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Photonic ring resonance is a versatile platform for performing multiplex immunoassays in real time

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ABSTRACT

Photonic ring resonance is a property of light where in certain circumstances specific wavelengths are trapped in a ring resonator. Sensors based on silicon photonic ring resonators function by detecting the interaction between light circulating inside the sensor and matter deposited on the sensor surface. Binding of biological material results in a localized change in refractive index on the sensor surface, which affects the circulating optical field extending beyond the sensor boundary. That is, the resonant wavelength will change when the refractive index of the medium around the ring resonator changes. Ring resonators can be fabricated onto small silicon chips, allowing development of a miniature multiplex array of ring based biosensors. This paper describes the properties of such a system when responding to the refractive index changed in a simple and precise way by changing the ionic strength of the surrounding media, and in a more useful way by the binding of macromolecules to the surface above the resonators. Specifically, a capture immunoassay is described that measures the change of resonant wavelength as a patient serum sample with anti-SS-A autoantibodies is flowed over a chip spotted with SS-A antigen and amplified with anti-IgG. The technology has been miniaturized and etched into a 4×6 mm silicon chip that can measure 32 different reactions in quadruplicate simultaneously. The variability between 128 rings on a chip as measured by 2 M salt assays averaged 0.6% CV. The output of the assays is the average shift per cluster of 4 rings, and the assays averaged 0.5% CV between clusters. The variability between chips averaged 1.8%. Running the same array on multiple instruments showed that after some improvements to the wavelength referencing system, the upper boundary of variation was 3% between 13 different instruments. The immunoassay displayed about 2% higher variability than the salt assays. There are several outstanding features of this system. The amount of antigen used on the chip for each test is around 200 picograms, only a few microliters of sample is necessary, and the assays take <10 min.

1. Introduction

1.1. Optical ring resonance

Optical ring resonance is a property of light that yields the removal of specific wavelengths when light enters a circular waveguide, called a ring resonator. Specifically, wavelengths of light that are exactly equal to the circumference of the ring divided by an integer, times the refractive index of the surrounding media, will become trapped and resonate within the ring, while all other wavelengths of light can leave the resonator (Iqbal et al. 2010; Ksendzov and Lin 2005; De Vos et al. 2007; Kwon and Steier 2008; Luchansky et al. 2010) (Fig. 1a). The resonant wavelengths that are trapped in the ring leave a negative peak in the spectrum of light leaving the ring. See Appendix A for a more detailed technical description of ring resonance and the principles of operation of the Maverick instrument.

The waveguide can be made in such a way that a portion of the light energy extends beyond the surface of the waveguide in the form of an evanescent tail that interacts with the material in the immediate proximity of the waveguide. Any matter that changes the index of refraction will change the resonant wavelengths in the ring resonator. It follows that when the refractive index of the surrounding media changes, the wavelengths of light that remain trapped in the ring resonator will change accordingly. The resonant wavelengths will shift proportionately higher as more matter is deposited above the ring (Fig. 1b). Thus, binding of material including protein and DNA can be detected directly since they have higher refractive indices than water (Washburn et al. 2010; Qavi et al. 2011). To enhance and amplify the signal, polystyrene beads (Luchansky et al. 2011; Iqbal et al. 2015) or enzymatic deposition of an insoluble precipitate (Kindt et al. 2013) above the rings can be used.

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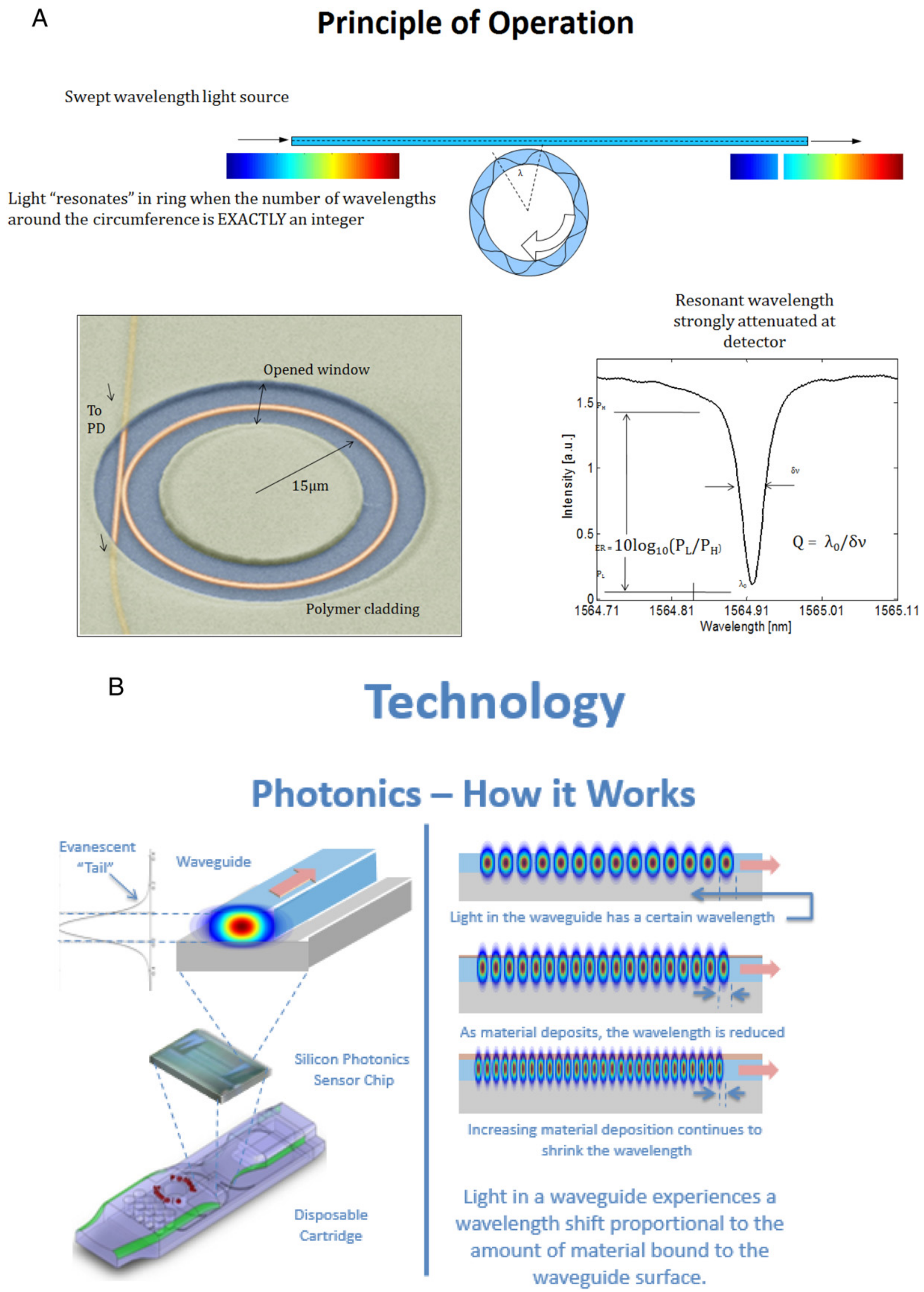

Fig. 1. (A) shows an electron micrograph of a linear waveguide and a ring resonator with open cladding around the ring on a chip, as well as representations showing how ring resonance traps a specific wavelength of light. B. At the bottom of the left side of B is a cartoon of a single chip disposable cartridge containing all reagents for the assay, with the chip above it and a representation of a waveguide on the chip at the top. On the right side of B is a representation of how light in a ring resonator "feels" compressed as material binds to the analyte on the ring.