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Label-free detection of ovarian cancer biomarkers using whispering gallery mode imaging

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ABSTRACT

Small optical microresonators that support whispering gallery mode (WGM) resonances are emerging as powerful new platforms for biosensing. These resonators respond to changes in refractive index and potentially offer many advantages for label-free sensing. Recently we reported an approach for detecting WGM resonances based on fluorescence imaging and demonstrated its utility by quantifying the ovarian cancer marker CA-125 in buffer. Here we extend those measurements by reporting a simplified approach for launching WGM resonances using excitation light coupled into a Dove prism. The enhanced phase matching enables significant improvements in signal-to-noise, revealing the mode structure present in each resonator. As with all label-free biosensing techniques, non-specific interactions can be limiting. Here we show that standard blocking protocols reduce non-specific interactions sufficiently to enable CA-125 quantification in serum samples. Finally, fluorescence imaging of WGM resonances offers the potential for large scale multiplexed detection which is demonstrated here by simultaneously exciting and imaging over 120 microsphere resonators. For multiplexed applications, analyte identity can be encoded in the resonator size and/or location. By encoding analyte identity into microresonator size, we simultaneously quantify the putative ovarian cancer markers osteopontin (38 μm diameter sphere), CA-125 (53 μm diameter sphere), and prolactin (63 μm diameter sphere) in a single PBS assay. Together, these results show that fluorescence imaging of WGM resonances offers a promising new approach for the highly multiplexed detection of biomarkers in complex biological fluids.

1. Introduction

Ovarian cancer is the most fatal of all the gynecologic cancers and unfortunately the most prevalent (Yap et al., 2009). In North America, ovarian cancer remains the fifth leading cause of cancer related deaths among the female population due to the high fatality rate. Over 60% of those diagnosed with ovarian cancer will eventually die from the disease (Chambers and Vanderhyden, 2006). This high fatality rate reflects difficulties in diagnosing ovarian cancer in stage I where survival rates are greatly improved. Given the lack of effective screening tools to identify ovarian cancer early, the statistics for the disease have changed little over the last three decades despite ongoing advancements in treatment (Chambers and Vanderhyden, 2006; National Institutes of Health, 2011).

Ovarian cancer is challenging to diagnose in the initial stages due to a lack of symptoms early in the disease progression (Jacobs and Menon, 2004). This often results in delayed diagnosis, which enables the cancer to spread from the ovaries where treatments are substantially less effective. For example, women diagnosed in stage I of the disease have 5 year survival rates over ~90%, while those diagnosed in stage three or later have survival rates less than ~20% (Chambers and Vanderhyden, 2006; Jacobs and Menon, 2004; Nossov et al., 2008). This illustrates the importance of early diagnostics which has motivated the ongoing search for biomarkers that could eventually lead to sensitive and specific screens of the disease.

Numerous serum biomarker candidates associated with ovarian cancer have been identified, providing promising targets for the development of assays to aid in early diagnostics (Hwang et al., 2009; Jacobs and Menon, 2004; Levina et al., 2009; Terry et al., 2004). Currently, a widely employed assay involves the detection of cancer antigen 125 (CA-125) (Karlan and McIntosh, 2007; Nossov et al., 2008). Serum concentrations of CA-125 are elevated in 60% of women with ovarian cancer and 80% of those with disseminated ovarian cancer (Nossov et al., 2008). However, this marker is also elevated in 1% of healthy women, 3% of women

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with benign ovarian diseases, and 6% of women with physiological conditions such as menstruation or pregnancy (Bast et al., 1998; Farghaly, 1992; Kenemans et al., 1993; Saksela, 1993; Welander, 1992). This marker alone, therefore, is usually considered insufficient for an early screen and is predominantly used to confirm a diagnosis or monitor the effects of treatment (Karlan and McIntosh, 2007). An effective early screen with the requisite sensitivity and specificity, therefore, will likely require the detection and quantification of multiple biomarkers (Badgwell and Bast, 2007; Suh et al., 2010; Woolas et al., 1995; Yurkovetsky et al., 2010). Fortunately, new biomarker targets continue to be identified and promising protein candidates as well as small, noncoding RNAs have been associated with ovarian cancer (Anastasi et al., 2010; Balch et al., 2009; Dahiya et al., 2008; Dobrzycka et al., 2009; Iorio et al., 2007; Nakae et al., 2006; Suh et al., 2010). The goal, therefore, is to develop an inexpensive, general platform for the sensitive, multiplexed detection of ovarian cancer biomarkers that can be easily adapted as new protein and non-protein targets are identified.

Assays that exploit refractive index changes as the sensing signal offer promising general routes for the development of multiplexed biomarker screens (Fan et al., 2008). These techniques enable the homogeneous detection of proteins, nucleic acids, or other markers of disease without the use of labels, making them attractive for both point-of-care and clinical applications (Gopinath, 2010; Shevchenko et al., 2011). In particular, recent work using optical resonators for label-free biomarker detection has shown considerable promise as platforms for assay development (Gopinath, 2010; He et al., 2011; Huang and Guo, 2011; Luchansky and Bailey, 2012; Qavi and Bailey, 2010; Serpenguzel et al., 1995; Shevchenko et al., 2011; Soria et al., 2011; Wilson et al., 2012).

Light coupled into small, high index structures such as glass microspheres can undergo continuous total internal reflection, leading to the confinement of light within the sphere. When the roundtrip distance within the sphere is an integer multiple of the coupled wavelength, the light coherently drives itself leading to resonances known as whispering gallery modes (WGMs). These resonances occur when the following relationship is satisfied (Gorodetsky and Ilchenko, 1999; Knight et al., 1997).

$$\lambda_r = \frac{2\pi r n_{eff}}{m} \quad (1)$$

In Eq. (1), λ_r is the resonant wavelength, r is the radius of the cavity, n_{eff} is the effective refractive index surrounding the sphere, and m is an integer. For sensing applications, binding of biomarkers to recognition elements attached to the surface of a resonator will alter n_{eff}, leading to measureable shifts in the resonant wavelength as shown in Eq. (1) (Soria et al., 2011; Suter and Fan, 2009).

High index glass microspheres, glass capillaries, and microfabricated planar resonator arrays have all been used in WGM sensing applications (Arnold et al., 2003; Carlborg et al., 2010; Hanumegowda et al., 2006, 2005; Ren et al., 2007; Suter and Fan, 2009; Vollmer et al., 2003; Washburn et al., 2009a, 2009b; Zhu et al., 2009). In particular, microsphere resonators offer an appealing platform for assay development since they are inexpensive, commercially available or easily fabricated, exist in a variety of sizes and materials, and are easily functionalized. Moreover, microspheres have exquisitely smooth surfaces, low material absorption, and minimal reflection losses which lead to quality (Q) factors that can be orders of magnitude larger than other resonator designs (Chiasera et al., 2010; Gorodetsky and Ilchenko, 1999; Gorodetsky et al., 1996). Large Q values translate into long effective path lengths and narrow resonances, both of which are highly desirable in sensing applications. Microsphere resonators, however, present challenges for integration into multiplexed sensing schemes when traditional, transmission based approaches are used for measuring WGM resonances (Fan et al., 2007). In an effort to combine their favorable optical attributes with multiplexed sensing capabilities, we recently reported a fluorescence imaging scheme for simultaneously measuring individual WGM resonances from a field of microspheres (Huckabay and Dunn, 2011).

The approach takes advantage of the evanescent field generated at the sphere surface when a WGM resonance is excited. By functionalizing the surface of the sphere with a fluorescent dye, distinct fluorescence rings are observed when a particular sphere comes into resonance. Using fluorescence imaging, large numbers of spheres can be simultaneously characterized as the excitation wavelength is scanned, providing a new approach for multiplexed detection. Previously, we showed that total internal reflection fluorescence microscopy (TIRF-M) could be used to launch and measure WGMs in a field of microresonators. This approach was validated by characterizing the concentration of CA-125 and tumor necrosis factor α (TNF-α) in buffer solution (Huckabay and Dunn, 2011). However, this approach suffered from a limited field of view, poor excitation efficiency for launching WGMs into the resonators, and required expensive TIRF instrumentation and optics.

Here we extend the previous measurements by reporting a simplified arrangement that provides a flexible and efficient coupling scheme enabling much larger fields of spheres to be excited. As shown by others, prism couplers can efficiently launch light into microresonators (Gorodetsky and Ilchenko, 1994, 1999; Rowland and Love, 1993). A Dove prism is used to launch WGMs in a collection of microspheres which simplifies the experimental design, lowers the cost, and enables improved phase matching conditions. As with all label-free approaches, non-specific interactions are a concern and can interfere with the desired signal. Using standard blocking protocols, we show that CA-125 doped into human serum samples can be quantified, thus showing that this approach can be applied in complex media. For multiplexed analyte detection, the larger field of view provided by Dove prism coupling enables large collections of resonators to be excited, which we show by imaging over 120 microsphere resonators simultaneously. Since analyte identity can be encoded into resonator size and/or location, the potential multiplexing capabilities of this imaging approach is significant. To illustrate the multiplexing capabilities, we encode the analyte identity into the resonator size to simultaneously quantify CA-125 and two other putative markers of ovarian cancer, prolactin and osteopontin (OPN), in a single PBS assay (Hwang et al., 2009; Levina et al., 2009; Kim et al., 2002). Finally, with the improved signal-to-noise arising from the enhanced phase matching, we show that the mode structure present in each resonator can be measured using the fluorescence imaging approach. These measurements enable the angular mode numbers to be characterized and may eventually lead to new approaches for characterizing analyte binding.

2. Experimental

2.1. Materials

Barium-titanate glass microspheres were obtained from Mo-Sci Corporation (Rolla, MO). Anti-CA-125 mouse monoclonal antibodies, anti-osteopontin monoclonal antibodies, and osteopontin protein were purchased from Abcam (Cambridge, MA). CA-125 ovarian tumor marker antigen was obtained from MP Biomedicals (Solon, OH) and the anti-prolactin monoclonal antibodies and prolactin protein were purchased from R&D