High-Q microsphere biosensor - analysis for adsorption of rodlike bacteria

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Abstract: Theory is developed for frequency shift and linewidth-broadening induced by rodlike bacteria bound to micro-optical resonators. Optical shift of whispering gallery modes (WGMs) is modeled by introducing a form factor that accounts for random horizontal orientation of cylindrical bacteria bound by their high refractive index cell walls. Linewidth-broadening is estimated from scattering losses. Analytic results are confirmed by measurement using E.Coli as model system (~10^2 bacteria/mm^2 sensitivity), establishing the WGM biosensor as sensitive technique for detection and analysis of micro-organisms.

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References and links


1. Introduction

Photon recirculation in optical microcavities significantly enhances interaction of the propagating field with the resonator material as well as interaction of the evanescent field with the surrounding medium. The evanescent field provides possibility to exploit giant photon lifetimes in spherical and toroidal microcavities (quality factor up to Q~10^9, [1]) for ultrasensitive detection of liquid samples [2,3] and biomolecules [4-12]. The detection method, based on measuring shift of resonance frequency, is particularly adept at recognition of biomolecules directly adsorbed on the cavity surface. Label-free biomolecular detection [4,9,12] has been demonstrated with sensitivity that rivals state-of-the-art surface plasmon resonance (SPR) [13,14]. Experiments are complemented by analytic theory [4-7] which relates changes in resonance frequency to evanescently induced polarization of nanometer-sized molecules. Detection of biomolecules from optical shift of resonant microcavities [4-6] promises single molecule sensitivity which has recently been achieved in microtoroidal resonators [12] where heat generated from adsorbed molecules exposed to a large circulating power provides added sensitivity. Here, we examine microcavities operated in the low-power regime where the established theory [4-7] is applicable for analysis of a wide range of biomolecules, polymers and viral particles that are small compared to the probing (visible or near-infrared) wavelength. Microcavity sensors, however, may also provide a useful tool to study biologically important surface coatings and aggregates which are formed from larger particles such as micrometer-sized bacteria [15-18]. The established theory [5-7] is then no longer valid since a form-factor is necessary to account for well-defined size, shape and orientation of inhomogeneous, micron-sized bacterial particles, and one can no longer disregard scattering in the analysis.

Here we develop theory that analyzes perturbations to optical microcavity sensors induced by random horizontal adsorption of bacteria with rodlike shape, a shape ubiquitous for various important bacterial and viral species [15-18]. Analytic theory is derived using Born-and Rayleigh-Gans approximations, and we introduce a form factor to model cylindrical bacteria shape with high refractive index cell wall (n~1.42) and low refractive index protoplasm (n~1.35). Theoretical results are confirmed in measurements taken with E.coli bacteria as model system. Adsorption of the micrometer-sized bacteria induces not only a large shift of resonance frequency, but also a significant increase in linewidth due to scattering; both effects are measured in good agreement with predictions. We also estimate the effective cell-wall
area of \( E. coli \) in contact with the resonator surface and confirm observation of cylindrical \( E. coli \) bound with its axis aligned parallel (horizontal) to the cavity surface.

The electromagnetic field \((\mathbf{E}, \mathbf{B})\) of a WGM (Fig. 1(a)), triggered in a dielectric microsphere by evanescent coupling from an optical fiber [4], is determined by the macroscopic Maxwell equations in a dielectric medium. For harmonic time dependence \( e^{-i\omega t} \) they read (we are using the Lorentz-Heaviside unit with the speed of light \( c \) set to one):

\[
\begin{align*}
\nabla \cdot \mathbf{D} &= 0 \\
\nabla \times \mathbf{E} &= i\omega \mathbf{B} \\
\n\nabla \cdot \mathbf{B} &= 0 \\
\n\nabla \times \mathbf{B} &= -i\omega \mathbf{D}
\end{align*}
\]  

(1)

The electric displacement vector is \( \mathbf{D} = \mathbf{\varepsilon E} \), where dielectric constant is given by

\[
\mathbf{\varepsilon}(\mathbf{r}) = \mathbf{\varepsilon}_{\text{background}}(\mathbf{r}) + \mathbf{\delta\varepsilon}(\mathbf{r})
\]

(2)

with \( \mathbf{\varepsilon}_{\text{background}} \) accounting for the background (cavity and solution) and \( \mathbf{\delta\varepsilon} \) accounting for the perturbation caused by adsorbed particles such as bacteria. We have

\[
\mathbf{\varepsilon}_{\text{background}} = \begin{cases} 
\n^2 \text{cav} & \text{for } r < R; \\
\n^2 \text{sol} & \text{for } r > R.
\end{cases}
\]

(3)

where \( R \) is the radius of the cavity, \( n^2 \text{cav} \)(\( n^2 \text{sol} \)) is the index of refraction of the cavity (solution) and \( \mathbf{\delta\varepsilon} \) is function of \( r \). For the experiment discussed in this paper, we have \( n^2 \text{cav} > n^2 \text{sol}, 0 < \mathbf{\delta\varepsilon}(\mathbf{r}) < n^2 \text{sol} \).

Fig. 1. (a) A whispering gallery mode (WGM) is excited at \( \approx 1.3 \) \( \mu \)m wavelength in a silica microsphere resonator. The mode is confined by total internal reflection and produces an exponentially decaying evanescent field. Adsorption of rodlike \( E. coli \) bacteria perturb the field and cause observable shift of resonance frequency as well as broadening of linewidth. Theory is developed for cylindrical bacteria bound with their axis aligned parallel (horizontal) to the surface but otherwise at arbitrary orientation. \( n^2 \text{cav}, n^2 \text{sol}, n^2 \text{wall}, n^2 \text{int} \) are the refractive indices of the cavity, the surrounding aqueous solution, the bacteria cell wall and interior (protoplasm) of the bacteria; respectively. (b) Fluorescent image of green fluorescent protein (GFP)-labeled bacteria. The micrograph confirms the orientation of the bound bacteria with cylindrical axis aligned parallel to the microsphere surface. Only few bacteria (arrows) are bound at their tip.
The WGM corresponds to the propagation of a light along the inner wall of the cavity because of the totally internal reflection under the resonance condition

\[
\lambda_{cav} \approx \frac{\pi \lambda}{2n_{cav}}
\]

with \(\lambda_{cav}\) the wavelength inside the cavity and \(l\) a large integer. The intensity of a WGM is highly concentrated in the vicinity of the wall of the cavity, and its polarization can be either transverse electric (TE) or transverse magnetic (TM) [19]. The WGM couples to the adsorbed particles via its evanescent field. Because the energy density of an electromagnetic wave is proportional to \(\varepsilon E^2\), the excess dielectric constant of the adsorbed particles ‘pulls’ the energy distribution of a WGM slightly outward. This in turn increases the optical length of the WGM and induces a red shift of its wavelength. The spectral width is also broadened by the radiation of the adsorbed particles driven by evanescent field.

The wavelength shift will be calculated in the following section, and the line width will be estimated in the section 3. In this section, we shall also describe the experiment and compare the result with theory. The section 5 concludes the paper.

2. The wavelength shift

The shift of the WGM wavelength is calculated by means of the Born approximation to the solution of the Maxwell equations Eq. (1) [20] and we shall outline the major steps for a TE mode below. The treatment of a TM mode together with some technical details will be deferred to the Appendices A and B.

To the leading order approximation, the dielectric excess \(\delta\varepsilon\) of Eq. (2) is replaced with its average over the ensemble distribution of the adsorbed particles, \(\overline{\delta\varepsilon} \ll n_{sol}^2\), which occupies a thin shell outside the cavity. For a typical bacteria cell of an index of refraction \(n_w\) for the cell wall and an index of refraction \(n_{int}\) for the protoplasm we have

\[
\overline{\delta\varepsilon} = \varepsilon \sigma [\Delta\varepsilon + (\Delta\varepsilon' - \Delta\varepsilon)A'(A)],
\]

where \(\Delta\varepsilon = n_w^2 - n_{sol}^2\), \(\Delta\varepsilon' = n_{int}^2 - n_{sol}^2\), \(A(A')\) is the average cross sectional area of the outer(inner) surface of the wall cut by a sphere of radius \(r\), and \(\sigma\) is the average surface density of the cells. Upon replacing \(\overline{\delta\varepsilon}\) of Eq. (3) by \(\delta\varepsilon\), the dielectric function becomes spherically symmetric with respect to the center of the resonance cavity and the standard multipole analysis applies.

The electromagnetic field of a TE mode of the order \((l,m)\) is given by:

\[
E_{lm}(r) = g_l(\omega r)X_{lm}(\hat{r})
\]

\[
B_{lm}(r) = -\frac{i}{\omega} \nabla \times E_{lm}(r),
\]

where the vector spherical harmonics

\[
X_{lm}(\hat{r}) = -\frac{i}{\sqrt{l(l+1)}} \hat{r} \times \nabla Y_{lm}(\hat{r})
\]

with \(Y_{lm}(\hat{r})\) the ordinary spherical harmonics. Substituting into the Maxwell equation Eq. (1), we find that the function \(g_l\) satisfies the ordinary differential equation

\[
-\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{dg_l}{dr} \right) + \frac{l(l+1)}{r^2} g_l - \varepsilon \omega^2 g_l = 0
\]
with \( e = e_{\text{background}} + \bar{\delta}e \). It follows from the joining condition of the electromagnetic field across a dielectric discontinuity that both \( g_i \) and its derivative are continuous functions of \( r \).

In the absence of adsorbed particles, the Eq. (6) becomes

\[
-\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d g_i^{(0)}}{dr} \right) + \frac{l(l+1)}{r^2} g_i^{(0)} - e_{\text{background}} \omega^2 g_i^{(0)} = 0
\]

(7)

The solution to the Eq. (7) can be expressed in terms of spherical Bessel functions, i.e.

\[
g_i^{(0)} = \begin{cases} 
A_j \left( n_{\text{cav}} \omega r \right) & \text{for } r < R \\
\left( j_i \left( n_{\text{sol}} \omega r \right) \cos \delta_i^{(0)} + n_i \left( n_{\text{sol}} \omega r \right) \sin \delta_i^{(0)} \right) & \text{for } r \geq R
\end{cases}
\]

(8)

For \( r \to \infty \), we have

\[
g_i^{(0)} \equiv \frac{1}{n_{\text{sol}} \omega r} \sin \left( n_{\text{sol}} \omega r - \frac{1}{2} l \pi + \delta_i^{(0)} \right).
\]

(9)

The quantity \( \delta_i^{(0)} \) is the phase shift of the TE mode scattered by the cavity and is determined by the continuity of \( g_i^{(0)} \) and its derivative across the wall of the cavity. We have

\[
e^{2i\delta_i^{(0)}} = \frac{D_i^* (\omega)}{D_i (\omega)}
\]

(10)

where

\[
D_i (\omega) = \left| \begin{array}{cc}
j_i (\zeta) & h_i^{(1)} (z) \\
\bar{g}_i (\zeta) & \zeta h_i^{(1)} (z)
\end{array} \right|
\]

(11)

with \( \zeta = n_{\text{cav}} \omega R \), \( z = n_{\text{sol}} \omega R \) and \( h_i^{(1)} (z) = j_i (z) + in_i (z) \). A WGM corresponds to

\[
n_{\text{cav}} \omega R = \nu + O(\nu^2)
\]

(12)

with \( \nu = l + \frac{1}{2} \gg 1 \). The explicit form of the \( O(\nu^2) \) term can be found in [19]. In the immediate vicinity outside the wall, the spherical Bessel functions take the approximate form (see Appendix A for the derivation)

\[
j_i (n_{\text{cav}} \omega r) \equiv j_i (z)e^{\sqrt{n_{\text{cav}}^2 - n_{\text{sol}}^2} \omega (r - R)}
\]

(13)

and

\[
n_i (n_{\text{cav}} \omega r) \equiv n_i (z)e^{-\sqrt{n_{\text{cav}}^2 - n_{\text{sol}}^2} \omega (r - R)}
\]

(14)

for \( 0 < r - R \ll R \). The \( O(\nu^\frac{1}{3}) \) term on RHS of Eq. (12) is chosen to satisfy the resonance condition, i.e. \( \delta_i^{(0)} = \frac{\pi}{2} \). Then the solution immediately outside the wall consists of only exponential decay behavior, corresponding to a trapped field configuration by the totally internal reflection. It was shown that the width of the resonance is extremely narrow.

In the presence of adsorbed particles, the large \( r \) behavior of the solution to Eq. (6) remains given by Eq. (9) but with a modified phase shift \( \delta_i = \delta_i^{(0)} + \Delta \delta_i \). The WGM frequency is
shifted accordingly in order for $\delta_i = \frac{\pi}{2}$. As long as $|\Delta \delta_i| < 1$, the amount of shift is given by

$$\Delta \omega = -\frac{\Delta \delta_i}{\frac{d \delta_i^{(0)}}{d \omega}}$$  \hspace{1cm} (15)

with the RHS evaluated at the unperturbed WGM frequency. To calculate $\Delta \delta_i$, we multiply Eq. (6) by $g_i^{(0)}$ and Eq. (7) by $g_i$. Then we take their difference and integrate the result with respect to $r$, i.e.

$$\int_0^\infty dr r^2 \left[ g_i^{(0)} \times \text{Eq.6} - g_i \times \text{Eq.7} \right]$$  \hspace{1cm} (16)

Upon using the asymptotic behavior Eq. (9) and that of $g_i$, we end up with

$$\sin \Delta \delta_i = n_{\text{sol}} \omega l \int \frac{drr^2 \Delta \delta(r) g_i^{(0)}(r)}{\mathcal{R}} g_i(r),$$  \hspace{1cm} (17)

which is rigorous up to now. To the leading order perturbation, we may approximate $\sin \Delta \delta_i \equiv \Delta \delta_i$ and replace $g_i$ of the integrand on RHS by $g_i^{(0)} = n_i (n_{\text{sol}} \omega r)$. Substituting the approximate expression thus obtained for $\Delta \delta_i$ and the explicit expression for $\frac{d \delta_i^{(0)}}{d \omega}$ derived in the Appendix A Eq. (15), we find that

$$\frac{\Delta \lambda}{\lambda} = -\frac{\Delta \omega}{\omega} = \frac{n_{\text{sol}} \omega l}{\mathcal{R}} \int \frac{drr^2 \Delta \delta(r) n_i^2 (n_{\text{sol}} \omega r)}{z(z^2 - z^2)n_i^2(z)}$$  \hspace{1cm} (18)

Substituting Eq. (4) into Eq. (18), and the parallel expression for a TM mode developed in the Appendix B, we end up with:

$$\left( \frac{\Delta \lambda}{\lambda} \right) = \frac{\sigma[\Delta \epsilon + (\Delta \epsilon' - \Delta \epsilon) \nu']}{(n_c^2 - n_{\text{sol}}^2)R} \rho \kappa$$  \hspace{1cm} (19)

where $\nu$ is the total volume of the cell and $\nu'$ is that of the interior. The fractional wavelength shift expressed by Eq. (19) is dimensionless and will not change if the speed of light is not set to one. The polarization factor $\rho = 1$ for TE mode and $\rho = 2 - (n_{\text{sol}} / n_c)^2 \approx 1.18$ for TM mode. The form factor $\kappa$ relates the evanescent field penetration depth with the size of the bacteria. In the long wavelength limit, where the penetration depth is much larger than the bacteria diameter, $\kappa \rightarrow 1$ and the formula is then equivalent to the perturbative dipole approximation derived in (5-7). The higher value of the TM mode is caused by the jump of the electric field amplitude across the cavity wall [7].

For our analysis, we model the E.coli as a cylindrical cell with its axis aligned parallel (horizontal) to the surface of the cavity upon binding. We have $\nu = 2\pi a b^2$ and $\nu' = 2\pi a' b'^2$ with $a$ the half length of the cylinder and $b$ the radius. The primed quantities stand for the corresponding size of the interior. The form factor $\kappa$ in this case is given by
\[ \kappa = \frac{\Delta \epsilon f(z) + (\Delta \epsilon - \Delta \epsilon') f(z')}{\Delta \epsilon' + (\Delta \epsilon' - \Delta \epsilon)v'} e^{-z} \]  

(20)

where \( z = \frac{4\pi b}{\lambda} \sqrt{n_{\text{sol}}^2 - 1} \) and \( z' = \frac{4\pi b'}{\lambda} \sqrt{n_{\text{sol}}^2 - 1} \) with \( \lambda \) the wavelength in the solution. The function \( f(x) \) is related to the modified Bessel function as \( f(x) = \frac{2}{x} I_1(x) \).

3. The line width

To estimate the line width broadening, we employ the Rayleigh-Gans approximation. Let us consider first an adsorbed particle located at \( \mathbf{R} \). In the coordinate system with its origin at the center of the spherical cavity, \( \mathbf{R} \equiv \hat{R} \mathbf{R} \). The induced dipole density inside the particle is given by

\[ \mathbf{d}(\mathbf{r}) = \partial \mathbf{c}(\mathbf{r}) \mathbf{E}_0(\mathbf{r}) \]  

(21)

with \( \mathbf{E}_0(\mathbf{r}) \) the incident evanescent field. Neglecting the reflection at the cavity wall, the secondary electromagnetic field from the induced dipole density in the radiation zone reads

\[ \mathbf{E}_{\text{rad.}}(\mathbf{r}) = -\frac{\omega^2}{4\pi} r e^{ikr} n \times [\mathbf{n} \times \mathbf{p}(\mathbf{n})] \]  

(22)

and

\[ \mathbf{B}_{\text{rad.}}(\mathbf{r}) = -n_{\text{sol}} \mathbf{n} \times \mathbf{E}_{\text{rad.}}(\mathbf{r}) \]  

(23)

where \( \mathbf{n} = \frac{\mathbf{r}}{r} \) is the unit vector in the direction of \( \mathbf{r} \).

\[ \mathbf{p}(\mathbf{n}) = \int d^3 r' e^{-in_{\text{sol}} \mathbf{r} \cdot \mathbf{r}'} \mathbf{d}(\mathbf{r}') \]  

(24)

with the integral restricted within the volume of the particle. The radiated power from the particle is then

\[ P(\hat{R}) = \lim_{r \to 0} \frac{1}{2} r^2 \int d^2 \mathbf{n} \mathbf{E}(\mathbf{r}) \times \mathbf{B}^*(\mathbf{r}) = \frac{n_{\text{sol}}^3 \omega^4}{32\pi^2} \int d^2 \mathbf{n} (\mathbf{p} \cdot \mathbf{p}^* - |\mathbf{n} \cdot \mathbf{p}|^2). \]

(25)

Averaging with respect to the orientation of the particle and summing up the contribution from all adsorbed particles, we obtain the total power radiation

\[ P = \sigma R^2 \int d^2 \hat{R} \overline{P(\hat{R})} \]  

(26)

and the line width \( \Gamma = \frac{P}{W} \), where \( W \) is the total energy of the WGM considered. For the geometry considered in the last section

\[ \Gamma = \frac{16\pi^3 \sigma [\Delta \epsilon v + (\Delta \epsilon' - \Delta \epsilon)v] \rho \eta}{3n_{\text{sol}}(n_{\text{cav}}^2 - n_{\text{sol}}^2) \lambda^4 R} \]  

(27)

with \( \eta \) another form factor whose explicit expression is displayed in the Appendix C. The line width of Eq. (27) carries a dimension of 1/time but the right hand side of Eq. (27) carries a dimension of 1/length. Therefore it will acquire a factor \( c \) if we do not set \( c=1 \).
4. Experiment

WGMs are excited in a silica microsphere ~340 μm in diameter (Fig. 1(a)). The microspheres are fabricated by melting the tip of an optical fiber in a butane/nitrous-oxide flame and selected by size after fabrication. Surface tension produces a sphere on a stem structure [4]. WGM are excited along the equator of the sphere by coupling from tapered optical fiber which is thinned by hydrofluoric acid erosion to a final diameter ~2-4 μm at the region where it evanescently couples to the microsphere cavity. The resonances are detected with an InGaAs photodiode as Lorentzian-shaped dips in the fiber transmission spectrum obtained by scanning a tunable coherent source (~1.311 μm center wavelength) coupled to one fiber end. Spectra containing 1000 points per scan are recorded every 10 ms at a resolution of 0.1 pm/point with a computer running Labview program. The resonance wavelength is determined from the minimum of the resonant line by polynomial fitting algorithm. The microsphere is immersed in a liquid sample cell filled with phosphate buffered saline (PBS) solution equilibrated at room temperature [4]. After acquiring baseline spectra, a bacterial PBS suspension is injected into the sample cell from where the bacteria start to adsorb to the microsphere surface.

Prior to use, the ampicillin resistant *E.coli* K12 bacteria are grown to saturation in Liquid Broth growth medium where most of them appear un-flagellated. They are then concentrated in an Eppendorf tube and washed three times in PBS by alternating sedimentation (~8000 rpm) and re-suspended with a micro-pipette. To promote bacterial adsorption, the silica microsphere is modified with a poly-l-lysine coating. For lysine coating, the microsphere is first cleaned for 4 minutes in an oxygen plasma sterilizer (Harrick). It is then immersed in a hanging drop of 0.01% poy-l-lyisine solution (Sigma) until dry, rinsed 5 minutes in PBS and immediately mounted in the sample cell. A charge coupled device (CCD) camera is fixed on an upright microscope and monitors bacterial adhesion by imaging of the microsphere surface with a 40x long working distance objective. Free-floating bacteria are unambiguously identified from the bound ones by discerning their Brownian motion. Bacterial surface densities are determined by counting the number of bacteria (typically between 0 and 100 counts) identified in the imaged surface area of ~10^5 cm^2, which is only a fraction of the ~0.36 mm^2 total sphere surface. The concentration of the bacteria is adjusted so that adsorption increases surface density at initial rate of ~5 × 10^3 cells / (cm^2 × seconds). The orientation and random distribution of the bacteria is confirmed by high-resolution images of green-fluorescent protein labeled bacteria (Fig. 1(b)). It is seen that the cylindrical bacteria are mostly bound with their axes aligned parallel (horizontal) to the surface. A few exceptions are indicated by arrows. Bacteria are irreversibly bound, and no unbinding events were observed over the time course of this experiment. We also would like to point out that our measurements do not discriminate between live and dead bacteria cells. It is, however, unlikely that the bacteria experience significant radiation damage upon binding since >94% of the mode energy resides in the silica cavity [5] and since the laser is not locked to a critically coupled resonance.

Figure 2 shows a series of transmission spectra obtained for successive time points (100 s intervals) during adsorption of *E.coli*. For each spectrum, the surface density of bound bacteria is determined from microscopic images. The overall wavelength shift is then plotted versus the surface density (data points in Fig. 3). The lines show the plots of analytic predictions (Eq. (19)) using the following parameters [21-25]: *E.coli* cylinder radius b = 0.4 μm and cylinder half length a = 1 μm, which corresponds to an average *E.coli* volume ~ 1 μm^3. The cell wall thickness is estimated at a-a’ = b-b’ = 42 nm, n_w =1.42, n_int. = 1.35 (which results in an average refractive index of *E.coli* n ≈ 1.37); n_sol = 1.33 and n_cav = 1.467, center wavelength λ = 1.311 μm, microcavity radius R = 170 μm. It is seen that the theory fits reasonably well with the measurement. The plot shows results of Eq. (19) for both polarizations (TE and TM).
Fig. 2. Shift of resonance wavelength and broadening of linewidth of a WGM due to adsorption of E.coli bacteria. The spectra were recorded in 100 s intervals. In parallel, bacteria surface densities where determined from optical micrographs.

In Fig. 3 we also plotted Eq. (19) in the long wavelength limit, where the size to wavelength ratio is small and the form factor $\kappa=1$ (only TE mode is shown). Given a wavelength shift that can be detected with sensitivity $\sim 1/50$ of the linewidth [4,9] and given the Q measured $\sim 4.3 \times 10^5$ for low bacterial surface density (Fig. 2), we estimate the current detection limit of our setup at $\sim 1.2 \times 10^2$ E.coli/mm$^2$, which corresponds to $\sim 44$ bacteria bound to the $\sim 0.36$ mm$^2$ total area of the sphere. With the average dry weight of an E.coli bacteria $\sim 2.8 \times 10^{-13}$ g, this corresponds to sensitivity for bacterial dry-mass loading $\sim 3.4 \times 10^{-11}$ g/mm$^2$.

In order to confirm the orientation of the E.coli bacteria on the resonators surface it would be interesting to estimate the effective footprint area which can be calculated by solving a thin film problem similar to [8]. If we consider a thin patch (thickness, t) of excess dielectric...
constant $\Delta\varepsilon = n_w^2 - n_{sol}^2$, and foot print area $A_{fp}$ to represent the effective *E.coli* cell wall patch that is in contact with the silica microsphere cavity (radius $R$ and refractive index $n_{cav}$), we have the starting point for a simple model. Beyond this patch, but contained within its foot print, is protoplasm having an excess dielectric constant $\Delta\varepsilon' = n_{int}^2 - n_{sol}^2$. Of course there are many patches on the surface (surface density $\sigma$), and the evanescent intensity penetrates only a characteristic length $L \approx (\lambda/4\pi)(n_{cav}^2 - n_{sol}^2)^{1/2}$. With this picture the wavelength shift for a TE mode is

$$\frac{\delta\lambda}{\lambda} = \frac{A_{fp}\sigma_b L}{(n_{cav}^2 - n_{sol}^2)R} \left[ 1 - \frac{e^{-t/L}}{n_w^2 - n_{sol}^2} + \frac{e^{-t/L}}{n_{int}^2 - n_{sol}^2} \right]$$

This equation is easy to solve for $A_{fp}$. For the case at hand, we calculate the effective footprint area of *E.coli* at $A_{fp} \approx 0.16 \, \mu m^2$, only about $1/10$th of its largest cylindrical area projection ($2a \times 2b = 2 \, \mu m \times 0.8 \, \mu m$). In comparison, for *E.coli* bound to the surface by one of its disc-shaped side walls, we would expect a much larger footprint $\pi b^2 \approx 0.5 \, \mu m^2$. The result is therefore consistent with the observation of *E.coli* bound with its axis aligned parallel (horizontal) to the surface (Fig. 1).

The coupling between the evanescent field and the adsorbed particles broadens the spectral line of a WGM, which is a second order effect in $\Delta\varepsilon$ and $\Delta\varepsilon'$ (Eq. (27)). Figure 4 shows the increase in line width, the slope of which can be estimated from the incoherent radiations from the adsorbed *E.coli* driven by the evanescent field of the incident WGM, Eq. (27). As in the case of the wavelength shift, we have $\eta = 1$ in the long wavelength limit. The agreement with the observation is up to the order of magnitude.

![Fig. 4. Measured fractional linewidth shift versus E.coli surface density (data points). The lines are plots of the analytic results for TE and TM modes. Also plotted is the calculated increase of fractional linewidth in the long wavelength limit (κ=1, TE mode).](image)

**5. Discussion and conclusion**

In conclusion, we have shown experimentally that adhesion of cylindrical bacteria at random horizontal orientation on high-Q optical microsphere sensors perturb resonance frequency as well as linewidth. The magnitude of the perturbations can be derived using Born-and Rayleigh-Gans approximations by introducing a form factor to account for the cylindrical bacteria shape. In the long wavelength limit, the theory is equivalent to the already established perturbative dipole approximation valid for nanometer-sized biomolecules. Horizontal
alignment of bound bacteria is confirmed from fluorescent images and consistent with an estimate of cell wall area in contact with the resonator. The resonance frequency shifts linearly with increase of bacteria surface density which establishes microsphere sensors as sensitive tool to detect bacteria and large viral species, label-free and in real time. The sensitivity of the current setup is measured at \(1.2 \times 10^2 \text{E.coli/mm}^2\), which corresponds to \(\sim 34\) pg/mm\(^2\) dry-mass loading or a total of 44 bacteria bound to the sphere surface at random location. With increase of Q-factor, decrease of mode volume and operation at favorable wavelength [26,27], such measurements can be performed on the single cell level; possibly in multiplexed, high-throughput experiments. Modification of the cavity surface with antibodies will allow for the specific detection of bacterial and viral species of ubiquitous cylindrical shape.

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**Appendix A**

*The Asymptotic behavior of spherical Bessel functions Eqs. (13) and (14):*

Combining the definition of a spherical Bessel function and the Debye formula for a Bessel function of a large argument and a large order [28], we have

\[
j_l(\nu \text{sech}\alpha) \equiv \frac{1}{2\nu} \left( \text{sech}\alpha \tanh\alpha \right)^{\frac{1}{2}} e^{\nu(\tanh\alpha - \alpha)} \quad (A1)
\]

and

\[
n_l(\nu \text{sech}\alpha) \equiv -\frac{1}{2\nu} \left( \text{sech}\alpha \tanh\alpha \right)^{\frac{1}{2}} e^{\nu(\alpha - \tanh\alpha)} . \quad (A2)
\]

We have

\[
\text{sech}\alpha = \frac{n_{\text{sol}} \omega r}{\nu} = \frac{n_{\text{sol}}}{n_{\text{cav}}} \left( 1 + \frac{r - R}{R} \right)
\]

at a WGM. In a vicinity just outside the cavity wall, \(0 < r - R < R\),

\[
\alpha \equiv \alpha_0 - \frac{r - R}{R} \coth\alpha_0 \quad (A4)
\]

where \(\text{sech}\alpha_0 = n_{\text{sol}}/n_{\text{cav}}\) and \(\nu \text{sech}\alpha_0 = n_{\text{sol}} \omega R\). Substituting Eq. (A4) into Eqs. (A1) and (A2), we derive Eqs. (13) and (14). Note that the second term of Eq. (A4) has to be retained in the exponent because of the large multiplicative factor \(\nu\).

*The Calculation of \(\frac{d\delta_{l}^{(0)}}{d\omega}\) at a WGM of TE polarization:*

It follows from Eq. (10) and Eq. (11) that at the resonance

\[
\begin{bmatrix}
j_l(\zeta) & n_l(z) \\
\end{bmatrix}
\begin{bmatrix}
j_{l}^{*}(\zeta) \\
\end{bmatrix} = 0
\]

(A5)

and then

\[
D_{l}(\omega) = D_{l}^{*}(\omega) \quad (A6)
\]
Therefore, the derivative of $\delta_i$ with respect to $\omega$ at the resonance reads

$$\frac{d\delta_i^{(0)}}{d\omega} = -\frac{d}{d\omega} \begin{vmatrix} j_i(\xi) & n_i(z) \\ j_i'(\xi) & n_i'(z) \end{vmatrix} j_i'(\xi)\frac{n_i(z)}{n_i'(z)}$$

(A7)

Eq. (A5) implies that

$$j_i(\xi) = \frac{\mathcal{N}_i(z)}{zn_i(z)} j_i'(\xi).$$

(A8)

For the denominator of Eq. (A7), we have

$$\begin{vmatrix} j_i(\xi) & j_i(z) \\ \mathcal{G}_i'(\xi) & j_i'(z) \end{vmatrix} = zj_i(\xi) j_i'(z) - \mathcal{G}_i'(\xi) j_i(z) = -\xi j_i(\xi) n_i(z)$$

\[= -\frac{\xi j_i(\xi)}{z^2 n_i(z)} \]

(A9)

where the Wronskian of spherical Bessel functions

$$\begin{vmatrix} j_i(\xi) & n_i(z) \\ j_i'(\xi) & n_i'(z) \end{vmatrix} = \frac{1}{z^2}$$

is substituted in the last step. Using the spherical Bessel equation

$$\frac{1}{z} [z j_i'(z)]'' + \left[ 1 - \frac{l(l+1)}{z^2} \right] j_i(z) = 0$$

(A11)

for $j_i(z)$ and the same equation for $n_i(z)$, we find

$$\left[ \mathcal{G}_i'(\xi) \right]' = -j_i'(\xi) + \xi \left[ \frac{l(l+1)}{\xi^2} - 1 \right] j_i(\xi)$$

(A12)

and

$$\left[ zn_i'(z) \right]' = -n_i'(z) + z \left[ \frac{l(l+1)}{z^2} - 1 \right] n_i(z).$$

(A13)

The numerator of Eq. (A7) becomes

$$\frac{d}{d\omega} \begin{vmatrix} j_i(\xi) n_i(z) \\ \mathcal{G}_i'(\xi) zn_i'(z) \end{vmatrix} = \frac{1}{\omega} \begin{vmatrix} j_i(\xi) & zn_i'(z) \\ \mathcal{G}_i'(\xi) & zn_i'(z) \end{vmatrix}$$

\[= (\xi^2 - z^2) j_i(\xi) n_i(z) = (\xi^2 - z^2) \frac{\mathcal{G}_i'(\xi)}{zn_i'(z)} n_i^2(z) \]

(A14)

where the resonance condition Eq. (A5) is used in the last two steps. Combining Eq. (A9) and Eq. (A14), we obtain that
\[
\frac{d\delta_i^{(0)}}{d\omega} = \frac{1}{\omega} z(\zeta^2 - z^2)n_i^2(z) \quad (A15)
\]

**Appendix B**

The electromagnetic field of a TM mode of the order \((l,m)\) reads:
\[
\begin{align*}
B_{lm}(r) &= f_i(\omega r)X_{lm}(\hat{r}) \\
E_{lm}(r) &= \frac{i}{\epsilon \omega} \nabla \times B_{lm}(r)
\end{align*}
\]  
(B1)

The Maxwell equation implies that the radial function \(f_i\) satisfies the equation
\[
-\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{df_i}{dr} \right) + \frac{l(l+1)}{r^2} f_i - \epsilon \omega^2 f_i + \frac{1}{\epsilon} \frac{d}{dr} \left( \frac{1}{r} \frac{df_i}{dr} \right) f_i = 0
\]  
(B2)

subject to the joining condition at \(r = R\),
\[
f_i \bigg|_{r=R^-} = f_i \bigg|_{r=R^+}
\]  
(B3)

and
\[
\frac{1}{\epsilon_{cav.}} \frac{d}{dr} (rf_i) \bigg|_{r=R^-} = \frac{1}{\epsilon_{sol.}} \frac{d}{dr} (rf_i) \bigg|_{r=R^+}
\]  
(B4)

Repeating the procedure for a TE mode in the section 2, we find that
\[
\sin \Delta \delta_i = n_{sol.} \omega \int_0^\infty dr f_i^{(0)} \left[ \omega^2 \overline{\delta \epsilon_i} f_i - \frac{1}{\epsilon r} \frac{d}{dr} (rf_i) \right]
\]  
(B5)

where \(f_i^{(0)}\) is the radial function in the absence of \(\overline{\delta \epsilon}\). Perturbatively, we may write
\[
\sin \Delta \delta_i \equiv \Delta \delta_i \equiv \frac{d\delta_i^{(0)}}{d\omega}\Delta \omega
\]
and set \(f_i = f_i^{(0)}\) and \(\epsilon = \epsilon_{sol.}\) on RHS, where the zeroth order phase shift of a TM mode is given by
\[
e^{2i\delta_i^{(0)}} = -\frac{D_i^r(\omega)}{D_i(\omega)}
\]  
(B6)

with
\[
D_i'(\omega) = \begin{bmatrix}
j_i(z) & h_i^{(1)}(z) \\
\frac{1}{\zeta} [\gamma_i(z)]' & \frac{1}{\zeta} [\gamma h_i^{(1)}(z)]'
\end{bmatrix}
\]  
(B7)

Upon integrating the \(2^{nd}\) term inside the bracket on RHS of Eq. (B5) and substituting the equation satisfied by \(f_i^{(0)}\), we end up with the formula
\[
\frac{\Delta \lambda}{\lambda} = -\frac{\Delta \omega}{\omega} \frac{1}{n_{sol.}} \frac{d\delta_i^{(0)}}{d\omega} \int_0^\infty dr f_i^{(0)} \left[ l(l+1) f_i^{(0)^2} + \left( \frac{d}{dr} (rf_i^{(0)}) \right)^2 \right].
\]  
(B8)
After a lengthy manipulation but straightforward with the aids of the approximations Eqs. (13) and (14), we find that the RHS. of Eq. (B8) is simply that of a TE mode multiplied by the factor $2 - \frac{n_{\text{sol}}}{n_{\text{cav}}}$.

**Appendix C**

The explicit expressions of the form factor of line width of a WGM of TE polarization is given by

$$\eta = \frac{3}{16} \int_0^{2\pi} \int_0^{\frac{\pi}{2}} d\alpha d\phi \cos \theta \frac{\Delta \nu g(\xi, \eta, \zeta) + (\Delta \nu' - \Delta \nu) v'(\xi', \eta', \zeta')}{\Delta \nu + (\Delta \nu' - \Delta \nu) v'} e^{-\frac{1}{2} \sin^2 \theta},$$

where $\xi = \frac{2\pi b}{\lambda} (\sin \theta \cos \phi - i \sinh \gamma)$,

$$\eta = \frac{2\pi b}{\lambda} \left[ - \cos \theta \sin \alpha + (\sin \theta \sin \phi - \cosh \gamma) \cos \alpha \right],$$

and

$$\zeta = \frac{2\pi a}{\lambda} \left[ \cos \theta \cos \alpha + (\sin \theta \sin \phi - \cosh \gamma) \sin \alpha \right] \text{ with } \cosh \gamma = \frac{n_{\text{cav}}}{n_{\text{sol}}}.$$ The quantities $\xi', \eta', \zeta'$ are obtained from $\xi, \eta, \zeta$ by replacing $a$ and $b$ with $a'$ and $b'$. The function $g$ is related to the Bessel function as $g(\xi, \eta, \zeta) = 2 J_1(\sqrt{\xi^2 + \eta^2}) \sin \zeta \frac{\xi}{\sqrt{\xi^2 + \eta^2}} \zeta$. 

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