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Biomolecular Analysis with Microring Resonators: Applications in Multiplexed Diagnostics and Interaction Screening

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Abstract

Silicon photonic microring resonators are a promising class of sensor whose value in bioanalytical applications has only begun to be explored. Utilized in the telecommunication industry for signal processing applications, microring resonators have more recently been re-tasked for biosensing due to their scalability, sensitivity, and versatility. Their sensing modality arises from light/matter interactions—light propagating through the microring and the resultant evanescent field extending beyond the structure is sensitive to the refractive index of the local environment, which modulates resonant wavelength of light supported by the cavity. This sensing capability has recently been utilized for the detection of numerous biological targets including proteins, nucleic acids, viruses, and small molecules. Herein we highlight some of the most exciting recent uses of this technology for biosensing applications, with an eye towards future developments in the field.

Introduction

Biomolecular detection technologies are invaluable in modern chemical biology, helping to advance fundamental studies of biophysical interactions and recognition, drug discovery, and the translation of new insights into clinical application. Not surprisingly, the literature is replete with emerging technologies offering enabling new capabilities, and the development of biosensing technologies has been a particularly active area of both academic research and industrial product development. Among the many different classes of transduction schemes, optical biosensors have been highly successful due to their diversity and generality [1]. In this short review we narrowly focus on one particular flavor of optical biosensor that has recently emerged as a promising technology both for fundamental interaction screening and in vitro diagnostic applications. Microcavity resonators, and in particular chip-integrated microring resonator arrays, have generated interest due to their amenability to scalable fabrication and demonstrated performance metrics. To maintain focus, and to meet length constraints, we focus our discussion entirely to microring resonator-based assays and developments within the past 5 years.

Microring resonators belong to a larger class of sensors known as whispering gallery resonators, a terminology that is fitting given the fact that these sensors are optical analogues of the whispering gallery acoustic phenomenon first explained by Sir Rayleigh following his observations in London's St. Paul's Cathedral. Optical microcavities support discrete modes

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in which light circumnavigates the structure and constructively interferes with the input source as described by Equation 1 [2],

$$m\lambda = 2\pi r n_{eff} \quad (\text{Eq. 1})$$

where an integer (m) multiple of the wavelength equals the circumference times the effective refractive index (n_{eff}).

Light from a laser source is coupled into the microstructure using diffractive grating couplers, or prism- or butt-end coupling via an adjacent linear waveguide structure or extruded fiber optic cable [3]. Under resonance conditions, light is coupled into the microstructure and propagates around the cavity via total internal reflection. A resulting evanescent optical field extends into the local environment, providing a mechanism for detecting binding-induced changes in local refractive index, as sampled by the optical mode. Importantly, light circulates the microcavities many times, giving effective path lengths much larger than the physical dimensions of the sensor itself. For linear waveguide sensors, sensitivity scales in part with path length and the photon recirculation in microcavities therefore provides advantages in terms of increased interactions with bound analytes. Microcavity resonators can vary greatly both in their material composition and geometry; common examples include microrings [4], slot-waveguide microrings [5], microdiscs [6], microspheres [7], microtoroids [2], and liquid core capillaries [8]. Of these, microrings are particularly amenable to scalable fabrication on account of their near planar geometry, which is compatible with widely used, batch microfabrication methods, or their integration into capillary structures. In terms of materials systems, polymer [9,10], silica, and silicon-based structures are the most common. In this review we focus on planar silicon and liquid core silica microring resonators, as these are the most common configurations. For a broader discussion of other optical microcavity-based sensors, the reader is referred to these reviews [11-13].

The growing interest in microring resonators for biosensing applications can be attributed to their unique combination of high performance sensing capabilities in a platform conducive to highly multiplexed, low cost measurements. The real-time data collection, label-free detection capabilities, and high sensitivity provide an alternative to assays requiring fluorescent or enzymatic tags, or those that are incapable of providing kinetic binding information. The potential for high level multiplexing using arrays of many 10s or hundreds of target-specific sensor elements also makes them attractive for clinical diagnostic assays, as well as for a range of bio(molecular) screening applications.

Diagnostic Assays

A compelling feature of microring resonators is their ability to act as “label-free” sensors that do not require fluorescent or enzymatic tags for detection. Labels can reduce the reliability, reproducibility, and accuracy of biomolecular assays by introducing signal bias [14], a consideration that is increasingly important as the size of target analytes is reduced. Microring sensors transduce the presence of analytes on the basis of binding-induced changes in the local refractive index, and since all biological molecules have refractive indices greater than water the sensors are universally responsive to all classes of biomolecular targets, given functionalization with appropriate capture agents.

Numerous label-free, microring-based assays have been developed for nucleic acids, including DNA [15-19], miRNA [20], and tmRNA [21]. Highlights of these studies include low detection limits (10 pM), single nucleotide polymorphism (SNP) discrimination [16], and analysis of DNA methylation patterns [17]. An impressive application of the technology paired methylation specific polymerase chain reaction (MS-PCR), bisulfite modifications,

and microring resonators to quantitate methylation frequency in genomic DNA from cancer cell lines [19].

An even larger body of work has focused on label-free protein analysis, leading to numerous label-free aptamer [22,23] and antibody-based assays on microring resonator detection platforms [18,24-32]. Initial proof-of-principle reports utilized simplified biological systems such as biotin-streptavidin or IgG:anti-IgG [5,10,28]; however, more recent work has focused on more relevant clinical biomarkers, frequently in multiplexed formats and in complex matrices. The first study in this vein demonstrated the detection of a colorectal cancer biomarker, carcinoembryonic antigen, in undiluted fetal bovine serum with a clinically-relevant detection limit (2 ng/mL) and very short assay time (10 min) [29]. In another notable study, Zhu et al. demonstrated the detection of CA15-3, a breast cancer biomarker, in human serum samples with a total assay time of 30 minutes. Importantly, this work featured a two-step blocking methodology that helped reduce nonspecific sensor fouling [26].

Given the powerful fabrication methods by which microring resonators can be created, multiplexed analysis is a key analytical advantage of the technology, particularly given the importance of multi-molecular signatures in the diagnosis and monitoring of many human diseases and disorders [33]. To date, multiplexed analyses of proteins and nucleic acids have only been realized in simplified assay formats; however, a number of clinically-relevant demonstrations of multiplexed analyses should be expected in the near future.

Despite the attractiveness of label-free analysis, the challenges associated with the complex matrices of clinical samples such as nonspecific fouling, cross-reactivity, and low-abundance biomarkers often necessitates the use of label-based, secondary detection schemes. Fortunately, the refractive index-based transduction of microring resonators is amenable to a plethora of tag-based signal enhancement modalities. To date, secondary antibodies and sub-micron beads have been used to enhance assay sensitivity and specificity [34-37]. Specifically, a sequence independent antibody that recognizes DNA:RNA heteroduplexes was used in the simultaneous detection of four miRNAs with a detection limit of 10 pM (350 fmol) [38]. Antibody labels were also used to monitor the secretion of four cytokines from mitogenically-stimulated, primary human T cells [34]. Importantly, secondary antibody-based immunoassays require two target-specific recognition events, which greatly increases the selectivity of measurements made in complex, clinically-relevant sample matrices. Sub-micron beads have also been employed as tertiary labels in a variety of biomolecular assays. The large size of these particles, relative to biomolecules, large surface area, and flexibility in surface chemistry render them highly amenable for refractive index signal enhancement strategies in microring resonator-based protein and nucleic acid assays [36,37]. Bead-based methods were employed recently for the analysis of C-reactive protein, a cardiac biomarker known to increase abundance by a factor of up to 10^5 during acute cardiac events. In recognition of the extreme challenge associated with this broad dynamic range, a three-step assay was developed that allowed quantitation across six orders of magnitude with a limit of detection in the 10s of pg/mL.

Virus and Whole Cell Detection

While the major thrust in microring resonator-based analyses have focused on assays for protein and nucleic acids, the versatility of the technology has also been extended to larger analytes such as virus particles and even whole cells. The binding of these analytes induces correspondingly large shifts in resonance frequencies, enabling rapid analysis with minimal sample processing.

Two recent reports describe relatively rapid (<1 hr) viral detection assays using different microring resonator configurations. These methods are much more rapid than serological methods for detection viruses and are simple alternatives to multi-step RT-PCR or ELISA assays. These developments are noteworthy because of the sometimes lengthy period between exposure and measurable immune response or infected phenotype, placing a premium on analysis time. It is anticipated that rapid viral analysis could have applications in agricultural monitoring, healthcare, and bioterrorism surveillance. Zhu et al. developed a sandwich-style immunoassay on an LCORR sensor to detect M13 bacteriophage over an impressive dynamic range of 7 orders of magnitude [39]. Using silicon photonic microrings, a 10 ng/mL detection limit was achieved for the detection of bean pod mottle virus from whole soybean leaf extract. [40]

Cell-based assays are of interest not only to further our understanding of cellular processes, but also represent an indirect method of sensing other analytes or conditions that affect cellular function and/or behavior. Wang and colleagues creatively exploited this capability using silicon-based microring sensors to monitor cell adhesion and growth events as well as motility perturbations resulting from the introduction of toxic compounds [41]. Gohring and colleagues detected CD4+ and CD8+ lymphocytes in white blood cell samples isolates from whole blood by coating a LCORR sensor with CD4+ and CD8+ specific antibodies [8]. This platform offered the benefit of near-single cell analysis, but also resulted in high ring to ring variability; however, accurate quantitation of cellular perturbations and cell counting was achieved with appropriate negative controls and data averaging. The authors envision this device as a companion diagnostic for anti-retroviral treatments of HIV+ patients.

Interaction Screening

The refractive index-based transduction methodology and multiplexing capability of microring resonators also make them highly promising to biomolecular screening and affinity characterization applications [22,31,42-44]. In contrast to endpoint-based methods, which only provide the thermodynamic binding constant, real-time monitoring technologies provide direct access to the kinetic rate constants that govern biomolecular interactions. Importantly, kinetic binding information can be used to unravel multi-step binding mechanisms, select for optimal capture agents, determine drug-target stability, and characterize interactions between competitive binders, among others.

Carbohydrate binding studies are well suited to ring resonator-based interrogation based on both the diversity of binding partners and wide range of binding affinities. Several exciting studies have coupled high density inkjet functionalization and novel linker chemistries to create robust carbohydrate arrays for glycomics applications. In one such study a silicon photonic sensor array was functionalized with various sugars via a piezoelectric inkjet printer for subsequent kinetic profiling of two carbohydrate binding proteins (lectins) [42]. A similar study immobilized glycans to organophosphonate-modified sensors via a divinyl sulfone moiety, enabling multiple surface regenerations and extended storage stability, and offering additional advantages over the more commonly used siloxane conjugation chemistry. This improved surface chemistry enabled the subsequent kinetic profiling of four lectins, as well as norovirus particles with a carbohydrate-dependent pathogenic mechanism [43]. The authors anticipate immediate applications in glycan based drug discovery, vaccine design, and carbohydrate-mediated host-virus interactions.

Microring resonator arrays have also been used to interrogate the binding affinity of protein capture agents. Notably, a panel of 12 antibodies against two protein targets was screened using a DNA-encoded surface conjugation methodology that enabled rapid surface regeneration and robust storage properties. In a kinetic titration assay format, the association and dissociation rate constants (k_a and k_d , respectively) and equilibrium dissociation

constant (K_D) were determined, and compatible antibody sandwich pairs were also revealed [31]. Aptamer-based capture agents have also been used on a microring resonator detection platform, allowing direct comparison in the performance of a DNA aptamer and monoclonal antibody against thrombin [22].

Another recent study probed the interactions of soluble proteins with cell membrane components by integrating the microring resonator arrays with phospholipid bilayer nanodiscs, a synthetic membrane construct [44]. The authors demonstrated the multiplexing capability of this detection approach by simultaneously quantitating four different protein-lipid, protein-carbohydrate, or protein-membrane protein interactions. This synergistic combination of technologies should allow facile screening of protein interactions with membrane-associated glycans, lipids, and membrane proteins.

Future Advances: Device & Fluidic Integration

Enormous advances within the past 5 years have firmly established microring resonator technology as a promising tool for many bioanalytical applications, and continued developments will further improve the translational capabilities of this measurement technology. Initial proof-of-principle studies using well-characterized model systems have given way to multiplexed assays of clinically-relevant targets in increasingly complex sample matrices. Continued advances will likely be propelled by 'lab on a chip' integration of complementary instrumental, fluidic, and assay developments. Fields such as drug discovery and epitope mapping are particularly well-positioned to benefit from the scalable, multiplexable, and real-time, kinetic screening capabilities offered by microring resonators, especially as advances in accompanying microfluidic fluid handling further decreases the amount of sample required for analysis.

Here we highlight two significant trends in microring resonator technology development: continued exploration of advanced geometries and the on-chip integration of optical components. A particularly promising geometry for increasing measurement sensitivity is that of 'cascaded microrings'. Claes, Bogaerts, and Bienstman developed and validated this construct in which two microrings are optically coupled so that a filter microring provides a spectrally-structured input for a sensor microring. When the two microrings are of slightly different sizes, the periodicities in resonances supported by each ring are slightly different, resulting in a Vernier scale effect in which their transmission dips periodically overlap. This creates a manifold of resonance peaks that can be collectively tracked and fitted, offering sensitivity advantages over monitoring single resonances, while retaining the fabrication benefits of a near-planar device geometry [45]. The authors subsequently coupled cascaded microring sensors with an arrayed waveguide grating spectral filter to enable the use of a low cost, broadband light source [46], a significant development given the substantial cost associated with high resolution tunable laser sources often utilized in microring resonator-based measurements. In a similar effort to remove the high cost of typical optical scanning instrumentation, Palit and colleagues integrated all optical components onto a single chip including the laser source, waveguides, microring sensors, and detector [47]. This is an impressive achievement in terms of device integration; however, future work will show whether the increased complexity in terms of on-chip architecture can match the performance capabilities achieved by passive sensor chips.

Perhaps the most major avenue in terms of advancing microring resonator bioanalysis will be efforts towards clinical translation, since the cost effective nature of many device geometries is well-suited to relatively low cost and high volume analytical applications. As highlighted above, there are numerous classes of targets that can be detected using this technology and significant efforts have already been invested in assay developments including improved sample pre-treatment, signal enhancement strategies, and robust anti-

fouling surface chemistries [43,48,49]. Technology advances will also be highly coupled with complementary technologies such as microfluidic liquid handling to reduce sample consumption [50], as well as automated printing of multiplexed sensor arrays [42] such that the multiplexing of biological functionalization matches that of sensor fabrication.

Conclusions

The recent emergence of microring resonators as a surface sensitive, label-free detection technique has led to a proliferation of research in the application of the technology to a range of biomolecular analysis applications. The combination of high sensitivity, application versatility, and robust and scalable fabrication approaches make this particular resonator geometry an attractive candidate for high performance bioanalytics. The past five years have seen rapid developments within the field, as literature reports have quickly progressed beyond overly simplified, proof-of-principle demonstrations to, for example, multiplexed biomarker detection from within complex, clinically-relevant sample matrices. This progression has been permitted by significant advances in signal enhancement strategies, improved surface chemistries, and the innovative utilization of novel reagents and methodologies. We anticipate that microring resonator technology will continue along this steep development trajectory as academic researchers and commercial efforts further demonstrate in vitro diagnostic applications, as well as exciting emerging applications including drug screening, biophysical interaction characterization, and epitope mapping.

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Highlights

- Microring resonators are promising biosensors
- Low-cost, multiplexed, and sensitive for biomarker based diagnostics
- Real-time detection is amenable to screening biomolecular interactions
- Expect continued integration of optical, fluidic, and assay elements

Figure 1.

(a) Analyte binding to surface-immobilized capture agents results in a red-shift in the resonant wavelength circulating in the microring structure. This signal can be monitored in time for quantitative analysis of binding or determination of interaction kinetics. Adapted with permission from [29]. Copyright (2009) American Chemical Society. (b) Schematic of the optofluidic ring resonator geometry illustrating the seamless integration of fluidic sample delivery and optical interrogation. Adapted with permission from [27]. Copyright (2010) Elsevier. (c) Scanning electron micrograph image of a silicon-on-insulator microring resonator and adjacent linear waveguide, both visible through an annular opening in a fluoropolymer cladding layer, which coats the rest of the substrate to reduce non-specific biomolecular interactions. (d) A cartridge assembly incorporating an array of silicon photonic microring resonators with a microfluidic delivery system. An individual resonator in a fluidic channel is shown in the inset. Adapted with permission from [5]. Copyright (2010) Royal Society of Chemistry.

Figure 2.

Multiplexed biomarker analysis demonstrations for both nucleic acid and protein diagnostic applications: (a) Multiplexed microRNA analysis, in which a resonance shift is only observed when target miRNA sequences (columns) hybridize onto specific microRNA array elements functionalized with complementary DNA capture agents (rows). Adapted with permission from [38]. Copyright (2011) American Chemical Society. (b) Quantitative analysis of five different protein biomarkers using an array of uniquely antibody functionalized microRNA resonators using only a 5 minute, label-free binding immunoassay format. Adapted with permission from [30]. Copyright (2010) American Chemical Society.

Figure 3.

(a) An artistic rendering of an array of microring resonators utilized for the biomolecular analysis of kinetic parameters of antibody and aptamer capture agents. (b) Binding of a protein biomarker to a aptamer-functionalized microring resonators results in a shift in the resulting resonance wavelength. Adapted with permission from [22]. Copyright (2011) Royal Society of Chemistry. (c) Protein-lipid and protein-protein interactions were quantitatively interrogated in a label-free binding format by combining microring resonators arrays with phospholipid bilayer nanodiscs, a cell membrane mimic. (d) The phospholipid composition can be tailored to control physisorption efficiency and modulate protein interactions. Adapted with permission from [44]. Copyright (2013) American Chemical Society. (e) Multiplexed kinetic titrations of DNA-encoded antibody libraries enabled the rapid screening of antibody capture agents to determine kinetic rate and equilibrium binding constants. Adapted with permission from [31]. Copyright (2011) American Chemical Society.

Figure 4.

(a) A schematic illustrating the generation of highly multiplexed silicon photonic microring arrays generated via piezoelectric spotting. Here, protein-glycan conjugates were printed with high spatial fidelity for use in subsequent lectin binding assays. Adapted with permission from [42]. Copyright (2011) Royal Society of Chemistry. (b) An illustration of a cascaded microring architecture, in which the filter microring output, which is occluded from the solution above, is optically coupled to the sensor microring input, resulting in 'packets' of spectral resonances (c) that can be interrogated using low-cost, broadband optical sources. Adapted with permission from [45]. Copyright (2011) Optical Society of America. (d&e) Microring resonators integrated within a digital microfluidic device wherein an array of actuation electrodes enables manipulation of individual droplets and improved spatial-temporal control of sample delivery. Adapted with permission from [50]. Copyright (2012) Springer-Verlag.