Detection of virus-like nanoparticles via scattering using a chip-scale optical biosensor

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A local evanescent array coupled biosensor is used to detect spherical polystyrene nanoparticles with diameters of 40 nm and 200 nm, whose sizes and refractive index are similar to virus particles. The sensitivity is ~1%/particle for 200 nm particles and 0.04%/particle for 40 nm particles. Mie scattering in an evanescent field theory is used to model the scattered light intensity for both sizes of nanoparticles. © 2012 American Institute of Physics. [http://dx.doi.org/10.1063/1.4758294]

In recent years, a number of viral diseases, such as bird flu and H1N1, have motivated increased interest in the development of new point-of-care virus detection techniques. 1,2 Timely diagnosis is necessary in order to quarantine the sick and provide effective antiviral treatment. Traditional viral diagnostic tools, such as enzyme-linked immunosorbent assay (ELISA), 3 restriction enzyme analysis (REA), 4 and real-time polymerase chain reaction (RT-PCR), 3 are effective but have several drawbacks that can inhibit widespread, rapid testing. These drawbacks include 2–10 days for cell culture, complicated sample preparation procedures, and requirements for immunoassay procedures within specialist laboratories. Compared with these established techniques, a desired biosensor for virus detection should be rapid, inexpensive, sensitive, and label-free. 5

Label-free photonic biosensors provide a relatively easy and inexpensive way to detect molecular interactions and quantify their kinetics. 7–9 One of the most popular detection principles is based on the evanescent field modulation. The binding of molecules in optical evanescent field causes the refractive index change in the binding area, which can be measured using the induced guided optical mode’s modulation. Many biosensors based on binding in evanescent fields provide very high sensitivities including surface plasmon resonance (SPR) biosensors 10–12 and micro ring resonator biosensors. 13,14 However, most of these sensors cannot simultaneously sense multiple analytes on a single waveguide since the modification of the transmitted optical mode’s phase or amplitude is measured at the end the waveguide or optical path.

A planar dielectric waveguide based local evanescent array coupled (LEAC) sensor has been proved to be a promising platform for point-of-care disease diagnostics, 15 and in previous work, 16–18 a LEAC biosensor has been employed to detect different antigen/antibodies complexes. The immunoassay mechanism of the LEAC sensor relies on specific binding of analytes or targets to one of several localized regions of immobilized biological molecular probes, such as antibodies or ssDNA, to modify the waveguide cross-section. The increased refractive index of the upper cladding shifts the local evanescent field up and hence a decreases photocurrent in the detector array under the waveguide. 15 When applied to the detection of virus particles, the LEAC sensor operates with an entirely different mechanism. Viruses can be captured on to the top surface of the LEAC waveguide via a variety of immobilized affinity receptors, including antibodies, aptamers, and engineered affinity proteins. Instead of the thin uniform adlayer (n = 1.55) formed by the immobilized antigen/antibody molecules, these captured virus particles form isolated “islands” on the waveguide. Due to the refractive index difference between the air (n = 1) and the particles (n = 1.55) in the evanescent field, light is scattered out of the guided mode. Some of the scattered light is incident on the detectors under the waveguide, leading to an increase in photocurrent in the region where particles are present, as shown in Fig. 1.

Compared to SPR biosensors or ring/disk resonator biosensors, LEAC sensor does not require a laser source but can be operated with LEDs that offer both noise and system simplification advantages. Furthermore, the LEAC biosensor is a non-resonant device with minimal temperature dependence. LEAC sensor is a silicon photonic device, which makes it easy to integrate photodetector and integrated circuit into a single silicon chip. An entire multianalyte LEAC system including optical source, detector array, integrated signal processing, and telemetry electronics with a simple capillary force driven microfluidics system should occupy a volume <1 cm3.

In order to understand the relationship between the scattered light intensity and the particle size, the scattering mechanism of small particles in an evanescent field is studied. The size parameter p is defined as p = nd/λ, where d is the diameter of the particle and λ is the wavelength of the incident light. For the 200 nm polystyrene nanoparticles (p = 0.96) used in our experiment, the Rayleigh approximation is not applicable, so Mie theory, 19 also called Lorenz–Mie theory, is used in the following calculations.

Since the virus particles are immobilized above the waveguide, the incident light in this case is the evanescent field that exponentially decays into the upper cladding with a decay constant, γ, calculated as γ = 2π2(neff2 − nupper2)1/2, where neff is the effective refractive index of the waveguide mode and nupper is the refractive index of the waveguide upper cladding.

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The averaged incident light intensity over the cross-sectional area of the particle perpendicular to the Poynting vector of the evanescent wave is

\[ I_0 = I_0 e^{-2\gamma r} \frac{n_p}{n_{\text{upper}}} \sin \theta_i \frac{I_1(2\gamma r)}{\gamma r}, \]

(2)

where \( I_1(2\gamma r) \) is the modified Bessel function of order 1 with argument \( 2\gamma r \). \( I_0 \) is incident evanescent wave intensity at \( z = 0 \).

From Eqs. (1) and (2), the relative amount of scattering can be quantified with the ratio of scattered power for particles of radius \( r \) compared to that for radius \( r_0 \)

\[ P(r) = e^{-2\gamma r} \frac{I_1(2\gamma r)}{\gamma r} \sum_{n=1}^{\infty} (2n+1) \left( |a_n(r)|^2 \Pi_n(\theta_i) + |b_n(r)|^2 \Gamma_n(\theta_i) \right) \]

\[ = e^{-2\gamma r_0} \frac{I_1(2\gamma r_0)}{\gamma r_0} \sum_{n=1}^{\infty} (2n+1) \left( |a_n(r_0)|^2 \Pi_n(\theta_i) + |b_n(r_0)|^2 \Gamma_n(\theta_i) \right). \]

(3)

For a wavelength of \( \lambda = 654 \text{ nm} \) and the single mode waveguide used in LEAC sensor,\(^{16}\) the normalized scattered power for particles with different diameters from 20 to 200 nm, which covers most of the virus sizes seen in nature, is plotted in Fig. 3. The scattered power is strongly dependent on the particle size. The scattered power of a single 200 nm particle is 39 times larger than a 40 nm particle.

Nanoparticle scattering was measured experimentally using LEAC biosensors fabricated using a commercial 0.35 \( \mu \text{m} \) CMOS process.\(^{15}\) A buried metal-semiconductor-metal photodetector array was integrated on the biosensor chip beneath a SiN/SiO2 waveguide.

For measurement purposes, light was coupled into the waveguide using end-fire coupling with 654 nm laser light through a single mode fiber (4/125 \( \mu \text{m} \) core/cladding diameter). A 16.8 Hz3V peak-to-peak AC signal with 2.6 V offset generated by a HP8116A function generator and was used to both modulate the 654 nm laser diode and serve as the reference signal for the lock-in amplifier. A PAR-5210 dual-phase lock-in amplifier was used to measure the photocurrents. Signal from a third needle that probed a fixed pad was used to normalize the signal collected from other pads, in order to account for change in the light power coupled to the waveguide resulting from fiber movement.
To verify the results shown in Fig. 3, we utilized the affinity interaction between biotin and NeutrAvidin to selectively capture both 40 and 200 nm fluorescently labeled NeutrAvidin coated polystyrene nanospheres onto the LEAC sensor. The polystyrene nanoparticles have similar optical properties to virus particles $n = 1.57,^{23}$ where the 40 and 200 nm particles represent the smaller and larger characteristic sizes for diagnostically relevant viruses. The biotinylated bovine serum albumin (BSA) was printed in discrete 288 µm diameter spots onto the LEAC waveguide using a microarray printer, after which the remaining surface was passivated in 45 mgmL⁻¹ BSA in PBS. These LEAC biosensors were then incubated in a solution containing NeutrAvidin coated particles (0.01, 0.005, or 0.001% solids) 45 mgmL⁻¹ BSA, and 1% tween-20 in PBS.

After measurement of the baseline photocurrent distribution of each buried photodetector, the LEAC sensor was incubated for 30 min in a solution containing 10-2-10-3% w/w NeutrAvidin coated, fluorescent microspheres (Invitrogen), 45 mgmL⁻¹ BSA, and 1% w/w tween-20 in PBS. The affinity existing between immobilized biotin on the waveguide and NeutrAvidin coated on the particles was utilized to mimic the affinity interaction among an immobilized biocognition element and its corresponding virus. After incubation, the sensor was sequentially rinsed and dried as described above. Figure 4(a) displays the selective capture of 200 nm diameter particles, where it can be seen that there is a clear boundary between the region printed with biotinylated BSA and the BSA blocked region that did not capture particles. Figure 4(b) displays the fluorescent intensity of the LEAC sensor surface after incubation with 200 nm particles, where it can be seen that the particles are isolated to the waveguide above the photodetectors 8-11. For each sensor, the number of particles immobilized to the waveguide above each photodetector was quantified using either SEM (for 40 and 200 nm diameter particles) or fluorescent microscopy (200 nm particles), where particles were counted if in physical contact with the waveguide. There were no significant differences between the SEM and microscopy methods pertaining to the 200 nm particles.

In this work, we utilize the photocurrent modulation ratio for the quantification of the scattering effects due to the presence of captured nanoparticles on the waveguide surface. The photocurrent modulation ratio is defined as $(I_p - I_0)/I_0$, where $I_0$ and $I_p$ are the photocurrents measured before and after nanoparticle incubation, respectively. Figure 4(c) plots the modulation ratio as a function of the photodetector number for photodetectors 4-15. Figure 4(c) also includes the number of particles captured onto the waveguide above each photodetector (seen in Fig. 4(b)), measured via fluorescent microscopy methods. It can be seen that on detectors 4-7, where there were less than 2 particles captured per photodetector region, the modulation ratio is approximately zero. The modulation ratio on detectors 8-11 sharply increases from evanescent scattering due to the presence of the polystyrene nanoparticles in higher densities. The raw modulation ratio drops to a negative value after detector 12, as increased levels of scattering in the particle immobilized region reduces the overall power of guided light in the waveguide.

![FIG. 4. (a) SEM picture of immobilized polystyrene nanoparticles with diameter of 200 nm. Dotted line shows a clear boundary between the region printed with biotinylated BSA and the BSA blocked region that did not capture particles. (b) Fluorescent microscope photo showing the patterned region on the chip. (c) Particle number distribution along the waveguide and the modulation ratio measured from different detectors. The particle diameter is 200 nm.](image)

To compensate for the decreased power measured at the $i$th detector due to increased levels of scattering in regions of high particle capture densities, we introduce a modulation correction factor, $K_i$,

$$K_i = e^{-\sum_{n=1}^{i-1} \ln(\Delta S_n) - \Delta S_n},$$

where $\Delta S_n$ is the propagation loss along the waveguide before the particle patterning. $\Delta S_n$ is the particle induced scattering ratio change on detector $n$, which can be defined as $\Delta S_n = N_n M_1 (1 - e^{-\Delta S_n})$. $N_n$ is the particle number on detector $n$ and $M_1$ is the modulation ratio on detector 1. Figure 4(c) also plots the corrected modulation ratio as a function of photodetector number.

Figure 5(a) shows the corrected modulation ratio vs. a particle count, where the data were collected from 11 photodetectors over 3 different LEAC biosensor chips. As expected, it can be seen that corrected modulation ratio is a linear function of $N$. The sensitivity for these 200 nm particles is approximately 1%/particle.

The relationship between the corrected modulation ratio and particle number for 40 nm particles is plotted in Fig. 5(b) for a single biosensor chip. The sensitivity for 40 nm particles is ~0.04%/particle. The sensitivity for 200 nm particle
is approximately 25 times larger than 40 nm particles. This experimental ratio is slightly smaller with respect to the results calculated using Eq. (3), where it is expected that 200 nm particles should provide a sensitivity 39 greater than that expected by the 40 nm particles. This difference may be due to the local field shift effect caused by the immobilized particles, which is not considered in the derivation of Eq. (3).

Polystyrene nanoparticles with two different diameters, 40 nm and 200 nm, were detected by a LEAC biosensor. The sensitivity for the 200 nm particle is ἅ 1%/particle and it is 0.04%/particle for the 40 nm particle. The particle scattering light intensity was calculated using the Mie scattering method in evanescent field described in reference, and the calculated ratio of scattered light intensity for 40 nm and 200 nm particles was 39, which is larger than the experimental ratio, 25. The experiment results show the potential of this biosensor in the virus detection application. Considering the top surface of the biosensor is SiNx, it would be an easy process to integrate microfluidic delivery of the virus particles to the LEAC biosensor. This will give the biosensor real-time monitoring ability and largely reduce the test time.

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