

Whispering gallery mode bio-sensor for label-free detection of single molecules: thermo-optic vs. reactive mechanism

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Abstract: Thermo-optic and reactive mechanisms for label-free sensing of bio-particles are compared theoretically for Whispering Gallery Mode (WGM) resonators (sphere, toroid) formed from silica and stimulated into a first order equatorial mode. Although it has been expected that a thermo-optic mechanism should “greatly enhance” wavelength shift signals [A.M. Armani *et al*, Science **317**, 783-787 (2007)] accompanying protein binding on a silica WGM cavity having high Q (10^8), for a combination of wavelength (680 nm), drive power (1 mW), and cavity size (43 μ m radius), our calculations find no such enhancement. The possible reasons for this disparity are discussed.

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1. Introduction

The research field of label-free Whispering Gallery Mode (WGM) Bio-sensing is now well established [1,2]. A reactive mechanism based on a refractive index perturbation on the WGM

frequency by bound particles [3] is responsible for almost all of the research thus far. The possibility of detecting single protein was first theorized based on this mechanism [3]. More recently researchers reported protein frequency shifts more than 1000 times the reactive projections, and claimed that these signals gave excellent theoretical agreement with a thermo-optic mechanism [4]. This paper was met with great fanfare [5,6]. Surprisingly, in the more than two years since the publication of Ref. [4] there has been no follow up. In what follows we investigate the physics of these two mechanisms, and conclude that the thermo-optic effect is far too small to account for the wavelength shifts measured in Ref. [4]. The possible reasons for this disparity are discussed.

2. Theory

Antigens are detected by a WGM sensor when antibodies immobilized on the sensor surface capture them, and interact with the resonant microcavity both reactively [3] and thermo-optically [4]. Comparison of the two interaction mechanisms is aided by carefully examining the WGM biosensor system.

Energy is injected into the WGM of a microsphere or toroid by evanescently coupling power P from an optical fiber. In the reactive mechanism a tiny change in phase occurs in the light orbit as the wave polarizes an antigen that has entered the evanescent field of the WGM. This interaction involves the real part of the polarizability of the antigen, $\text{Re}[\alpha]$. Since the interaction simply changes the local refractive index (RI), the phase shift and the resultant frequency shift of the resonant state are independent of the circulating power in the cavity. The thermo-optic mechanism as described in Ref. 4 is in stark contrast since its frequency shift is proportional to P . The latter mechanism works by heating the antigen through linear absorption and transferring some of this heat to the resonator. Local heating of the resonator is thereby proportional to the imaginary part of the polarizability, $\text{Im}[\alpha]$, and causes an additional change in RI with temperature T as characterized by the thermo-optic coefficient dn/dT . Our goal is to estimate the relative magnitude of the thermo-optic vs. reactive frequency shift for an antigen binding to an antibody immobilized on the equator of a silica microcavity driven into its equatorial mode, while in an aqueous environment.

We first choose a spherical WGM resonator since its high symmetry allows for analytical solutions. In fact, the first-order reactive perturbation has already been worked out [3,7] and shown to agree with experimental data [8,9]. As for the thermo-optic mechanism, it has only been described for a micro-torus for which an explicit solution has not been presented [4]. After arriving at generalized results for the micro-sphere we will return to a discussion of numerical results for the micro-torus.

We start by estimating the strength of the heat source h generated by absorption of energy from the WGM by the antigen molecule at position $\mathbf{r}_a$. This heat is produced when the electric field at the antigen at frequency ω , $\mathbf{E}(\mathbf{r}_a, t) = \mathbf{E}_0(\mathbf{r}_a) \exp(i\omega t)$, drives the out-of-phase component of the induced dipole moment $\mathbf{p}$; $h = \langle \mathbf{E}(\mathbf{r}_a, t) \cdot \partial \mathbf{p} / \partial t \rangle$, where $\langle \dots \rangle$ is the time average over one cycle. Since $\mathbf{p} = \alpha \mathbf{E}(\mathbf{r}_a, t)$, we find

$$h = \frac{1}{2} \omega \text{Im}[\alpha] |\mathbf{E}_0(\mathbf{r}_a)|^2. \quad (1)$$

Here we assume as in Ref. [4] that the “quantum efficiency” for generating heat is one (*i.e.* scattering is minimal). The imaginary component of the polarizability is proportional to the absorption cross-section σ of a given molecule [$\text{Im}[\alpha] = (\epsilon_0 n_m / k) \sigma$, where n_m is the environmental refractive index and k the free-space wave-vector] and can be estimated from the attenuation of light through a cuvette filled with the associated solution.

The next step is to relate $\mathbf{E}_0(\mathbf{r}_a)$ with the power driving the sphere. This is most easily done by using the energy stored in the resonator as an intermediary. As a consequence energy builds up in the sphere, until the power dissipated equals the power coupled in. In this steady state, the WGM will have the energy W_m given as

$$W_m = PQ / \omega, \quad (2)$$

where Q is the total quality factor of the resonator system and P is the power driving the mode. The relationship between W_m and $\mathbf{E}_0(\mathbf{r}_a)$ is found as follows.

Consider a WGM in a microsphere of radius R and RI = n_s in a medium of RI = n_m . Its modal energy is principally contained within the microsphere [3], and can be readily approximated as

$$W_m \cong \frac{1}{2} \varepsilon_0 n_s^2 \int |\mathbf{E}_0(\mathbf{r})|^2 dV, \quad (3)$$

where ε_0 is the vacuum permittivity, and the integral is carried out within the sphere. We will concentrate on the first order equatorial TE mode for which the azimuthal quantum number m and the angular momentum quantum number l are equal: $m = l$. This mode is the one most similar to the toroidal mode in Ref. 4 with only one polar angular lobe of intensity at the equator and one radial internal peak near the surface. For large l , the square modulus of the electric field within the sphere is

$$|\mathbf{E}_0(\mathbf{r})|^2 = c_m [j_l(n_s kr)]^2 |Y_l(\hat{\mathbf{r}})|^2 \quad (r \leq R), \quad (4)$$

where $j_l(z)$ is a spherical Bessel function, $r = |\mathbf{r}|$ ($r = 0$ at the sphere center), $\hat{\mathbf{r}} = \mathbf{r} / r$, Y_l is the spherical harmonic function associated with the equatorial mode, and c_m is a proportionality constant [3].

The field exterior to the sphere decays exponentially from the surface with a decay constant $\Gamma \cong k(n_s^2 - n_m^2)^{1/2}$. At the receptor of radius a on the x axis (Fig. 1),

$$|\mathbf{E}_0(\mathbf{r}_a)| = |\mathbf{E}_0(R\hat{\mathbf{x}})| \exp(-\Gamma a). \quad (5)$$

The volume integral in Eq. (3) can be separated into a product of the integral over solid angle Ω and the integral over r . Since Y_l is normalized with respect to Ω , Eq. (3) reduces to

$$W_m \cong \frac{1}{2} c_m \varepsilon_0 n_s^2 \int_0^R [j_l(n_s kr)]^2 r^2 dr. \quad (6)$$

On resonance, the radial integral in Eq. (6) may be asymptotically ($kR \gg 1$) related to the surface value of j_l^2 through $\int_0^R [j_l(n_s kr)]^2 r^2 dr \cong (R^3 / 2)(1 - n_m^2 n_s^{-2}) [j_l(n_s kR)]^2$ [3]. With this relationship Eq. (5) can now be recast as

$$|\mathbf{E}_0(\mathbf{r}_a)|^2 \cong \frac{4 |Y_l(\hat{\mathbf{x}})|^2 \exp(-2\Gamma a)}{\varepsilon_0 (n_s^2 - n_m^2) R^3} W_m. \quad (7)$$

The heat generated by receptor absorption (Eq. (1) can now be evaluated in terms of experimental parameters by utilizing Eqs. (2) and 7;

$$h = \frac{2 \text{Im}[\alpha / \varepsilon_0] |Y_l(\hat{\mathbf{x}})|^2 \exp(-2\Gamma a)}{(n_s^2 - n_m^2) R^3} PQ. \quad (8)$$

One clearly sees the major role of Q in heat generation. $|Y_l(\hat{\mathbf{x}})|^2$ is proportional to $l^{1/2}$ and $l \cong n_s kR$ for large l [3], indicating that the heat source grows considerably in strength as R is reduced. At some point the Q factor will fall due to increased diffraction. Next we calculate the temperature elevation caused by the heat source in Eq. (8).

The delta-function heat plume will be conducted both into the silica and the surrounding medium. Bio-sensing experiments are carried out over seconds. By contrast thermal relaxation

takes microseconds. So all we need to calculate is the steady state temperature distribution $T(\mathbf{r})$. This is most easily arrived at from the solution to

$$\kappa \nabla^2 T = -h \delta(\mathbf{r} - \mathbf{r}_a), \quad (9)$$

where κ is the thermal conductivity (κ_s and κ_m within the sphere and in the surroundings, respectively). Since $a \ll R$, a simple “flat earth” hypothesis is reasonable. By analogy with electrostatics the heat source may be treated as a point charge near a dielectric interface for which the solution appears in Ref.[10]. The “thermal charge” h and its image are used to satisfy the boundary conditions at the interface for the temperature and its normal derivative [11].

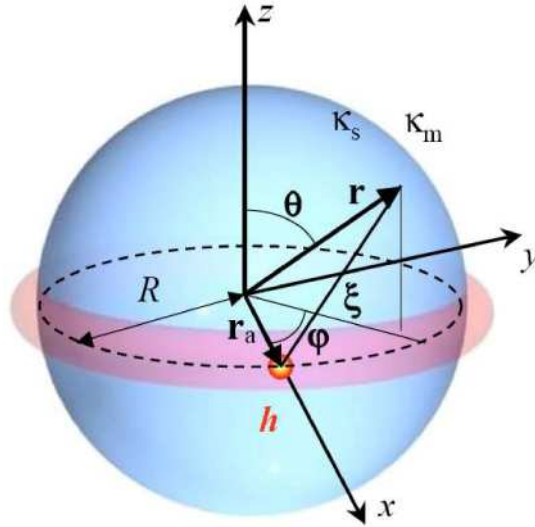


Fig. 1. Heat source h at the surface of a microsphere. Through thermal conduction, the source establishes spatial distribution of temperature.

The temperature elevation within the microsphere δT is simply a function of the distance ξ from the heat source, and is given as

$$\delta T = \frac{h}{2\pi\xi(\kappa_s + \kappa_m)}. \quad (10)$$

where

$$\xi \equiv [r^2 + (R + a)^2 - 2r(R + a)\sin\theta\cos\phi]^{1/2}. \quad (11)$$

The temperature increase in Eq. (10) causes a change in RI within the microsphere through the thermo-optic effect, $\delta n = (dn/dT)\delta T$. Altogether the RI change is

$$\delta n = \frac{dn/dT}{\pi\xi(\kappa_s + \kappa_m)} \frac{\text{Im}[\alpha/\epsilon_0]PQ}{(n_s^2 - n_m^2)R^3 c_{in}[j_l(n_s kR)]^2} |\mathbf{E}_0(\mathbf{r}_a)|^2. \quad (12)$$

where Eqs. (4), 5, 8 and 10 were used. This complicated equation provides all of the essential dependences. The distance ξ can be no smaller than a (~ 1 nm). We can use Eq. (12) to estimate the frequency shift $\delta\omega$. Based on the formalism developed for the reactive effect [7,12]:

$$\frac{\delta\omega}{\omega} = -\frac{\int \delta([n(\mathbf{r})]^2) \mathbf{E}(\mathbf{r}) \cdot \mathbf{E}_0^*(\mathbf{r}) dV}{2 \int [n(\mathbf{r})]^2 |\mathbf{E}_0(\mathbf{r})|^2 dV}. \quad (13)$$

where $[n(\mathbf{r})]^2$ is the relative permittivity at $\mathbf{r}$ which changes by $\delta([n(\mathbf{r})]^2)$, and $\mathbf{E}(\mathbf{r})$ is the field after the RI change. Equation (13) applies to both the thermo-optic and reactive cases. Since the perturbation in refractive index is in the numerator, and the denominators are identical for both cases, a comparison of the frequency shifts simply involves finding the ratio of the numerator for the thermo-optic case to the numerator for the reactive case. In what follows we will evaluate the numerator for each in turn, and label them N_t and N_r , respectively.

For the thermo-optic case it is convenient to write $\delta(n^2) = 2n\delta n$. For the TE mode, $\mathbf{E}$ is just $\mathbf{E}_0$ in the silica, and the numerator is

$$N_t = 2n_s \int \delta n(\mathbf{r}) |\mathbf{E}_0(\mathbf{r})|^2 dV. \quad (14)$$

We next express ζ in the spherical coordinates (see Fig. 1). Then, Eq. (14) becomes

$$N_t = \frac{\eta I_l}{R} \text{Im}[\alpha/\varepsilon_0] |\mathbf{E}_0(\mathbf{r}_a)|^2 PQ, \quad (15)$$

where

$$\eta \equiv \frac{2n_s (dn/dT)}{\pi(n_s^2 - n_m^2)(\kappa_s + \kappa_m)} \quad (16)$$

is determined by the materials only, and I_l is a dimensionless quantity defined as:

$$I_l \equiv [\psi_l(n_s kR)]^{-2} \int_0^R [\psi_l(n_s kr)]^2 dr \int d\Omega \xi^{-1} |Y_{ll}(\hat{\mathbf{r}})|^2, \quad (17)$$

where $\psi_l(z) = z j_l(z)$, and $d\Omega = \sin\theta d\theta d\phi$.

For the reactive case the antigen bound to the equator represents a local change in the polarization $\delta\mathbf{P} = \varepsilon_0 \delta(n^2) \mathbf{E}$ equivalent to its induced dipole moment per unit volume $\text{Re}[\alpha] \mathbf{E}_0 / V_a$. Consequently $\delta(n^2) \mathbf{E} = \text{Re}[\alpha / \varepsilon_0] \mathbf{E}_0 / V_a$, and integration over the antigen's volume V_a in the numerator of Eq. (13) gives

$$N_r = \text{Re}[\alpha / \varepsilon_0] |\mathbf{E}_0(\mathbf{r}_a)|^2. \quad (18)$$

All we have left to do is to compare the thermo-optic frequency shift $(\delta\omega)_t$ to the reactive frequency shift $(\delta\omega)_r$ through the ratio N_t/N_r ,

$$\frac{(\delta\omega)_t}{(\delta\omega)_r} = \frac{N_t}{N_r} = \frac{\eta I_l}{R} \frac{\text{Im}[\alpha / \varepsilon_0]}{\text{Re}[\alpha / \varepsilon_0]} PQ. \quad (19)$$

Equation (19) is surprisingly simple and ripe for evaluation. First let's deal with the factor η . For silica in water $n_s = 1.454$, $n_m = 1.329$, $\kappa_s + \kappa_m = 1.88 \text{ Wm}^{-1}\text{K}^{-1}$, $dn/dT = 1.3 \times 10^{-5} \text{ K}^{-1}$, and therefore $\eta = 1.84 \times 10^{-5} \text{ m/W}$. We will take R , P , and Q to be $43 \mu\text{m}$, 1 mW , and 10^8 respectively as our standard parameters, close to the parameters used in the paper by A. M. Armani *et al* [4]. For an antigen bound to the equator the dimensionless I_l factor is found to be 0.14. All that we require now is to find the "loss-tangent" ($\text{Im}[\alpha / \varepsilon_0] / \text{Re}[\alpha / \varepsilon_0]$) for a characteristic protein [*e.g.* Streptavidin, Bovine Serum Albumin (BSA)]. The real part $\text{Re}[\alpha / \varepsilon_0]$ is typically determined from the change in refractive index upon adding protein to solution, whereas the imaginary part $\text{Im}[\alpha / \varepsilon_0]$ is determined from the solution's optical absorption. BSA is a good choice for calculating the frequency shift ratio in Eq. (19) since

refractive index data allow us to determine that $\text{Re}[\alpha/\epsilon_0]=4.8\times 10^{-20}\text{cm}^3$ [3], and we have determined $\text{Im}[\alpha/\epsilon_0]$ at 680 nm from our measurements of absorbance in solution to be $1.3\times 10^{-24}\text{cm}^3$ [13]. We find the ratio of the thermo-optic to reactive frequency shift to be 0.16. Surprisingly, the thermo-optic effect is smaller than the reactive effect in the presence of 100W of circulating power! A similar result is found for Streptavidin, a protein used in Ref. 4.

This small thermo-optic effect in relation to the reactive effect for BSA for the standard parameters is particularly surprising considering the anticipated enhancement in Ref. 4. It should be pointed out that by taking the ratio in Eq. (19) we have eliminated the explicit dependence on mode volume; both the thermo-optic and reactive effects are inversely proportional to mode volume. In this way we have reduced the dependence on the type of WGM resonator. However there will be a small dependence in the integral I_l . This integral represents the surface normalized overlap between the thermal plume and the WGM ring of intensity. One can construct a similar integral for the toroidal case for which the wavefunctions are obtained from FEM (*e.g.*, Comsol) numerical solutions to the vector Helmholtz equation. The value for I_l obtained for the toroidal mode in Ref. [4], for our standard parameters (the overall radius of the toroidal resonator is 43 μm), is only slightly different than that for the equatorial mode of a micro-spheroid having the same overall size (*i.e.* 0.17 vs. 0.14). So the ratio of the thermo-optic to reactive shift for the toroid is 0.19. By using FEM we also calculated the ratio of the reactive shift for the toroid to that of the sphere to be 2.0. By putting all of these ratios together we find that the thermo-optic shift of the toroid to the reactive shift of the microspherical resonator for BSA should be 0.38. However the thermo-optic shifts calculated for the toroidal resonator in Ref. 4 are extraordinarily large in comparison to the corresponding reactive shifts that we calculate for a microspherical resonator having the same overall size [3]. For example, for Streptavidin this ratio is ~ 5000 . A large part of the explanation for this disparity lies in the reported molecular absorption cross-sections in Ref. 4.

For a Rayleigh sized particle the imaginary part of the polarizability $\text{Im}[\alpha]$ may be expressed in terms of an absorption cross section σ as we have pointed out earlier, and the thermo-optic shift would be proportional to σ . Although this is explicitly stated by Armani *et al* [4], we believe that the cross sections used in Ref. 4 to support the thermo-optic mechanism are “massively” inflated!

As an example we return to the case of the protein Streptavidin. The authors of Ref. 4 report a spectroscopic measured absorption cross-section for Streptavidin at 680 nm of $2\times 10^{-16}\text{cm}^2$. Our own absorbance measurements on a 0.1 mM solution of Streptavidin (Invitrogen 43-4302) find this cross-section to be $1.5\times 10^{-19}\text{cm}^2$, a factor of 1300 times smaller than that in Ref. [4]. This relatively small cross-section is not uncommon for protein in the red portion of the spectrum. The NIST standard for protein is BSA (Bovine Serum Albumin, SRM 927d) [13]. BSA has a molecular weight comparable to Streptavidin and from the NIST absorbance data at 600 nm, BSA’s absorption cross-section is $0.7\times 10^{-19}\text{cm}^2$. Measurements on 7% BSA (mass) in PBS (Phosphate Buffered Saline) agreed with NIST’s measurements and were used to validate our measurement procedure. Our Streptavidin cross section plugged into Armani’s theoretical equation would yield a wavelength shift less than one thousandth of what is calculated and measured in Ref. [4].

How about the non-protein cross sections? The Cy5-antigen used in Ref. 4 is reported to have a spectroscopic measured absorption cross section of $4\times 10^{-14}\text{cm}^2$ at 680 nm. Such a cross-section for a dye molecule with oscillator strength ~ 1 and having Cy5’s fluorescence decay rate and spectral width (in solution at room temperature) is unprecedented and unphysical [14]. Indeed, there is supporting data for a much smaller cross section. An absorption cross section at 680 nm for Cy5 molecule attached to DNA of $\sim 0.7\times 10^{-16}\text{cm}^2$ can be extracted from data in Ref. [15]. Finally from Invitrogen molar extinction data taken on the Cy5-antigen we have found the cross section at 680 nm to be 10^{-16}cm^2 . This Cy5-antigen cross section plugged into Armani’s theoretical equation would yield a wavelength shift about

1/400 of what is calculated and measured in Ref. [4]. This effect is expected to be further lowered due to excited state saturation as well as emission; saturation reduces the effective absorption cross section [14] and emission reduces the amount of absorbed energy converted to heating the surroundings.

3. Conclusion

We have provided theory that directly compares the thermo-optic to the reactive mechanisms for label-free biosensing with WGM resonators. We arrive at an expression that compares these two mechanisms for a given WGM resonator independent of mode volume. The application of this theory produces surprising conclusions with respect to Ref. 4.

For the conditions presented in Ref. 4 (*i.e.* standard parameters) our calculations show that the thermo-optic mechanism associated with linear absorption is smaller than its reactive counterpart, and cannot account for even one thousandth of the reported frequency shifts attributed to single protein binding.

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