

Protein detection by optical shift of a resonant microcavity

F. Vollmer,^{a)} D. Braun, and A. Libchaber

Center for Studies in Physics and Biology, Rockefeller University, New York, New York 10021

M. Khoshima, I. Teraoka, and S. Arnold

Microparticle Photophysics Lab (MP³ L), Polytechnic University, Brooklyn, New York 11201

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We present an optical biosensor with unprecedented sensitivity for detection of unlabeled molecules. Our device uses optical resonances in a dielectric microparticle (whispering gallery modes) as the physical transducing mechanism. The resonances are excited by evanescent coupling to an eroded optical fiber and detected as dips in the light intensity transmitted through the fiber at different wavelengths. Binding of proteins on the microparticle surface is measured from a shift in resonance wavelength. We demonstrate the sensitivity of our device by measuring adsorption of bovine serum albumin and we show its use as a biosensor by detecting streptavidin binding to biotin. © 2002 American Institute of Physics. [DOI: 10.1063/1.1482797]

During the last decade, optical biosensors capable of detecting unlabeled macromolecules¹⁻³ have become valuable tools in life sciences⁴⁻⁶ as well as drug discovery.^{7,8} Optimal biosensor design for detection of label-free macromolecules should be simple, sensitive, and particularly adept at tasks such as molecular recognition. High sensitivity is of critical importance, since one must often detect only a few molecules.

We present an optical biosensor for detection of label-free molecules with unprecedented sensitivity. Our biosensor derives its sensitivity from use of optical resonances in a transparent dielectric microparticle. The resonances are generated when light, confined by total internal reflection (TIR), orbits near the particle surface and returns in phase after each revolution. The frequencies of these so-called whispering gallery modes (WGMs), characterized by the number of wavelengths L within an orbit, are extremely sensitive to added dielectric material on the microparticles surface. It is estimated that an atomic thickness can lead to a detectable shift of a given resonance frequency.⁹

The sensitivity of our biosensor is measured using adsorption of bovine serum albumin (BSA) dissolved in phosphate buffered saline [(PBS) pH 7.4] on a silica glass microparticle bathed in PBS solution. Furthermore, we show the use of our device as a biosensor. We detect the binding of streptavidin to surface immobilized biotinylated BSA.

The silica glass microparticle is of spheroidal shape, with a diameter of $\sim 300 \mu\text{m}$ in the plane of the light orbit. It is fabricated by melting the tip of a stripped single-mode fiber with a butane/nitrous oxide microflame torch. The silica glass surface is chemically modified with 3-aminopropyltrimethoxysilane.¹⁰ Optical resonances of the silica glass microparticle are stimulated by evanescent coupling to an eroded optical fiber¹¹ as shown in Fig. 1(a). Light orbiting in the silica glass microparticle due to TIR produces an evanescent field just outside the glass surface. By overlapping this field with the evanescent field of a fiber core, light from the optical fiber can be coupled into optical reso-

nances of the microparticle. A stretch of approximately 1 cm of a single-mode optical fiber with a core diameter of $6.6 \mu\text{m}$ (FS-SC-6234, 3M) is stripped and then etched in hydrofluoric acid to a final diameter of $4 \mu\text{m}$, thus removing all the cladding and exposing the evanescent field. The microparticle is then positioned in contact with the etched part of the fiber. A sample cell is built around this fiber-sphere contact using two glass slides separated at 4 mm by spacers composed of two pairs of silicone rubber pads. The buffer solution is retained in the 1 ml sample cell by surface tension, and its temperature is measured by a thermocouple probe (5TC-GG-K-30-36, Omega). The sample cell is imaged on a charge coupled device microscope/camera from above [Fig. 1(b)].

A distributed feedback laser diode with a nominal wavelength of $\sim 1340 \text{ nm}$ (ML776H11F, Mitsubishi) is used as a tunable laser source. The laser current is scanned repeatedly between 10 and 38 mA through a saw-tooth shaped function. The periodically scanned output wavelength has a current

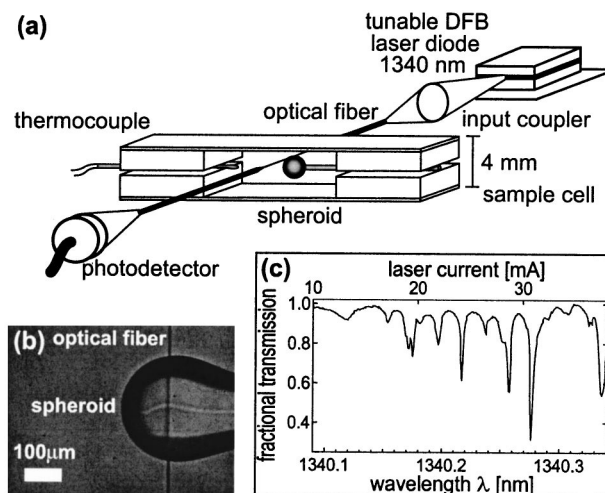


FIG. 1. (a) Experimental setup. Optical resonances of a spheroidal glass microparticle are excited by coupling the spheroid evanescently to an eroded single mode optical fiber. (b) Picture of the spheroid coupled to the eroded part of the optical fiber. (c) Resonance dips vs wavelength.

^{a)}Electronic mail: vollmef@rockvax.rockefeller.edu

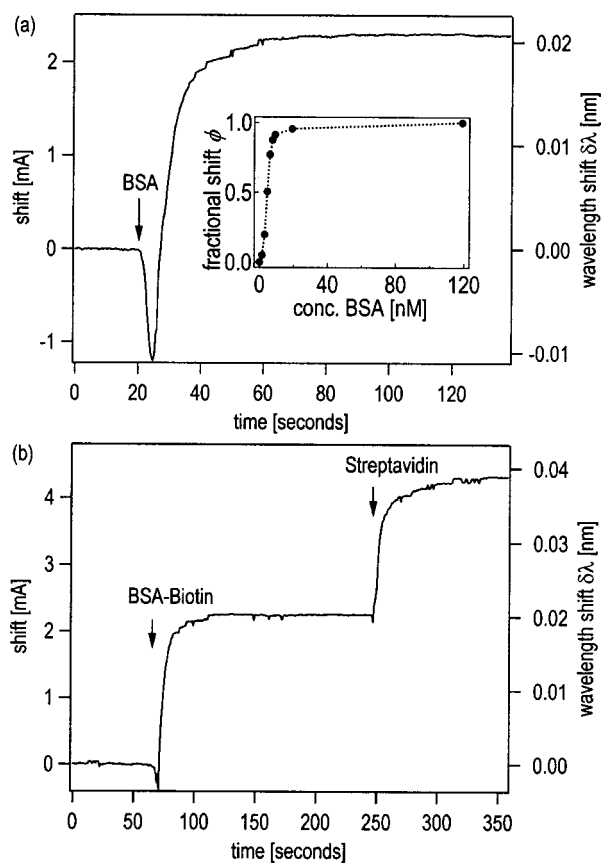


FIG. 2. (a) Current and corresponding wavelength shift of a resonance before and after injection of BSA into the sample cell. The overall shift is entirely due to BSA adsorption. The inset shows the fractional shift for different BSA concentrations. (b) Biosensing: Detection of streptavidin binding to previously surface immobilized BSA biotin.

dependence of 0.009 nm/mA. The intensity of the light transmitted through the optical fiber is detected and amplified using an InGaAs photodiode (PDA400, Thorlabs). The current source is driven by a LABVIEW (National Instruments) program which also tracks a resonant dip in the intensity of the transmitted light by detecting its position with a parabolic minimum fit.

Optical resonances of the microparticle are detected as dips in the transmitted intensity as a function of laser wavelength [Fig. 1(c)].¹² The Quality factor Q of a given resonance is $\sim 2 \times 10^6$.

Since accretion of new material increases the effective size of the silica glass microparticle, a resonance will shift to a longer wavelength to accommodate the larger circumference. This is shown in the adsorption of BSA molecules. Figure 2(a) shows the current and the corresponding wavelength shift of one resonance before and after injection of BSA in the sample cell to a final concentration of 1.5 μM . Following sample injection, the wavelength shift is first negative due to thermal contraction of the glass spheroid. This effect reverses rapidly as the resonance wavelength begins to increase and the shift saturates with time to an overall level of $\delta\lambda \approx +0.021$ nm. The overall shift in wavelength between before and after BSA injection at room temperature ($\sim 22.7^\circ\text{C}$) is entirely due to BSA adsorption on the microparticle.

The fractional shift at different BSA concentrations, defined as the ratio of $\delta\lambda$ to its value at saturation measured at

1.5 μM BSA, is shown in the inset of Fig. 2(a). This isotherm shows that the response saturates for concentrations as low as 20 nM. Consequently, we expect no more than one layer of BSA molecules is bound to the microparticle at saturation.

It is possible to derive an analytical theory for the perturbation caused by an individual BSA molecule, and calculate the effect of statistically distributed molecules with a surface density σ_s . In the asymptotic limit for $L \gg 1$, the perturbation is caused by the energy needed to polarize a nanosized protein molecule.¹³ The molecule needs only be characterized by its excess polarizability α_{ex} ,¹⁴ and the resulting shift is¹³

$$\frac{\delta\lambda}{\lambda} \approx \frac{\alpha_{\text{ex}}\sigma_s}{\epsilon_0(n_1^2 - n_2^2)R}, \quad (1)$$

where ϵ_0 is the vacuum permittivity, R is the orbital radius, and n_1 and n_2 are the refractive indices of the sphere and the buffer solution, respectively.

As a test of Eq. (1), we have used this relationship to estimate the surface density associated with the wavelength shift at saturation, which has been determined from several measurements $\delta\lambda = +0.021$ nm. With $\alpha_{\text{ex}} = 4\pi\epsilon_0 \times 3.85 \times 10^{-21}$ cm³ we find $\sigma_s = 1.7 \times 10^{12}$ cm⁻², and therefore the average surface area covered by one molecule $\sigma_s^{-1} = 5.9 \times 10^{-13}$ cm². We expect BSA to reduce its surface energy by binding with its largest planar projection. Crystallographic data for human serum albumin,¹⁵ which has a molecular weight almost identical to that of BSA (66 438 g/mol compared to 66 432 g/mol for BSA), reveals that this area is about 3.7×10^{-13} cm², which is $\approx 63\%$ of σ_s^{-1} , consistent with no more than one layer of adsorbed BSA molecules.

A surprising result is the low noise in the current signal before the BSA was added, i.e., the low standard deviation of 20 μA (corresponding to 0.18×10^{-3} nm). For a glass microparticle of 30 μm diameter, which can still host WGMs, a surface density of binding sites for a target analyte on the order of 2×10^{12} cm⁻², and with a dielectric constant similar to that of BSA in Eq. (1), we estimate the smallest detectable molecular weight to be ~ 50 Da (Dalton, equals g/mol), smaller than the 180 Da detection limit of surface plasmon resonance biosensors.¹⁶

For detecting layers of molecules by rather unspecific adsorption and desorption on a silanated glass microparticle, the sensitivity of our device is certainly limited by the maximum achievable density of surface functionalities binding the adsorbate, $\sigma_s = 5 \times 10^{14}$ cm⁻² for silane agents.¹⁷ Then, we should be able to detect a layer of entities as small as 0.2 Da, comparable to a proton.

Our device can be used as a versatile biosensor if the microparticle surface is modified with a biological recognition element, capable of specific binding to a target analyte. We use biotinylated BSA (BSA biotin, Sigma) as the recognition element which we immobilize on the aminosilanized microparticle surface by adsorption as described before for nonmodified BSA. Figure 2(b) shows the initial shift of an optical resonance wavelength after injection of BSA biotin to a final concentration of 0.01 mg/ml. Subsequent injection of streptavidin (recombinant from *E. Coli*, Sigma) to a final concentration of 0.01 mg/ml leads to a further overall shift of the

given optical resonance wavelength. This additional shift is due to binding of streptavidin to the surface immobilized biotin.

The ratio of the wavelength shift due to streptavidin binding compared to BSA biotin adsorption is 0.94. Taking into account the molecular weight ratio of the two binding partners and assuming similar dielectric constants for both proteins, we estimate a $\sim 1:1$ stoichiometry for the binding reaction.

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¹M. Malmqvist, *Nature (London)* **361**, 186 (1993).

²P. A. Lowe, T. J. Clark, R. J. Davies, P. R. Edwards, T. Kinning, and D. Yeung, *J. Mol. Recognit.* **11**, 194 (1998).

- ³R. L. Rich and D. G. Myszka, *Curr. Opin. Biotechnol.* **11**, 54 (2000).
- ⁴M. Malmqvist and R. Karlsson, *Curr. Opin. Chem. Biol.* **1**, 378 (1997).
- ⁵A. R. Mendelsohn and R. Brent, *Science* **284**, 1948 (1999).
- ⁶A. P. Turner, *Science* **290**, 1315 (2000).
- ⁷D. G. Myszka and R. L. Rich, *Pharm. Sci. Technol. Today* **3**, 310 (2000).
- ⁸R. Karlsson, M. Kullman-Magnusson, M. D. Hamalainen, A. Remaeus, K. Andersson, P. Borg, E. Gyzander, and J. Deinum, *Anal. Biochem.* **278**, 1 (2000).
- ⁹A. Serpenguezel, S. Arnold, and G. Griffel, *Opt. Lett.* **20**, 654 (1994).
- ¹⁰N. Zammateo, L. Jeanmart, S. Hamels, S. Courtois, P. Louette, L. Hevesi, and J. Remacle, *Anal. Biochem.* **280**, 143 (2000).
- ¹¹J. P. Laine, B. E. Little, H. A. Haus, *IEEE Photonics Technol. Lett.* **11**, 1429 (1999).
- ¹²G. Griffel, S. Arnold, D. Taskent, A. Serpenguezel, J. Connolly, and N. Morris, *Opt. Lett.* **21**, 695 (1996).
- ¹³S. Arnold, M. Khoshima, F. Vollmer, S. Holler, and I. Teraoka (unpublished).
- ¹⁴ $\alpha_{\text{ex}}/4\pi\epsilon_0 = (n/2\pi)(dn/dc)m$, where $n=1.332$ is the solvent refractive index, $dn/dc=0.184 \text{ cm}^3/\text{g}$ is the differential refractive index of a BSA solution in water, and $m=(66432 \text{ g/mol})/(6.02 \times 10^{23} \text{ mol})$ is the mass of a BSA molecule.
- ¹⁵D. C. Carter, X. M. He, S. H. Munson, P. D. Twigg, K. M. Gernert, M. B. Broom, and T. Y. Miller, *Science* **244**, 1195 (1989).
- ¹⁶R. Karlsson and R. Stahlberg, *Anal. Biochem.* **228**, 274 (1995).
- ¹⁷L. T. Zhuravlev, *Langmuir* **3**, 316 (1987).