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Biochemical Signal Detection in Miniaturized Fluidic Systems by Integrated Microresonator

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Abstract— **An optical sensor integrated into a polymer microfluidic chip is proposed as a low cost solution to highly parallel biochemical analysis. The sensor consists of a single high-finesse optical resonator for direct analytes detection. High quality silica microspheres (diameter ~300 μm) are easily produced and low-loss whispering gallery modes were excited through evanescent coupling at wavelengths near 1550 nm and 544 nm. The quality factor (Q) and ring down time of these modes is sensitive to minute changes in the microresonator environment thus making it an excellent candidate for a sensor. Instead of the traditional time domain studies, we determine quality factors and ring down times as long as 53.8±0.6 ns (Q ~ 10^6) from phase shift measurements using optical sources with sinusoidal intensity modulations of 300 kHz and below.**

I. BACKGROUND

THE manipulation and analysis of micro- to picolitre volumes of sample in a lab-on-a-chip environment has been the focus of significant research activity in recent years Much of this research has been directed towards superseding traditional analytical methods employed by the biomedical community. The goal has been to greatly reduce the cost of current analytical procedures, to decrease the complexity of sample manipulation, and to decrease the turn-around-time of (bio)medical diagnosis. A large variety of microfluidic manipulation and separation techniques have been developed to this end. Advances in polymeric material fabrication using hot embossing techniques promises even lower production costs in the future (Fig. 1). A key challenge to achieve economical, even disposable microfluidic systems is to provide adequate sensing capabilities at comparably low costs. Because of the very small sample volumes, the detection of separated components often requires the introduction of chromophores or fluorophores that selectively bind to a given analyte, which then allows for subsequent optical detection via absorption or fluorescence. Removal of this labeling step would simplify the process considerably and increase the versatility of the microfuidic platform.

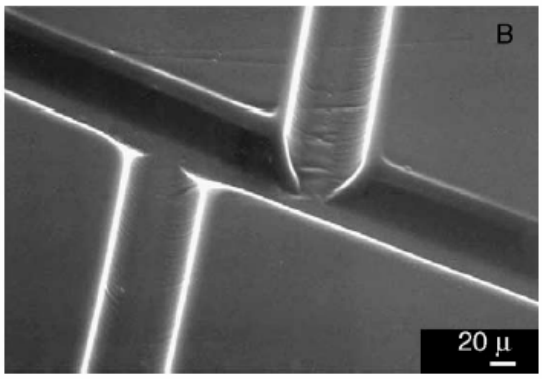

Fig. 1: SEM photomicrographs of injector region of microfluidic device embossed in polymer (PMMA) (adapted from ref. 1).

Optical resonators, in various forms, have been used for many years as a very sensitive optical detection method. Initially, optical resonators were constructed from pairs of high reflectivity mirrors and used in gas-phase spectroscopy and analysis [2-4]. More recently, monolithic resonators or resonators constructed from optical fibres have been employed [5-7]. If a pulse of light is injected into an optical resonator, the lifetime of that pulse will depend upon the net round-trip loss experienced by the pulse as it repeatedly traverses the optical cavity. A characteristic lifetime, τ, can be defined as the time taken for the intensity to be reduced to 1/e of its initial value. Loss mechanisms arise from both system imperfections and from the intentional absorption/scattering due to the gas or condensed matter system under study.

Silica microspheres, with diameters from tens to hundreds of microns, have been shown to act as very high quality optical resonators with Q factors ranging from 10^6 to 10^{10} [8]. These spheres are formed via surface tension from molten silica which gives rise to essentially atomically smooth surfaces. At appropriate wavelengths, absorption losses can be very low. Light injected through evanescent coupling to a microsphere can excite so-called 'whispering

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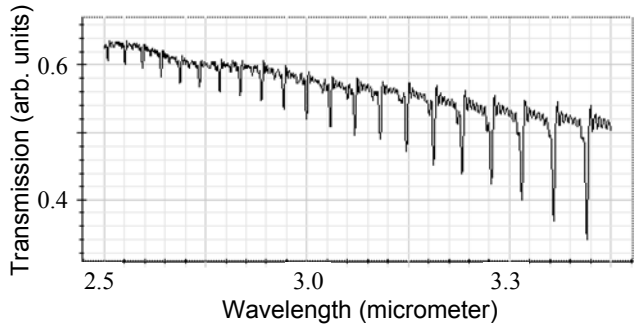

Fig. 2: Calculated transmission through a tapered fiber coupled to a 25 μm microresonator in air as a function of wavelength, determined by finite-element time domain simulations.

gallery modes' (WGM) within the resonator in which light is guided by total internal reflection. Such excitations only occur at those wavelengths for which an integral number of wavelengths circumscribe the sphere and thus, the WGM frequencies are dependent upon the diameter of the sphere (Fig. 2). The line widths of the allowed modes are determined by the total quality factor of the resonator system. The optical characteristics of silica microresonators suggest several possible means of incorporating them into microfluidic devices in the role of chemical sensors, since the interaction of a chemical species with the microresonator will influence the optical lifetime of the cavity. The electromagnetic field of the circulating light is not strictly confined within the resonator volume. An evanescent field, which decays rapidly with distance, extends beyond the geometrical confines of the resonator. This field is capable of interacting with absorbing or scattering species that are not physically contained within the resonator structure. A loss of energy from the evanescent field results in the reduction of the optical lifetime of light circulating within the resonator. These lifetimes can be measured using pulsed, tunable lasers to excite the WGM and sensitive photon detectors to measure the emitted light. Since, in effect, the beam interacts with the same small sample volume around the sphere through thousands or tens of thousands of round trips, minute changes to this environment have dramatic effects on the overall optical properties of the microresonator. By identifying specific optical signatures with biochemical species, the dynamic process of separating proteins into their constituent peptide blocks can be monitored and potentially controlled.

Alternatively CW light can also be coupled into optical resonators. To achieve efficient coupling with the resonator, the frequency of the light must match an eigenfrequency of the optical cavity. The allowed eigenfrequencies depend upon the physical dimensions of the resonator. The line width of these resonances decreases as the finesse of the cavity increases. When CW light is resonantly coupled to a high-finesse optical cavity, a very high optical intensity develops within its interior. This circulating high intensity light can be used to excite weak optical absorptions in species within the cavity [9]. In the case of solid-state resonators, the evanescent field surrounding the resonator is correspondingly enhanced. If the input light is intensity modulated sinusoidally, the cavity output will also be intensity modulated, but shifted in phase with respect to the input light (Fig. 3). This is due to the inherent response time of the resonator, related to its characteristic lifetime, τ. Any loss process that decreases the cavity optical lifetime will decrease the observed phase-shift. This phase-shift method has been applied to mirror type optical cavities as well as those consisting of fiber optic loops [7,10].

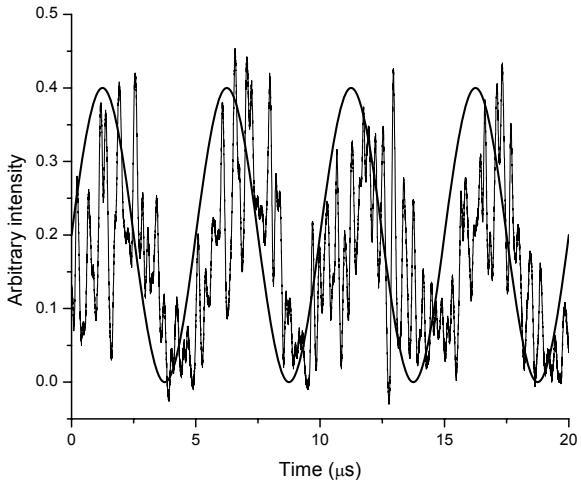

Fig. 3. Light emitted from a waveguide resonator compared to the waveform of the incoming light. The phase angle $\phi = \arctan(-\Omega\tau)$ is indicative of the photon life time. (adapted from ref. 7)

Microspheres can be functionalized with affinity reagents that will selectively adsorb analytes of interest. In the simplest instance, adsorption of an analyte onto the surface of a microsphere will increase its effective radius, thus causing a shift in the eigenfrequency spectrum of the microsphere [11]. Light of wavelength λ_0, which was resonant with the microsphere in the absence of the analyte, will no longer be optimally coupled into the cavity due to the analyte-induced shift of the resonant frequencies and the intensity of the circulating and emitted light will decrease. Also a reduction in optical lifetime at this wavelength will result. Adsorption of an analyte on the surface of a microsphere will also alter the local refractive index. The intensity of light scattered from a microresonator is a sensitive function of the local refractive index for wavelengths near resonance. Refractive index changes on the order of 10^{-4} are easily measured using microresonators and detection of changes as small as 10^{-9} is believed possible [12]. Recently, it was predicted that single protein adsorption on a silica microsphere is detectable at visible wavelengths [13].

Finally, optical absorption of the evanescent field surrounding a microresonator can also be exploited. If a microresonator is excited at an eigenfrequency that also happens to be resonant with an optical absorption of an analyte molecule, analyte molecules in close proximity to

the resonator will absorb energy from the evanescent field and thus reduce the optical lifetime of the cavity. When a resonator is excited with a CW source, the high field intensity that builds-up within the resonator will be reflected in an intense evanescent field. This intense evanescent field can be used to excite fluorophore markers with which analytes may be labeled [14].

II. EXPERIMENTS

High quality glass microspheres (diameter ~300 μm) are produced from standard telecom fiber (Corning SMF28) in the arc of a fiber fusion splicer (Fitel S182) (Fig. 4).

Fig. 4. View of 300 μm silica microresonator and eroded delivery fiber

Surface tension draws the molten silica into a spherical shape, while the remaining fiber stem provides a convenient means of holding and manipulating the microsphere. Two different evanescent wave coupling techniques have been successfully employed using an: i) eroded fiber, or ii) high index prism. Eroded fibers are created by careful etching of a single-mode fiber using hydrofluoric acid to produce a tapered transition from the nominal 125 μm diameter to a region 5 μm in diameter following the procedure of Haddock et al.[15]. This exposes the evanescent field of the fiber core. When the microsphere is placed in this region, light can efficiently couple from the fiber to the sphere when resonance conditions are met. The intensity of light transmitted through the fiber will be reduced when light couples from the fiber into the microsphere. Note that the image in Fig. 4 was recorded to show both the fiber and the microresonator. The actual excitation configuration is obtained by placing the microresonator above the fiber so that the excited modes are not affected by the stem. The transmission through the eroded fiber as a function of wavelength is determined using a tunable narrow band laser (200 kHz line width) operating near 1550 nm (Ando AQ4320D) and InGaAs detector (Thorlabs DET410). This source can support sinusoidal intensity modulations up to 300 kHz for phase delay measurements as described earlier (Fig. 3). More recent experiments also use tapered fibers produced by heating and stretching the fiber to achieve sub - 10 micron fiber diameters.

The second technique for evanescent wave coupling uses a beam internal to a high index prism. The beam is set incident at an angle above the critical angle to undergo total internal reflection at the prism surface. By focusing the internal beam to a tight spot on the prism surface, the evanescent field is localized to a small spatial region. Fig. 5 shows an image of the 300 μm microresonator being excited with green light from a HeNe laser (543.5nm). For phase delay measurements, the output intensity of the laser is

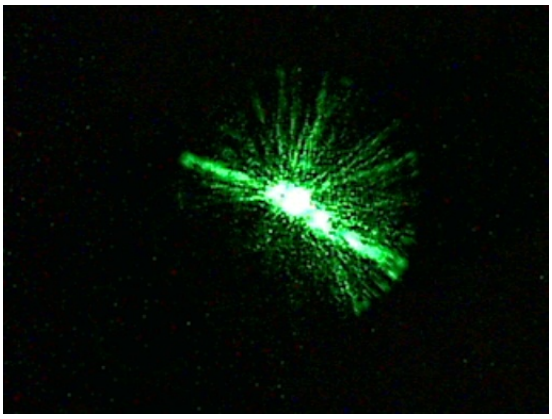

Fig. 5. Light scatter from microresonator excited using a high index prism. The prism is behind the microresonator with the internal beam incident from the top left of the image.

modulated by a photoelastic modulator at a 100 kHz repetition rate.

III. RESULTS

Experiments are ongoing but preliminary results of WGM coupling demonstrate that phase measurement is an effective technique for estimating cavity ring down time with these simple resonators. When a 300 μm silica microresonator is

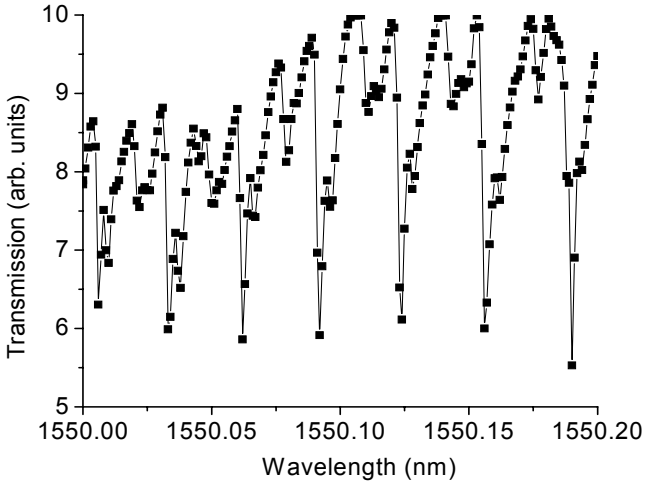

Fig. 6. Transmission through eroded fiber in proximity to 300 μm silica microresonator all contained in deuterium oxide. Lines between points are included as a guide to the eye.

brought in proximity to the eroded fiber, the transmission of the fiber develops distinct spectral features (Fig. 6). Data is recorded every 1 pm (the minimum step size for this source).

The irregular mode pattern is expected due to the imperfect shape of the microresonator which breaks the energy degeneracies of the modes. Both microresonator and fiber are immersed in deuterium oxide to more closely mimic the microfluidic environment. Water was not used due to overtone absorption at these wavelengths. A similar spectral pattern was observed when both fiber and microresonator located in air but with lower coupling efficiencies. This is likely due D_2O's higher refractive index corresponding to an extended evanescent field from the delivery fiber, resulting in better coupling to the resonator modes.

The cavity ring down time could be measured by tuning the optical source to one of the minima shown in Fig. 6 and pulsing the laser. To avoid the cost and complexity of a short pulse, tunable laser, we opted instead to modulate the intensity of the source and measure the phase lag of the output using a lock-in amplifier (Stanford Research Systems SR844). A background phase lag due to electronics and other system effects was removed by measuring the lag with the microresonator removed from the fiber. The resulting phase lag as a function of modulation frequency is shown in Fig. 7. Theory [10] considering the cavity ring down to be a single exponential decay predicts a simple relationship between phase lag(φ), modulation frequency (Ω, measured in rad/s) and ring down time(τ): $\tan(\varphi) = -\Omega\tau$

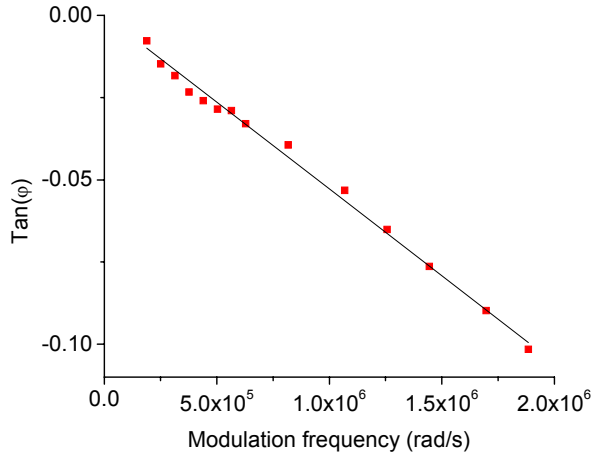

Fig. 7. Tan of phase lag (φ) of signal transmitted through a tapered fiber coupled to 300 μm silica microresonator. Straight line is a linear fit..

A linear fit to the data shown in Fig. 7 provides a slope of 53.8±0.6 ns. Note that the magnitude of slope can be easily ascertained with modulation frequencies as low as 6×10^5 rad/s (100 kHz). The corresponding time domain measurement would require pulses on the order of 10 ns corresponding to spectral bandwidths greater than 40 MHz. Also for optimal signal averaging, repetition rates of 1MHz or above would be desired. Clearly, the phase shift measurement requires a considerably less sophisticated source.

Measurements of photon lifetimes and in particular phase shift measurements complement the conventional methods of determining the quality factor of high finesse cavities. Typically the Q-factor is obtained by determination of the spectral width of a cavity resonance with a narrow band light source. Our method is different since a narrow band excitation is not necessary. Indeed one can show that even a microsphere displaying a pseudo-continuum of modes can be used as a sensor.

IV. SUMMARY

The goal of this research project is to use silica microsphere resonators as universal detectors for unlabelled proteins. Ultimately, these microresonators will be employed on microfluidic devices. Electroosmotic and electrophoretic methods can be used to achieve analytical separations in such a lab-on-a-chip environment and microresonators will provide for a rapid and inexpensive method for the detection of biomolecules through refractive index changes. Initial experiments show phase lag measurements as a straightforward technique to measure ns ring down times in microresonators made from standard telecom fiber. In one possible scheme, analytes can be selectively adsorbed onto the microresonators. The surfaces of silica microspheres can be derivatized with amino, carboxylate, or biotin groups that will allow for the immobilization of antibodies [11]. Free antigens will selectively bind to these derivatized surfaces and may thus be detected through their influence on the microsphere's optical properties. Particular resonators may be designated to detect specific analytes. Together, this promises a polymer-based, low-cost, high-speed, and disposable biochemical sensing platform where many protein separation processes can be dynamically monitored and controlled in parallel using only micro to picolitres of organic samples.

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