DNA Detection by Differential Perturbation of two Microsphere Cavities

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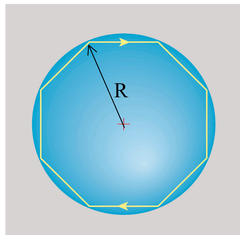
1 Abstract.

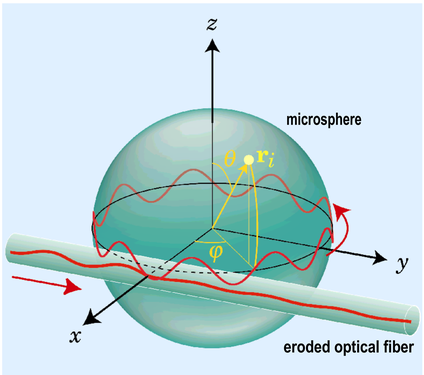
We report the detection of unlabeled DNA oligonucleotides on microsphere probes from a response of their photonic resonance modes. Narrow linewidth resonant modes (called whispering-gallery-modes, WGMs) occur from a light ray orbitting inside the microsphere due to total internal reflection. The resonance wavelength increases when dielectric material such as nucleic acid is added on the microparticles surface. Each microsphere is modified with an oligonucleotide probe of interest. The kinetics of an hybridization event is detected with millisecond time resolution from an increase of a sphere specific resonance wavelength. We demonstrate that the narrow, microsphere specific resonances are separated by several linewidths which allows multiplexing of signals from two (or more) different microparticle probes coupled to the same waveguide. We show that the differential signal of two microspheres can be used to identify a single nucleotide mismatch in a label-free 11-mer oligonucleotide. This approach to hybridization measurements is particularly sensitive, with the typical differential signal for a single nucleotide mismatch at 54:1.

2 Whispering Gallery Modes.

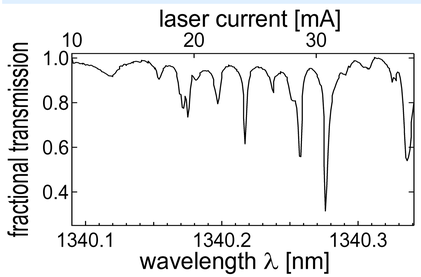
A light ray can be confined inside a dielectric sphere due to total internal reflection at the sphere surface. The long confinement time (high Q) allows the light to circumnavigate the sphere for many orbits.
If used for biosensing, such an optical resonance (WGM) enables the light to interact with the same analyte molecule for several thousand times. The resonance thus improves the detection limit by orders of magnitude as compared to existing single pass techniques. The ultimate detection limit of an optical cavity has been estimated to be on the order of a single, unlabeled macromolecule.



3 Detection Principle.

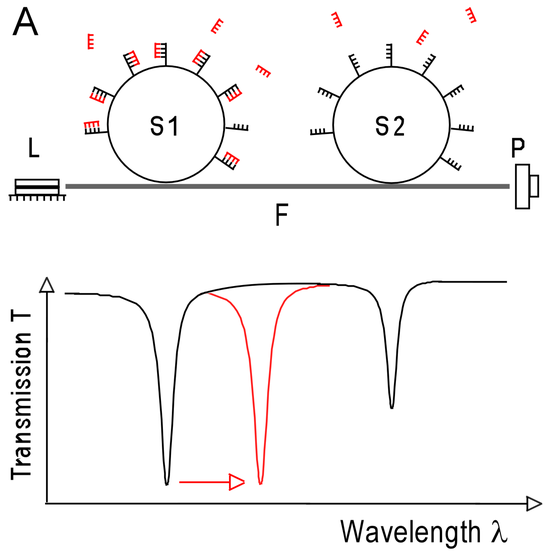


Resonances in glass microspheres can be excited by the light transmitted through an optical fiber.
Coupling of light between fiber and sphere occurs only for specific resonance wavelengths.
Experimentally, a resonance is detected as a dip in the spectrum of the light intensity transmitted through the fiber-sphere system.
Detection of biomolecules is possible due to the evanescent field which extends from the microsphere surface. Dielectric material such as DNA and protein molecules polarize when entering the evanescent field of the microsphere. This perturbation of the optical cavity leads to a red shift of a given resonance wavelength.

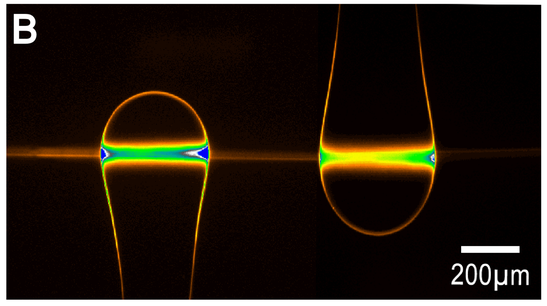


4 Setup

(A) Light from a tunable laserdiode L (1.3 µm) is transmitted through a single mode optical fiber F. Two silica microspheres S1 and S2 are evanescently coupled to the fiber. A photodetector P records the intensity at the other fiber end. Optical resonances from each sphere are identified as Lorentzian dips in the transmission spectrum. A hybridization event of a label-free oligonucleotide on sphere S1 is detected by an increase of the S1-specific resonance wavelength.



(B) Micrograph of two spheres coupled to the same optical fiber running horizontally through the center of the image. The image shows resonances of light orbitting inside each sphere.



5 Theory.

$$\frac{\delta\lambda}{\lambda} \approx \frac{\alpha_{ex}\sigma_s}{\varepsilon_0 (n_1^2 - n_2^2) R}$$

- λ ... wavelength (1.3 µm)
- $\delta\lambda$ ... wavelength shift
- α_{ex} ... excess polarizability of the bound protein
- σ_s ... surface density of the bound protein
- n_1, n_2 ... refractive indices of the sphere and the buffer solution, respectively
- R ... orbital radius
- ε_0 ... vacuum permittivity

References:
"Protein detection by optical shift of a resonanc microcavity", *Applied Physics Letters* 80 (**21**), 2002, 4057-4059
"Shift of Whispering Gallery Modes in Microspheres by Protein adsorption", *Optics Letters* 28 (**4**), 2003, 272-274

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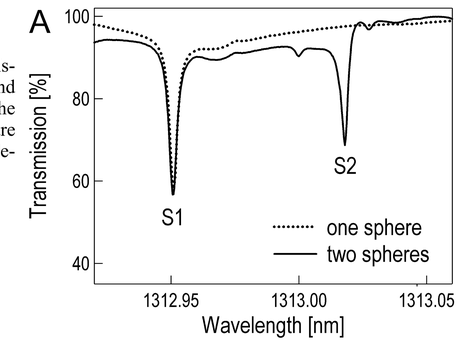
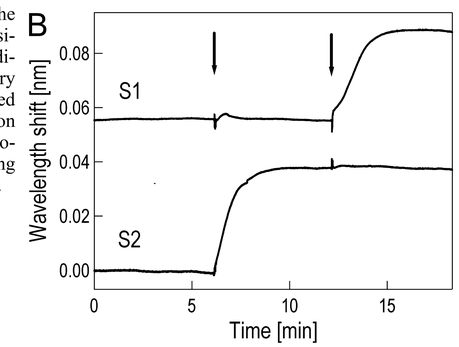
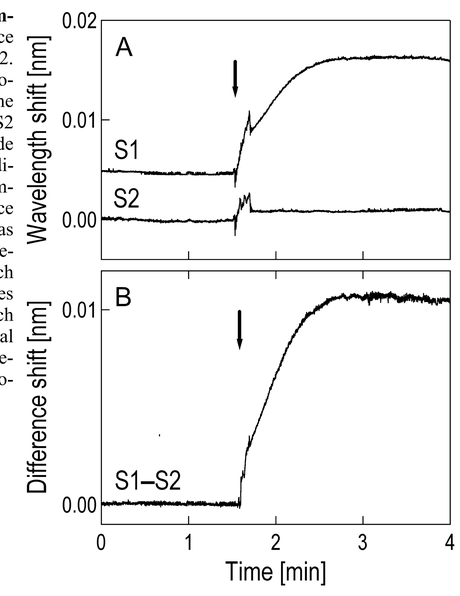
6 Results.

Sphere Multiplexing. (A) Transmission spectrum for one (dotted line) and two (solid line) spheres coupled to the same optical fiber. Both spheres are modified with 27-mer oligonucleotides.



Shift of Resonances. (B) Shows the time trace of the two resonance positions from S1 and S2. The arrows indicate when the two complementary DNA oligonucleotides were injected into the sample solution. Hybridization saturates within minutes and the resonance wavelength of the corresponding sphere increased about .038 nm each.



Single nucleotide mismatch discrimination. (A) Time traces of resonance wavelengths in two spheres S1 and S2. S1 was modified with a 11-mer oligonucleotide (CTATCTCAGTC). The oligonucleotide immobilized on S2 differed by a single nucleotide (CTAT**A**TCAGTC). The arrow indicates when the oligonucleotide complementary to the sequence immobilized on sphere S1 was injected. In equilibrium, the wavelength shift for the perfect match sequence was approximately ten times as large as the shift for the mismatch sequence. (B) The difference signal allows one to identify a single nucleotide mismatch with a high signal-to-noise of 54.