Experimental demonstration of application of ring down measurement approach to microcavities for biosensing

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ABSTRACT

Cavity ring down measurement approach is a promising technique for biosensing as it is insensitive to intensity fluctuations of a laser source. This technique in conjunction with ultra high Q microcavities have a great potential for ultra sensitive biosensing. Until now, most work on microcavity biosensors has been based on measurement of the resonant frequency shift induced by binding event on surface of the microcavity. Such measurements suffer from the noise due to intensity fluctuations of the laser source. However, the binding event will also introduce shift in quality factor of the microcavity, which can be tracked by using cavity ring down spectroscopy. In this work, we report on experimental demonstration of application of ring down measurement approach to microcavities for biosensing by tracking disassociation phase of a biotin-streptavidin reaction. These measurements were performed by using a bioconjugated ultra high Q microtoroidal cavity immersed in a liquid microacquarium. We found that disassociation curves agree with previously reported results on the protein kinetics measurements.

Keywords: Cavity ring down, microcavity, biosensing

1. INTRODUCTION

Optical biosensors are widely used for detection of biomarkers. Various platforms have been demonstrated for label free, real time and integrated optical biosensors.[1] Among these platforms, microcavity biosensors with ultra high quality factors ($Q \sim 10^7 - 10^9$) have the potential to offer highly sensitive diagnostic platforms. Because of their large photon life time, the circulating light will sample the binding event many times and as a result higher sensitivity can be achieved as compared to the other approaches. In 2001, Ilchenko and Maleki demonstrated the first microcavity sensor,[2] since then researchers have demonstrated a variety of the microcavity biosensors for detecting various biological events.[3,4]

In a microcavity biosensor, the binding event causes a shift in both the resonant wavelength and the quality factor (Q). However, in most of the demonstrated microcavity biosensors so far, the resonant wavelength is tracked as a function of the binding event and in few instances,[5,6] the quality factor was measured by linewidth measurements for various concentrations of the analytes. All of these configurations suffer from noise due to the intensity fluctuations of the laser source. In linewidth measurements, an additional noise source due to fitting of the Lorentzian curves to the resonant peaks is also present. These noises can be eliminated by applying cavity ring down spectroscopy to microcavity biosensors.

Cavity ring down spectroscopy (CRDS) is a widely used technique in gaseous phase for sensing absorption of gasses[7] and is a promising technique for biosensing applications.[8] This technique, by definition, is insensitive to the intensity fluctuations of the laser source as the rate of decay of the ring down time signal is measured. Thus by applying CRDS to the microcavities in a biosensor, better noise immunity, and thus higher sensitivity,

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can be achieved. The goal of this work is to provide an experimental demonstration of application of ring down measurement approach to microcavities for biosensing.

In our work, we show simultaneous measurement of the quality factor and the wavelength shift by using phase shift cavity ring down spectroscopy (PS-CRDS) in a biosensor by tracking changes in these parameters for disassociation phase of biotin-streptavidin system in a liquid flow cell containing a bioconjugated microtoroid. We found that the disassociation curves agree with the previously reported results.

This paper begins with the background remarks on microcavities and the cavity ring down spectroscopy in section 2. The process flow to fabricate the toroidal microcavities is shown in section 3. In section 4, various steps involved in the surface functionalization of the microtoriod with the Biotin are provided. The experimental setup and results are presented in section 5 and finally conclusions are outlined in section 6.

2. THEORETICAL BACKGROUND

In this section, fundamentals behind microcavities and the cavity ring down spectroscopy are briefly reviewed.

2.1 Whispering Gallery Mode Microcavities

In microcavities, optical mode (whispering gallery mode , WGM) does not occupy the whole volume of the cavity; but it travels along the circumference of the cavity in such a way that major portion of the mode lies inside the cavity (figure 1). A tapered optical fiber[9] along with a tunable laser source can be used to couple the light inside the cavity. Depending upon the input wavelength range and the cavity geometry, only specific wavelengths known as resonant wavelengths($\lambda_{resonant}$) can be coupled. The transmission spectrum of the cavity coupled with the tapered fiber contains the Lorentzian dips which are characterized by their resonant wavelength and FWHM ($\Delta\lambda$) values. For biosensing, small portion of the WGM lies outside the cavity that interacts with a biological event and changes the resonant wavelength and the quality factor. These changes are then tracked as a function of the binding event.

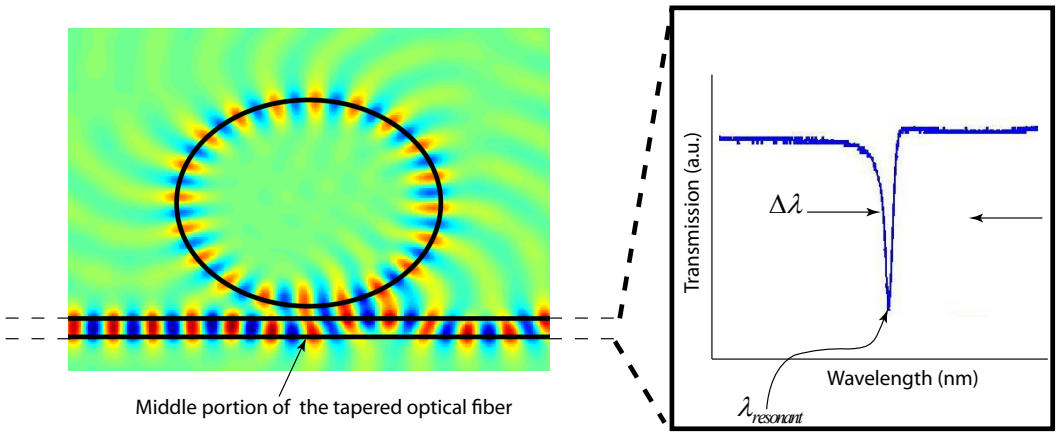


Figure 1. A microcavity coupled with a tapered optical fiber. A typical transmission spectrum is also shown at the fiber output. For clarity, a microcavity with the low quality factor is shown.

The Quality factor (Q) of a microcavity is a measure of the photon life time (or ring down time) of the cavity. Various loss mechanisms influence the Q of a microcavity such as losses due to the WGM radiation, absorption of the surroundings, intrinsic material loss, scattering from the surface inhomogeneities, surface contaminants, and the external coupling. Therefore overall Q of a microcavity is given by:

$$Q_{total} = \frac{1}{Q_{wgm}^{-1} + Q_{surroundings}^{-1} + Q_{material}^{-1} + Q_{scattering}^{-1} + Q_{contaminants}^{-1} + Q_{external}^{-1}} \quad (1)$$

The Q of a cavity can be determined either by linewidth measurements i.e. $\lambda_{resonant}/\Delta\lambda$ or by using cavity ring down spectroscopy as described in the next section.

2.2 Cavity Ring Down Spectroscopy (CRDS)

In CRDS, a laser pulse is injected into a cavity and then decay of the pulse is monitored on the oscilloscope (figure 2). The fitting algorithms are then applied to the detected light for extraction of the ring down time.[10]

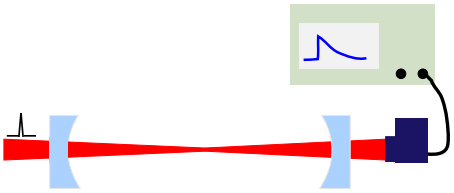


Figure 2. CRDS Principle

The losses present in the cavity influences the ring down time. For a microcavity, the decay rate is given by:

$$\frac{1}{\tau} = \frac{2\pi c}{\lambda_{resonant} Q_{total}} \tag{2}$$

where
τ is the total decay time(ring down time), Q_{total} is defined in 1.

CRDS was first demonstrated in 1984[11]for measuring the reflectance of mirrors of a free space cavity. The first application of CRDS for sensing absorption of gas appeared in 1988[12] and since then many modifications of the original CRDS have been proposed.[7] In this work, we have used phase shift cavity ring down spectroscopy (PS-CRDS), a variant of the original CRDS, for biosensing and is briefly described in the next section.

2.3 Phase Shift-Cavity Ring Down Spectroscopy (PS-CRDS)

The CRDS requires an exponential fitting of the detector output. However, this requirement can be removed by using the PS-CRDS. In the PS-CRDS, continuous wave laser source is intensity modulated according to the equation 3:

$$I = I_o(1 + \alpha_{input} \sin \omega t) \tag{3}$$

where α is the modulation depth and ω is the modulation frequency.
This intensity modulated light is then injected into a cavity. Due to the intensity modulation, output light from the cavity has a phase shift with respect to the input light.[13] This phase shift is related to the cavity decay time according to the equation 4:

$$\tan \phi = -\omega\tau \tag{4}$$

Moreover, modulation depth of the output light is also related to the cavity decay time:

$$\alpha_{output} = \frac{\alpha_{input}}{\sqrt{1 + \omega^2\tau^2}} \tag{5}$$

Figure 3 shows the graphical relationship between the input and output waves. Therefore both ϕ and α_{output} can be correlated to the binding events for biosensing.

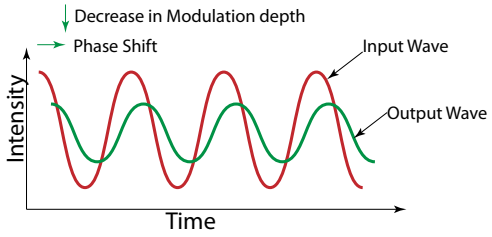
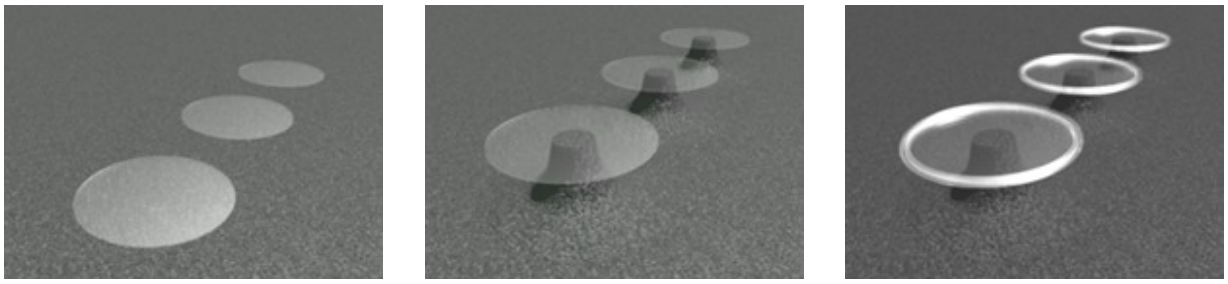


Figure 3. PS-CRDS Principle

3. MICROCAVITY FABRICATION

The microtoroids are fabricated on $2\mu m$ thermally grown silica on silicon wafers. First, $150\mu m$ in diameter silica disks are patterned on the silicon wafer by photolithography techniques and buffered HF etching at room temperature. Next, XeF_2 gas at $2800mmHg$ would isotropically undercut silicon which will result on forming silica microdisks on top of silicon pillars. Finally, they are reflowed by a 50W CO_2 laser (Synrad) to form the microtoroids with major (minor) diameter of $105\mu m$ ($5\mu m$).[14] The fabrication process is shown in figure 4.



(a) Patterning circular silica pads using photolithography and buffered oxide etching

(b) XeF_2 etching to form microdisks

(c) CO_2 reflow of the microdisks to form microtoroids

Figure 4. Fabrication process of a microtoroidal cavity

4. DEVICE FUNCTIONALIZATION

In order to functionalize the surface of devices, we followed a previously detailed NHS-ester bioconjugation process.[15] First, the silica surface of microtorid is terminated with hydroxyl groups through oxygen plasma treatment. Next, the hydroxylated microtoroids are aminated through chemical vapor deposition of 3-aminopropyltrimethoxysilane (APTMS). Finally, the devices are incubated in solution of NHS-Biotin in dimethylsulfoxide (DMSO) at room temperature where biotin will attach to the surface of cavities through stable amide bonds. A scanning electron micrograph of a biotin functionalized microtoroid is shown in figure 5.

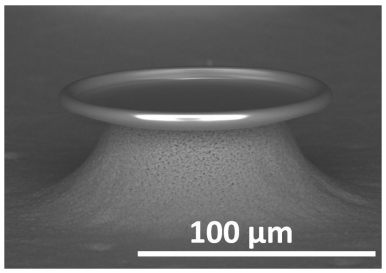


Figure 5. . A scanning electron micrograph of a biotin functionalized microtoroid

5. EXPERIMENTAL SETUP AND RESULTS

The experimental setup is shown in figure 6. A tapered fiber is used to couple the light to a microtoroidal cavity functionalized with Biotin. The two function generators, FG_1(100mHz, 1V peak-to-peak, triangular wave) , and FG_2 (13MHz, 4V peak-to-peak, sinusoid) are simultaneously modulating the wavelength and intensity of the tunable laser (New Focus, Velocity Scan 633nm tunable laser) respectively. The shift in phase between the reference sinusoid and the sinusoid at the fiber output is continuously recorded by the oscilloscope. The quality factor and the resonant wavelength are then extracted by further signal processing. The biconjugated microcavity is immersed in a PBS microacquarium and the streptavidin is injected into the aquarium at the rate of $50\mu L/min$ for 7 min. After switching off the syringe pump, both the quality factor and the resonant wavelength are tracked as a function of disassociation phase of the biotin-streptavidin reaction. The results are

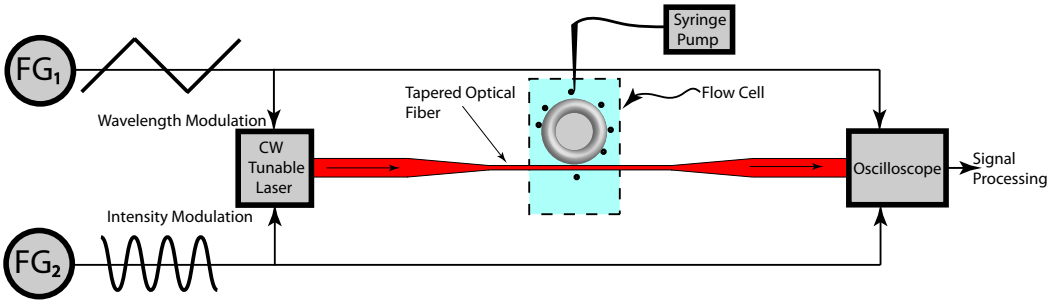
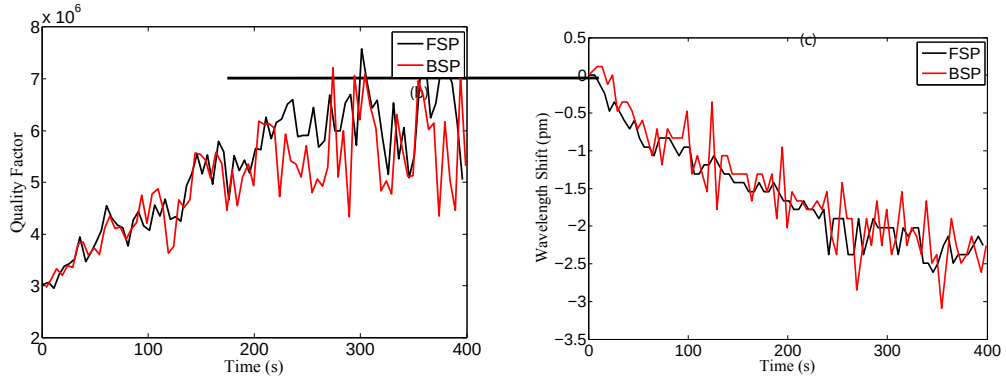


Figure 6. Experimental Setup, FG - Function Generator

shown in figure 7. In figure 7b, negative values of the wavelength signify the shift of the resonant peak towards blue which agrees with the previously reported result.[4] Since the unbinding of streptavidin from the microtoroid will increase the Q of the cavity, that is why an increasing trend in Q is obtained experimentally (figure 7a).



(a) Quality factor (b) Frequency Shifts

Figure 7. Experimental parameters. FSP (Forward scanning peak) represent the resonant peak in the increasing voltage part of the triangular wave whereas the BSP (Backward scanning peak) represent the resonant peak in the decreasing voltage part of the triangular wave

6. CONCLUSIONS

This work presents a proof of concept for utilizing cavity ring down spectroscopy in conjunction with ultra high Q microcavities for biosensing applications. Because of its immunity to the intensity fluctuation of the laser source, the microcavity ring down biosensor has the potential to offer high sensitivity as compared to its frequency domain counterpart. This sensor has prospects of finding real time and label free sensing applications in health care, and agricultural sectors.

7. ACKNOWLEDGEMENTS

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