidly bind monolayers of streptavidin protein (cf. Fig. 7(a)) and antibodies, and these layers are stable for many hours under fluid flow and during additional functionalization steps (e.g. attachment of DNA probes). Rabbit polyclonal antisera were purified through protein G columns and the isolated PAb used for functionalization of the sensor surface. The antibody solutions were prepared with a PAb concentration of 1 μM . The sensors were then functionalized with antibodies by placing PAb solution on each sensor element using a GeSiM Nano-plotter 2.1 spotting machine. This tool is able to accurately place 100 picoliter drops in an area of approximately 100 μm diameter on each sensor element. A total of five drops were placed on each sensor element, to ensure dense antibody coverage, and chips were allowed to dry. Finally, before carrying out the binding measurements, the chips were rinsed in a phosphate buffered saline (PBS) solution through the fluidic channel to remove any loosely bound PAb. This step eliminates sensor signal drift arising from detachment of weakly bound PAb probe molecules. The 16 sensor elements on a chip were divided into five groups of three, and each group was spotted with one type of antibody. The one remaining sensor that remains protected by the 2 μm SU-8 cladding layer is used as a reference sensor to monitor temperature drifts and laser wavelength calibration.

Samples were prepared by culturing each *E. coli* serotype reference strain in brain heart infusion broth to late log phase growth. The bacteria were then heat killed (2h at 100°C) and cells removed from culture broth by centrifugation. While the resulting isolate contained intact whole cells, we expect the sample preparation procedure will have caused some degree of cell breakdown or lysis, freeing cellular material into the liquid. The bacteria were suspended in a PBS solution at a concentration of approximately 10^8 CFU (colony forming unit) per mL, as determined by dilution series and plate counts of the original broth culture. The bacteria serotype was determined by passing this solution over the functionalized sensor chip.

In the binding experiments a second antibody amplification step is added to the test protocol. Previous SPR based work [25] on bacteria capture has shown that antibody amplification can increase the level of detection, and provides an additional confirmation of the identity of the captured antigen. After the initial exposure of the sensors to sample fluid, a pure PBS solution containing only one type of PAb for a single O-antigen is flowed through the chip. The PAb concentration in the amplification solution is 0.5 μM . If bacteria or the associated antigens have become attached to the sensors in the initial capture step, the free antibodies in solution will bind to the immobilized antigens, thereby increasing the total bound molecular mass on the surface and inducing an additional sensor response. The antibody amplification step not only improves the detection limit, but also enhances specificity of the test result. Since the amplification fluid contains only the antibody and no other protein, the amplification response is usually very specific to a given serotype even when non-specific binding occurs during the initial capture step. Specificity is of course the

most critical criteria in serotyping application, where the rate of false positives/negatives must be minimized. The minimum level of detection is of less relevance since some form of sample enrichment is necessary in most testing applications, even if for no other reason than to prove that the bacteria were alive at the time of sample collection.

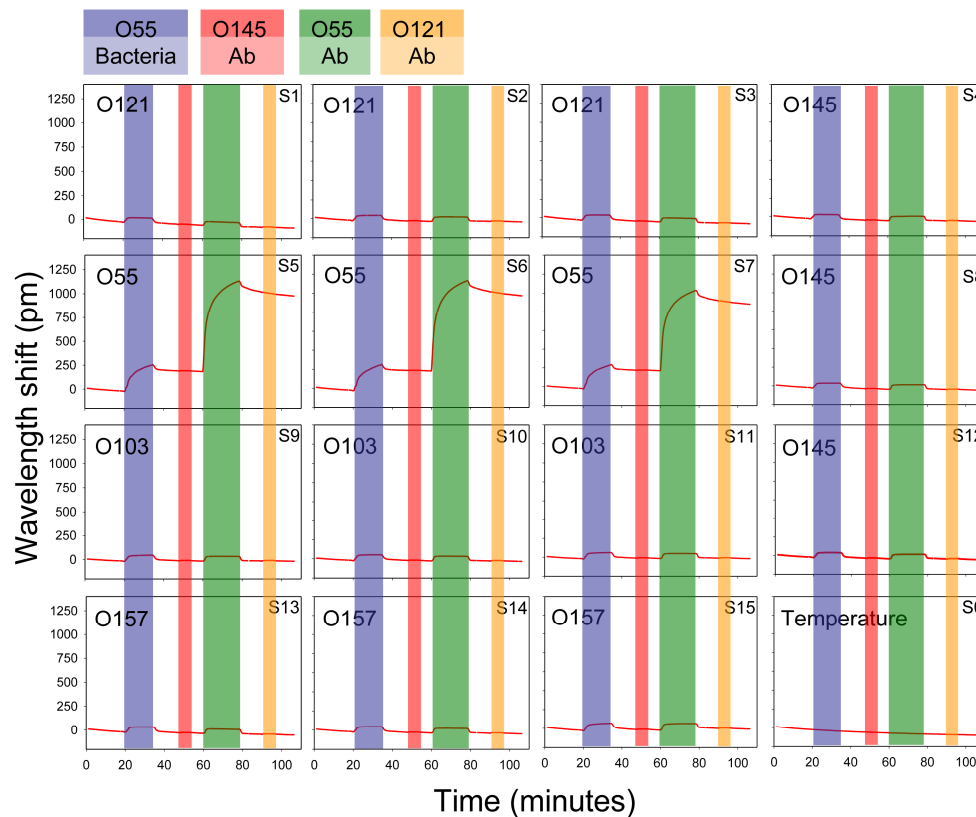


Fig. 8. Molecular binding curves from a 16 sensor PWEF array functionalized with antibodies to *E. coli* serotypes O121, O145, O55, O103, and O157, as indicated in the upper left of each panel. The colored bands delineate the time intervals during which the array was exposed to solutions containing O55 bacteria or various antibodies as indicated in the legend at the top. The bacteria concentration is 10^8 CFU/ml. During the intervals between colored bands pure PBS solution was flowing through the chip. The Sensor S0 (lower right) was isolated from the liquid by a 2- μ m-thick SU-8 layer and used to compensate temperature-induced drifts.

Figure 8 shows the sensor response curves for all 16 sensors on a chip in units of resonance wavelength shift, which is directly proportional to molecular mass on the sensor surface. Sample flow rate through the chip fluid channel is set at 20 μ L per minute. When the chip is exposed to liquid containing O55 bacteria (i.e. the time interval between 20 and 35 minutes in Fig. 8), an irreversible capture response is obtained on only the three sensors functionalized with O55 specific PAb antibodies (sensors S5, S6 and S7). During exposure to the bacteria sample the non-O55 sensor response also shifts slightly, but the shift disappears almost immediately after the bacteria sample flow is stopped and the sensors are rinsed with PBS. This signal plateau during sample exposure may be due to small differences between the sample fluid refractive index and the refractive index of pure PBS rinse solution.

To carry out the additional antibody amplification test as outlined above, the sensor chip is sequentially exposed to three different serotyping antibody (O145, O55, and O121) solutions. Only the O55 amplification solution causes a strong binding response, and only on the three O55 functionalized sensors. This result confirms that the captured antigen on sensors

S5, S6 and S7 is of the O55 bacterial serotype. Similar experiments have been carried out for samples containing other *E. coli* serotypes and probe antibodies, with comparable results. This work will be reported elsewhere.

The nature of the entity that is captured on the sensor surface is an important question for assay and sample preparation design. Possible scenarios include the capture of whole cells, fragments of lysed bacteria, and individual LPS molecules or LPS molecular micelles that contain the O-antigen. The speed and continuity of the sensor response in the presence of the bacteria sample suggests that the bound target is in the form of molecules or very small fragments of cellular material. The diffusion length for a particle after an elapsed time t is given by $L_D^2 = 2Dt$, where the diffusion constant for a particle of radius r can be estimated using the Stokes-Einstein equation

$$D = k_B T / 6\pi\eta r \quad (2)$$

here k_B is Boltzman's constant, the temperature is assumed to be 300 K and the viscosity of water is $\eta = 0.001$ Pa·s. A bacteria sized particle of radius $r = 1$ μm will have a diffusion length of $L_D = 50$ μm , corresponding to the fluid channel thickness, after about 100 minutes. Similarly, it should take nearly 20 minutes to reach a diffusion length matching the interparticle spacing of about 21 μm at a cell concentration of 10^8 CFU/ml. The capture of whole bacteria should thus be a slow process unless additional means of transporting bacteria to the surface using electrical or acoustic manipulation are used [26,27]. The results in Fig. 7 show an immediate response approaching saturation in less than 10 minutes. An estimate of the wavelength shift of a ring resonator sensor predicts that one bacteria (length 1 μm , assumed cell refractive index $n \sim 1.5$) binding to the sensor should produce an easily observable step of 20 pm. Whole cell binding should therefore produce a random step-like response as cells accumulate on the sensor. In practice, only smooth binding curves are observed even for low bacteria concentrations. These considerations suggest that the captured entity is a fast diffusing particle much smaller than a cell. Microscopic examination shows very few (<10) cells attached to the sensor waveguide after an experiment. The conclusion is that the sample preparation process lyses (breaks apart) a significant fraction of the bacteria population, releasing the O-antigen containing molecules or fragments into solution, where they can diffuse rapidly through the sensor channel volume. Cell lysis during sample preparation has previously been noted to reduce the minimum level of detection (MLD) by more than one order of magnitude in SPR based optical detection [25].

In the assay results presented here the bacteria concentration is set at 10^8 CFU/mL. The MLD in similar tests is approximately 10^6 CFU/mL, comparable with MLDs noted for dead cells in SPR studies [25]. The MLD is dependent on many factors including quality of the antibodies, strength of probe-to-surface attachment, and method of sample preparation. In this work the bacteria in the sample fluid were heat treated to kill the bacteria and produce O-antigen sample, but ultimately other methods of sample preparation may provide a greater concentration of viable antigen per CFU. While the cell wall O-antigens are not affected by boiling, the flagellar H-antigens are denatured by heat treatment. Since H-antigen serotyping is also an important additional source of information for strain typing, it would eventually be useful to adopt a sample preparation procedure that allows both H and O antigen typing on the same chip. Clearly adapting the PWEF platform to a full *E. coli* serotyping assay for food testing and other applications will require extensive work on antibody assessment and selection, as well as sample preparation methods to ensure maximum antigen extraction yet maintain the immunoreactivity of respective antigens.

However, the experiment reported here does demonstrate that the PWEF microarray platform can perform rapid multiplexed antibody based serotyping tests to identify *E. coli* and other pathogens, using a low cost disposable chip. This type of affinity binding test can be faster and provide multiplexed throughput, be more quantitative, and can use significantly less antibodies than agglutination based serotyping methods.

5. Summary

This paper describes a photonic wire evanescent field waveguide sensor micro-array platform and associated instrumentation. The instrument is capable of reading existing 16 and 128 sensor array chips, but there is no practical barrier to a much larger number of sensors in an array. The sensor chips employ 260 nm thick silicon photonic wire ring resonators as the detection element. A number of innovations distinguish this sensor platform. The ring resonators are formed using a dense Archimedean spiral configuration that allow long sensor waveguides to be contained within a small spot less than 100 μm diameter circular area. The sensor chip layout is therefore compatible in size with commercial microfluidic spotting tools. The long sensor length enhances sensor response while optimizing chip surface area contributing to the sensor response. The optical coupling employs a novel subwavelength patterned grating coupler design that allows the sensor waveguide and grating couplers to be fabricated in a single etch and lithography step, thereby considerably simplifying fabrication and reducing manufacturing cost. Chip fabrication on eight inch wafers has been demonstrated using deep-UV lithography. Microfluidic channels are fabricated monolithically on-chip, and sensor functionalization is carried out using automated microfluidic spotting tools. As a result there is no need for the manual application and removal of different sets of microfluidic blocks and masks (e.g. PDMS) in functionalizing the sensors and preparing the chip for measurement. An automated reader instrument has been developed that allows the chip to be aligned and connected to microfluidic sample delivery circuit within one minute. Finally, the application of the PWEF microarray for *E. coli* bacteria serotyping has been demonstrated using a 16 sensor array to identify any one of five *E. coli* serotypes in a sample.

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