Photonic Crystal Microarray Sensing of Cancer Cell Line Lysates

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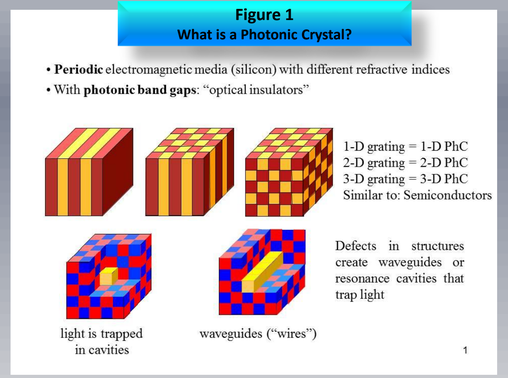
ABSTRACT

We experimentally demonstrate the ability of photonic crystal microcavity-based biosensors to specifically detect the EMT transcription factor, ZEB1, in minute volumes of sample. Two-dimensional photonic crystals in silicon-on insulator (SOI) have recently demonstrated the ability to confine and guide slow light on length scales of the wavelength of light. This has led to high sensitivity as well as miniaturization into compact sensors for chemical and bio-sensing. The precise wavelength of infrared light trapped by the resonance cavity is influenced in a very sensitive fashion by binding interactions between biomolecules attached to the silicon surface. In essence, the binding interaction alters the refractive index of the solution in contact with the sensor and this is detected as a shift in the resonance wavelength trapped by the device. When the sensor surface is derivatized with a specific antibody, the binding of the corresponding antigen from a complex whole-cell lysate will generate a resonance shift, revealing the presence of the antigen. The sensor cavity used here has a surface area of ~11 mm^2 and contacts approximately 13 nL of lysate. The device was able to reliably detect ZEB1 binding in diluted samples of H358 NSCLC cells containing ~10 cells/μL. Specificity was demonstrated using a sandwich assay in which a second antibody was introduced following the initial binding and washing steps. The resonance wavelength was then super-shifted, but only when the second reagent was a different ZEB1-specific antibody that recognized a separate epitope from the first. These photonic crystal waveguides can be multiplexed on a single chip yielding a useful platform for highly sensitive and rapid detection of several biomolecules simultaneously in minute samples.

BACKGROUND

1) Micro-scale detection of proteins is highly desirable in order to maximize biomarker analysis in small biopsies.
2) ZEB1 is an important EMT inducer in lung cancer linked to aggressive tumors
3) Photonic Crystal (PC) devices trap "slow light" in resonance cavities dimensionally equivalent to a single mitochondrion: 0.5x4 μm.
4) Exact wavelength of light trapped is very sensitive to refractive index.
5) Refractive index is altered by surface interactions with molecules.
6) Antibodies linked to PC resonance cavity will bind antigens resulting in shifted wavelengths.
7) PC-based sensing devices are used by the military and others for detecting explosives, chemical warfare agents, methane, etc.

Figure 1
What is a Photonic Crystal?



PCs are made from layers of silicon on insulator (SiO_2)

HYPOTHESIS

Photonic Crystal resonance cavities can specifically detect a single protein in whole cell lysates prepared from a lung cancer cell line.

Figure 2
Fabrication of Photonic Crystal waveguides & resonance cavities

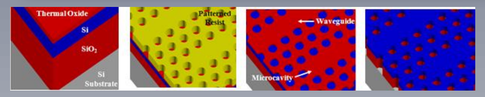


Figure 3
SEM of Photonic Crystal

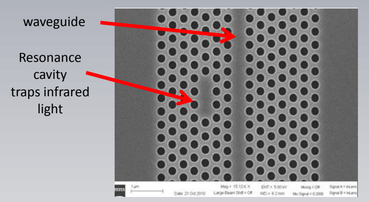
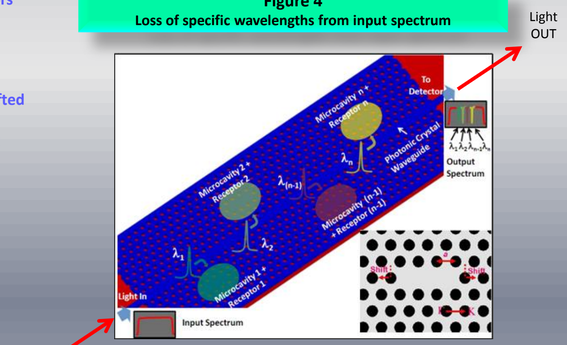


Figure 4
Loss of specific wavelengths from input spectrum



Light passes through waveguide; certain wavelengths are trapped

Figure 5
Resonance cavity traps single wavelength

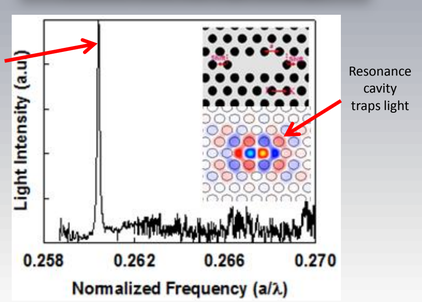
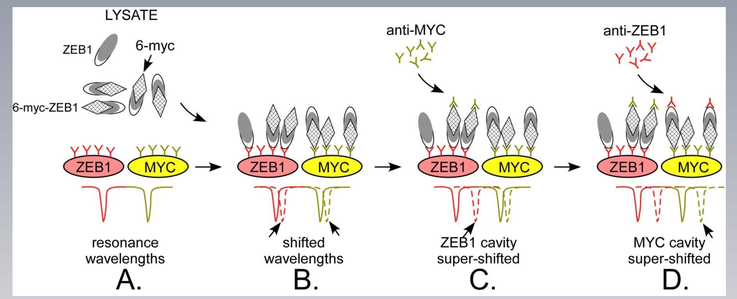


Figure 6
Principle of Operation

Proteins bound to a resonance cavity change the exact wavelength of light trapped. Wavelength shifts can be measured very precisely.



(A) Two resonance cavities (red and yellow ovals) are derivatized with antibodies for ZEB1 or MYC tag. The baseline resonance wavelengths trapped by each cavity are indicated.
(B) Lysate containing 6-myc-ZEB1 is introduced: binding of ZEB1 causes right-ward shift in resonance wavelengths (dotted curves).
(C) Introduction of anti-MYC antibody creates a "sandwich" configuration causing the anti-ZEB1 but not anti-MYC cavity to undergo a super-shift (arrow).
(D) Conversely, introduction of anti-ZEB antibody now causes the anti-MYC cavity to be super-shifted.

Reciprocal super-shifts can be used to un-ambiguously detect primary antigens, fusion proteins, phospho-proteins, etc .

Figure 7
Western Blot Detection of 6-myc-ZEB1 in NCI-H358 Lung Cancer Cells

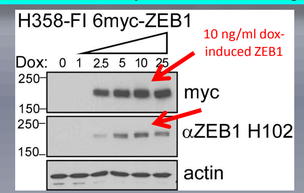
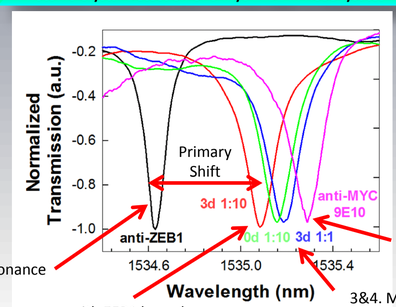


Figure 8
Photonic Crystal Detection of 6-myc-ZEB1 in H358 lysates



1. Baseline resonance
2. Shifted resonance with ZEB1 bound
3&4. More shift with more ZEB1, no shift with negative lysates
5. Super-shift with anti-MYC second antibody

Figure 9
Four Cavities on One Device

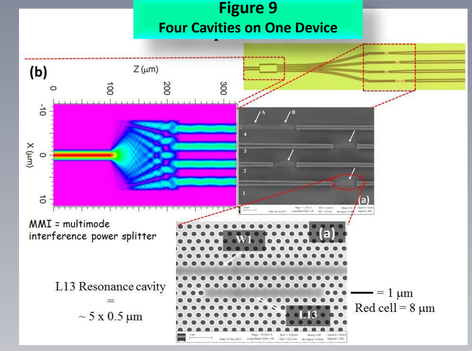
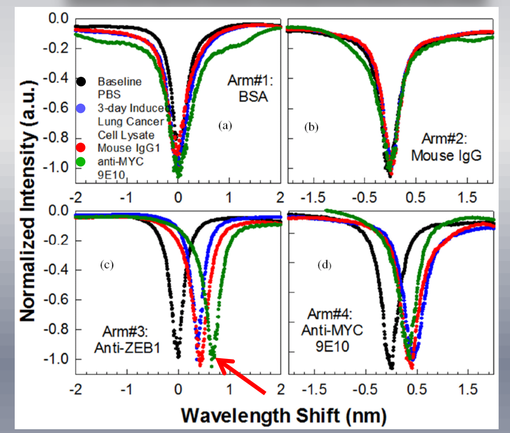


Figure 10
Control and Specific Cavities Unambiguously Detect 6-myc-ZEB1



Four arms of the Quadruplexed PC include:
ARM 1 = BSA
ARM 2 = Mouse IgG
ARM 3 = anti-ZEB1
ARM 4 = anti-MYC 9E10

Spectra for each arm are for PBS (black); H358-6myc-ZEB1 lysate (blue); mouse IgG (red) and anti-MYC 9E10 (green). Primary shifts are observed for anti-ZEB and anti-MYC cavities; super-shift (arrow) is observed only for ARM#3 with anti-MYC antibody.

CONCLUSIONS:
(A) Photonic Crystal devices can detect specific proteins from complex cell lysates.
(B) Less than 10 cells per μL can be detected.
(C) Applications include fusion proteins in leukemia, post-translational modifications, measurement of multiple protein changes in very small samples (<100 cells).

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