

## Chapter 7

# Conclusions and Future Work

### 7.1 Summary

Though whispering gallery mode optical resonators were first characterized over twenty years ago, it is important to remember that they have only recently been put to use as chemical and biological sensors. During this developmental stage of the technology, it is critical that researchers continue to scrutinize all available data and models in order to avoid propagating misconceptions or errors that would delay progress toward a useful instrument. Because of their potential to help answer scientific questions, it is easy to forget that the goal for those who use any technology as useful and promising as WGM sensors should always be to make it more available and reliable. With this in mind, I present here my work to examine the various transport phenomena that contribute to the limiting behavior of WGM sensors and to explore ways researchers may take advantage of their extraordinary potential.

I begin by introducing the field of biosensors and how WGM sensing represents the state of the art in this arena. Explaining the principles behind how they may be used for biosensing, I discuss the details of their fabrication and incorporation into an apparatus to perform sensing experiments. This involves carefully positioning a waveguide with respect to a resonator and enclosing this assembly in a flow cell. I share some of the lessons I learned in assembling the equipment required for this delicate process and describe some of the techniques I have come to adopt as these details seldom appear in literature (to the great dismay of those beginning in this field).

Drawing conclusions with an analytical tool like the WGM sensor requires a fundamental understanding of how it operates. This understanding is the sieve through which artifacts may be identified and meaningful data may be extracted. With this in mind I present an exhaustive analysis of the physical models proposed in the literature to describe and predict sensor behavior. I reveal that a variety of misinterpretations of the relevant physical processes have contributed to the misconception that this behavior was well-understood, and go on to present a roadmap for calculating the resonance shift resulting from the interaction between a single biomolecule and the WGM. Though the immediate goal of such a calculation is to validate via theory the single-molecule sensitivity observed previously by Armani et al. [1], the study is intended as an aid and impetus for all researchers in the field to carefully consider the effect of experimental parameters. By applying current understanding and physical models, our results predict that these devices are have a sensitivity too low by a factor of  $10^3 - 10^4$  to explain these experimental observations.

The physical processes previously assumed to be relevant to WGM sensing experiments are inadequate. I go on to propose the role that nonlinear optical phenomena may play in ultra high- $Q$  WGM biosensor sensitivity, focusing in particular on 2-photon absorption. The likely occurrence of this effect would suggests that a single protein molecule adsorbed to a WGM resonator gives off much more heat when intensely illuminated on-resonance than previously estimated. Including 2-photon absorption in our physical model predicts single-molecule resonance shifts comparable to those observed in Ref. [1].

The extraordinary single-molecule sensing data mentioned above also inspired a rigorous consideration of fluid flow and its effect on observed binding kinetics. In the ultralow concentration limit, the analysis of this process can draw heavily from the filtration literature where particle capture from dilute air streams has been described extensively. My work shows how concentration boundary layers that form around objects in flow can greatly enhance the rate at which single molecules or other discrete entities will be adsorb to a sensor. Thin boundary layers yield increased flux of analyte to the sensor surface, and I demonstrate how these layers become thinner for objects with smaller radius or for higher uniform flow rates. Of particular note is the phenomena predicted for microtoroidal resonators, which benefit from anisotropic boundary layer formation due to their minor and major radii. Spheres have only a single characteristic radius and experience larger,

symmetric boundary layers and may therefore demonstrate slower adsorption rates than microtoroids, an important result considering the fact that the WGM sensing literature includes results for both geometries. These results have important implications for the design of flow systems and resonator geometries for sensing applications that must conserve sample, as in the case of medical diagnostics.

I further explore the advantages of WGM sensors over other technologies for biomolecular detection applications by demonstrating quantitative sensing of the small molecule 8-isoprostane (8ip) at sub-picomolar concentrations. This species is used as a biomarker for oxidative stress in the body, but has proven to be a particularly attractive target for probing the conditions in the respiratory track. Sampling a patient's airways has always involved invasive and often dangerous bronchoalveolar lavage techniques, but recent advances in collecting and analyzing exhaled breath condensate (EBC) provide a new and potentially far less traumatizing method. The biomarker 8ip is present at concentrations too close to the detection limits of other analytical techniques to resolve key details in the data collected. I demonstrate that while WGM biosensors using 633 nm resonant light may be able to detect at concentrations as low as 10-100 fM of 8ip in buffer, a more affordable and robust chirp laser using 1310 nm resonant light can still match the sensitivity of current analytical techniques applied for this purpose.

## 7.2 Future Work

There is still a great deal of work to be done to prepare WGM sensing for the transition into a viable instrument. True quantitative sensing will require either a model to account for *all* experimental parameters (likely a computational nightmare to implement) or a convenient method for calibration. Moreover, there is a need for experimental demonstration of how the resonance shift scales with these parameters, including  $Q$ ,  $\frac{d\lambda}{dt}$ , and  $P_D$ . Identifying parameters that do not significantly influence the sensor response would decrease the number of constraints that exist when designing and scaling up sensor fabrication processes. How the sensor response changes according to analyte physical properties, such as the molecular weight,  $M_w$ , the thermo-optic coefficient,  $\frac{dn}{dT_a}$ , and the hydrophobicity, will also be useful when evaluating applications for

these devices. For example, protein biomarkers can be detected at lower concentrations than small molecule (e.g., 8ip) biomarkers because of the difference in molecular weight.

While the ultimate task of how to fabricate these virtually imperfection-free devices with as little variability as possible may appear daunting, the solution may lie in rethinking the entire laser-waveguide-resonator design. Already devices with integrated waveguides [155, 156] and narrow-linewidth tunable lasers without external cavities [154] have been developed. A great deal of work lies ahead incorporating these features into a single instrument, possibly even a single device.

These lines of research represent the next logical steps for anyone in the field of WGM sensing to build upon the results I present here. However, my work has focused on questions particularly well-suited for investigation by a chemical engineer, work that matches the expertise and skill set of those in the Flagan Lab. With that in mind I wish to highlight some of the interesting chemical engineering questions that arise from my work and describe what steps might be taken to explore these topics.

### **Additional Complexity in Mass-Transfer Analysis**

Mass-transfer effects play a role in the analysis of all biosensor data because the transient response will always depend on two concurrent processes that occur on independent timescales—reaction and diffusion. As my work on boundary layer formation and enhancement of WGM sensor response demonstrates, even in the presence of fluid flow it is diffusion that is ultimately responsible for the flux of analyte to the sensor surface. In the case of boundary layer development, that diffusion occurs over a shorter distance than if there was no flow.

My current work treats analyte binding as though it were particle capture by a filter fiber in flow. This simplification includes the convenient assumption that binding occurs immediately and irreversibly, however that is not the case for WGM sensing experiments. An antibody-functionalized resonator exhibits a binding equilibrium with the analyte in the solution, with finite association and dissociation rates. A full analysis of mass-transfer effects for these experiments requires that this feature be included in the model.

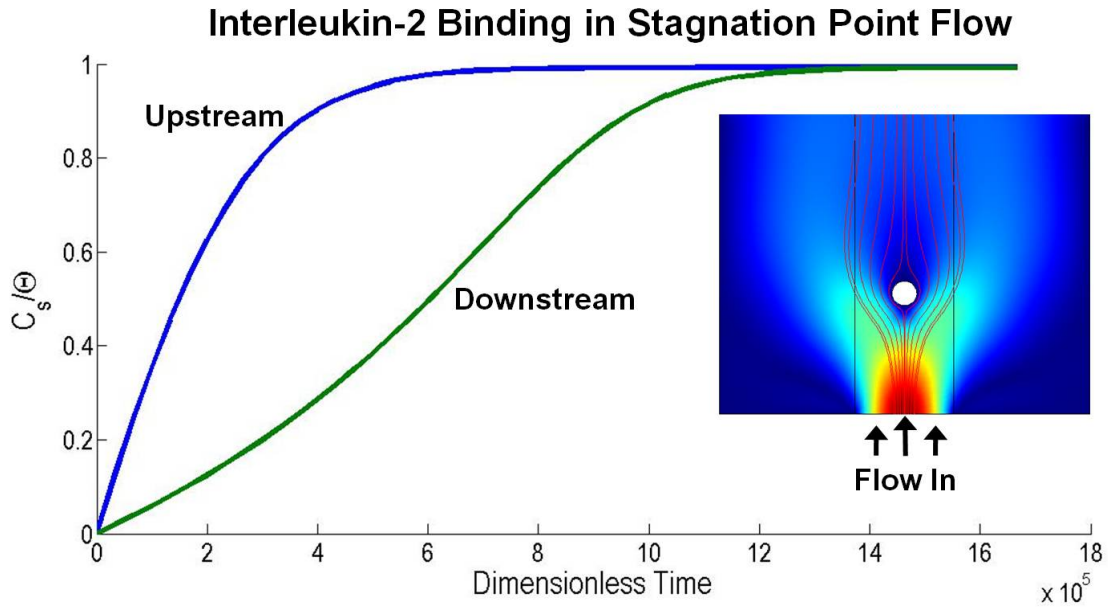
I have already seen from my modeling in Chapter 6 how the boundary layers that form around a WGM

biosensor in flow are smaller upstream than downstream. This produces a molecular flux that is greater at the most upstream point of the sensor (on the equatorial plane) than the most downstream point. For my purposes here I will refer to these points as nodes. Preliminary 2-dimensional modeling of flow around a cylinder, including characteristic binding affinities between interleukin-2 (analyte) and its antibody (targeting species), shows that the antibodies saturate at the upstream node faster than they do at the downstream one. Fig. 7.1 demonstrates this trend and gives greater detail about the surface concentration profile of bound interleukin-2 over time.

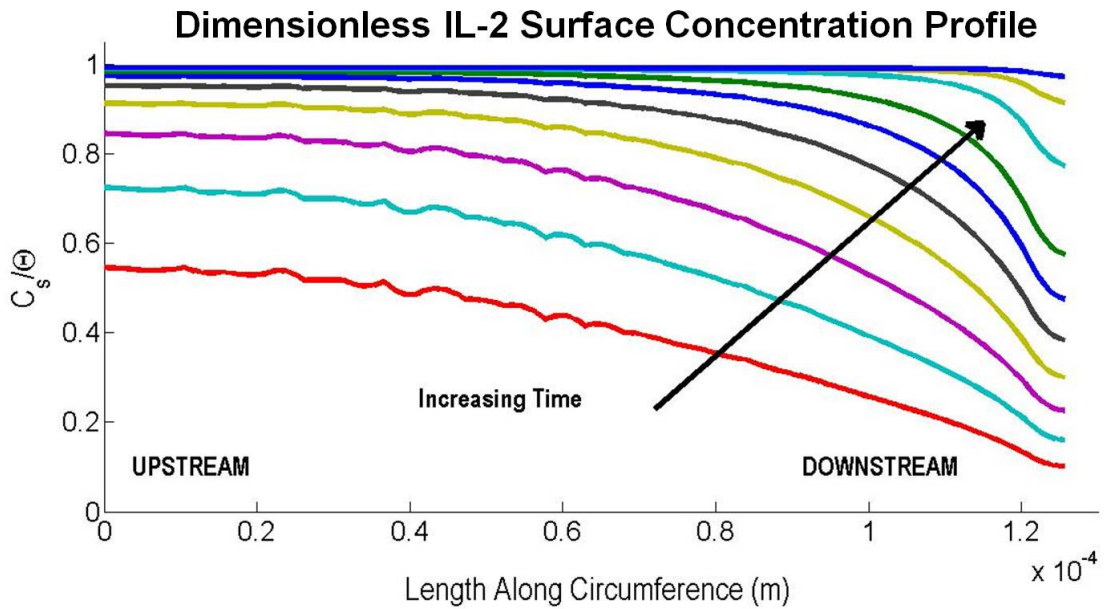
The WGM biosensor is typically used to report the shift in resonant wavelength, a quantity integrated over the entire portion of the device where material may interact with resonant light. Alternatively, the quality factor may be used to gather localized information about binding because of its dependence on surface scattering. The presence of any inhomogeneity will serve as a point of scattering and reduce the  $Q$ . If binding at the upstream node is significantly faster than at the downstream node, a "hot spot" may be created upstream and scatter light. Similarly, a "cold spot" at the downstream node could scatter light due to the low density of adsorbed material, which likely based on how abruptly such a cold spot appears to develop along the resonator surface in Fig. 7.1b.

The anisotropic binding that results from stagnation point flow directly affects the shape of the transient response curve because the resonant wavelength only stops shifting when the entire system is at equilibrium, which occurs only when adsorption balances dissociation rates everywhere on the device. Fitting a function to this curve is a crucial step in measuring the binding reaction rate constant for a given targeting species and analyte pair. Any distortion to this shape will make the curve fitting process less accurate. It appears from Fig. 7.1b that the difference between the surface concentration at the upstream and downstream nodes is never large enough where the binding at one point is limited by transport while the other is limited by reaction rate.

I think there is a great deal to be learned from continuing this work, especially because such a change in  $Q$  with analyte adsorption pattern implies that the sensitivity of the device is another transient quantity in a collection of properties that are already complicated functions of time. This means that the following



(a)



(b)

Figure 7.1: Modeling results for stagnation point flow around a cylinder with adsorption of IL-2 to its antibody. Upstream flowrate is  $100 \mu\text{L}/\text{min}$ . (a) Dimensionless surface concentration of bound IL-2 at upstream node and downstream nodes as a function of dimensionless time (with respect to characteristic desorption timescale). Flow geometry as depicted in inset, with red lines depicting streamlines and cylinder diameter of  $80 \mu\text{m}$ . (b) Dimensionless surface concentration of bound IL-2 as a function of arc length from the upstream node ( $x = 0 \text{ m}$ ) to the downstream node ( $x = 1.26 \times 10^{-4} \text{ m}$ ). Each curve corresponds to a single point in time.

quantities may vary as the tunable laser is swept across a resonant wavelength: the coupled power, the temperature of the resonator and surrounding medium, the refractive index of the resonator and surrounding medium, the mode structure, and the quality factor. Perhaps more interestingly, however, is the possibility that flow could be used to generate a scattering source efficient enough to break the degeneracy of the counter-propagating modes and yield a "split" resonance as seen in Fig. 3.11. These modes have been used to extract additional information about the scattering species, including particle size and number [96]. Some resonators feature imperfections that naturally cause this mode-splitting, but the use of careful flow patterns to create such a mode on demand could provide a more reliable means to explore how this sensing technique can be further applied.

Since any investigation of stagnation point flow effects on the adsorption profile of the analyte would mean sacrificing the convenient axial symmetry of the optical mode, continuing this work requires a full 3-dimensional description of the resonant light rather than the 2-dimensional version provided by the popular Oxborrow method [103]. All modeling results presented here have been calculated using COMSOL, a finite element analysis multiphysics program. Incorporating the binding equilibrium into such a model would be a relatively straightforward application of weak form surface reaction equations to describe the surface-bound species. The full 3-dimensional mode simulation is a task I am far less familiar with and may pose the greater challenge. Such a mathematical model would enable the investigation of the exact binding response, although including a heat transfer model to describe how temperature changes (turning the refractive index, now considered a material function solely of temperature, into a scalar variable of position as well) is so thoroughly complex that it may push the Flagan Laboratory's computation server to its limits.

While using this model to explore the significance of experimental parameters like injection port size and position relative the resonator would be quite useful, a more realistic approach to these questions may be through simple experiments. This would be necessary to confirm the existence of scattering-induced change in  $Q$  as a result of stagnation point flow, and could also be used to determine how the sensor transient response would change if multiple injection ports were used to alter the traditional flow pattern. This flow pattern could help eliminate any change in  $Q$  due to scattering by hot or cold spots and would prove

particularly useful when designing a flow cell for a WGM sensor instrument.

## Optical Effects on Surface Reactions

For all sensor geometries, including the planar SPR sensor, one key assumption that is made when interpreting data or analyzing mass-transfer effects is that the affinity between the targeting species and the analyte is well approximated by the value measured for the freely diffusing species using other analytical methods and standard conditions. This assumption might be inaccurately applied to the WGM biosensor because of the unusually high electric fields to which these biomolecules are exposed when  $Q > 10^7$  resonators are used and because of the temperature changes that may be occurring due to absorption. One or both of these factors could have significant effects on the apparent surface reaction rates, and I consider each here separately.

It is well established that nearly all materials exposed to a large gradient in electric field will experience what is referred to as an *optical gradient force*. This force results from the material's inherent ability to polarize in the presence of a field, which creates a dipole that may be thought of as two isolated charges. Each charge will experience equal and opposite force in the presence of a uniform electric field, however this is not the case when a gradient in the electric field  $\Delta\mathbf{E}$  exists. The net force  $\mathbf{F}$  on the dipole in this case can be expressed as

$$\mathbf{F} = \frac{1}{2}\alpha\nabla\mathbf{E}^2, \quad (7.1)$$

where  $\alpha$  is the polarizability of the molecule or particle. In this way a material will always be pulled toward areas of greatest electric field strength, a feature exploited by optical micro- and nanomanipulation instruments called *optical tweezers* [157].

This force is relevant to how these WGM biosensors may be used to measure adsorption and desorption reaction rates because of the evanescent field that decays exponentially from the surface of the device. The gradient created by this decaying field provides a force attracting material to the surface, as has been demonstrated by Arnold et al. [106]. In that study, the authors were able to form an optical trap by capturing partially sulfonated polystyrene beads (particle radius  $a = 375$  nm) in the potential well that resulted from



the attractive interaction of the optical gradient force and the repulsive interaction of the Coulombic force between the unfunctionalized silica resonator and particle, which each had a negative character in solution. These experiments were designed to demonstrate the existence of this phenomena, employing large particles to ensure that they would experience sufficient force to be trapped. WGM sensing experiments can use resonators with higher  $Q$  values ( $10^8$  compared to the values of  $1.5 \times 10^6$  as used in Ref. [106]) that produce larger gradients in the electric fields that scale as  $|\nabla\mathbf{E}| \sim Q^{1/2}$ , such that some microtoroidal WGM biosensors could generate optical gradient forces an order of magnitude stronger for a given species than those experienced using the spherical devices in Ref. [106].

In the presence of such an optical force, analyte molecules that are bound to the targeting species will find it particularly difficult to migrate away from the sensor. In fact, the vibrations due to thermal excitation of a molecule that typically lead to dissociation may no longer be isotropic, but would instead favor the bound state by virtue of the excluded (or, at least, less favorable) direction of motion. This effect could have implications on heat transfer, which occurs via molecular collision, and may lead to apparent thermal and mass diffusion coefficients that do not match their bulk values. Indeed, anecdotal evidence for role of the optical gradient force in WGM biosensing comes from a series of experiments performed in the laboratory of Professor Armani at the University of Southern California, conducted by graduate students Ce Shi and Maria Chistiakova [2].

In those experiments, a solution containing both dilute streptavidin protein as well as streptavidin-functionalized polystyrene nanoparticles (radius  $a = 25$  nm) were exposed to a biotin-functionalized WGM resonator in buffer. The results for this two-species sensing experiment featured an unstable equilibrium in the surface adsorption reaction, which was replaced by a different equilibrium. The result resembled the response depicted in Fig. 7.2 and likely relates to the fact that the streptavidin diffuses much faster than these nanoparticles and likely coated much of the resonator at first, with the balance of the surface being covered with polystyrene nanoparticles. Over time, however, the streptavidin may have dissociated and diffused away while the PS nanoparticles were trapped at the surface by optical forces, essentially rendering their adsorption reaction irreversible. As the smaller streptavidin molecules relinquished their binding sites

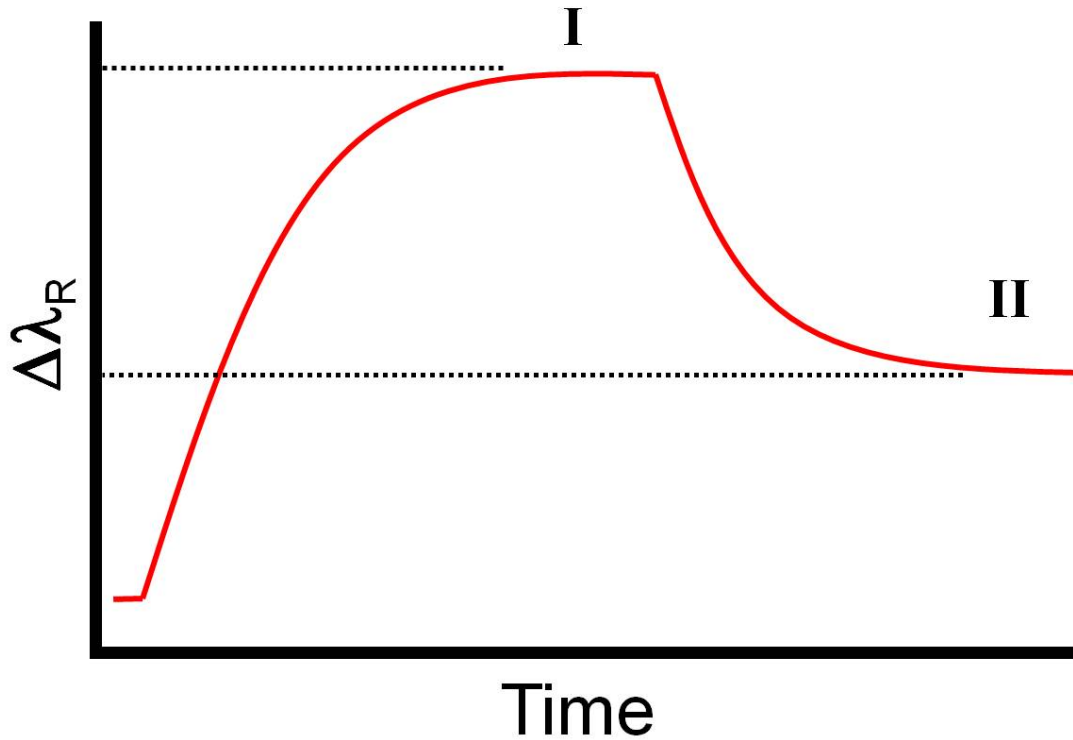


Figure 7.2: This graph shows how the WGM biosensor response appeared for detection of a mixture of streptavidin protein and streptavidin-coated polystyrene nanoparticles (radius  $a = 25\text{nm}$ ) with a biotin-functionalized device. This is not actual data. Note the existence of two equilibria: the first (I) where the surface is populated with bound protein and nanoparticles, and the second (II) where the smaller streptavidin has dissociated and been mostly replaced by nanoparticles.

to the nanoparticles, a new equilibrium coverage may have been achieved that included almost entirely nanoparticles (some streptavidin molecules may have been “trapped” via steric effects).

As mentioned above, the second way in which the intense electromagnetic fields may affect the surface reaction between the analyte its targeting species it through absorption. There are a range of phenomena through which material on the surface may absorb or interact with light that lead to this material dissipating heat to its surroundings. We address some of these processes in earlier chapters, but as a brief review I will note that linear absorption, nonlinear absorption, two- and three-photon absorption, stimulated Raman scattering, and Brillouin scattering all introduce vibrational energy to a molecule that is converted locally into heat. Each process has a characteristic efficiency, and some of them are only relevant at extremely high optical powers. Regardless, the resonator itself as well as the surrounding medium (i.e., water) absorb

sufficient light even at low coupled powers and moderate quality factors ( $Q \gtrsim 10^5$ ) that nonlinearities occur during sensing experiments. This “thermal broadening” effect comes from the fact that power is increasingly coupled into the device during a sweep through wavelength space, warming the materials and inducing a concurrent resonance shift such that the sweep seems to be *chasing* the resonant wavelength and the dip trace acquired by the oscilloscope no longer has the symmetrical Lorentzian shape found in the absence of absorption.

The bulk warming provided by the the silica and water is enough to change the affinity of the analyte with its targeting species. The rate constant  $k_{rxn}$  used to describe a chemical reaction is a function of the temperature  $T$ , with most obeying an Arrhenius law where  $k_{rxn} = Ae^{-\frac{E_a}{RT}}$  and  $A$  is a constant,  $E_a$  is the activation energy, and  $R$  is the gas constant. Warming a reaction typically drives it faster, although it is very difficult to determine *a priori* if the adsorption or the dissociation reaction will be affected more. Therefore, one can only say with certainty that the dissociation equilibrium coefficient  $K_D$  will be a function of temperature.

When species are present that absorb much more efficiently than their surrounding materials (i.e., water and silica), such as an oligomeric protein, the pulses of heat may be sufficient for the molecule to breakdown either into its component pieces or at some weak bond. These events represent side reactions that may be irrelevant at standard laboratory temperatures that become significant when heat is injected locally and is unable to diffuse very quickly on the timescale of the pulse. Additionally, the specificity of the targeting species may change due to side reactions involving molecules structurally or chemically similar to the analyte that are present in the flow cell. What is certain, however, is that adding heat to the complex WGM sensing system can have any number of effects that must be taken into account when optimizing the application of this emerging tool.

Measuring the exact significance of these effects is difficult because they are coupled and would have overlapping effect. Increasing the coupled power to increase the optical gradient force would also increase the temperature of the materials. In order to observe the isolated importance of temperature, one could immobilize on the sensor (in addition to the targeting species) a species that absorbs very well at around

400-500 nm but does not exhibit any fluorescence. Most proteins absorb weakly at these wavelengths and therefore would not act as effective heat sources, so a specially designed fluorescence quencher molecule may be a good option. The trick would be to excite modes in the resonator with red *and* blue or green light simultaneously. The low-wavelength light would stimulate a heat source while the red light would be used to excite the resonance used for sensing. This is in lieu of using far-field illumination at the low wavelength because it would be difficult to get an exact measure of the amount of power actually getting to the quencher heat sources. Moreover, scanning the low-wavelength laser adds an additional variable which may be helpful to use while studying the effect of continuous or intermittent heating on the surface reaction. This experiment hinges upon the existence of a fiber-coupled laser at these wavelengths with moderate linewidth and the possibility of tuning. This should pose little challenge as New Focus is currently developing a Velocity<sup>TM</sup> tunable laser with  $< 500$  nm wavelength.

Another interesting experiment to study the optical gradient force would be to vary the size of the nanoparticles at low coupled power in the under-coupled regime. By monitoring the frequency of dissociation events one may be able to establish a cutoff size at which optical gradient forces are relevant while minimizing variability in heat injected into the system via absorption. Alternatively, one could investigate the role of laser scan rate on optical gradient force and minimum particle size required for it to matter. Here it is helpful to remember that the optical gradient force is only applied while on or near resonance. As such, one critical threshold in this behavior may occur when the characteristic time it will take a particle to escape the evanescent field is equal to the time between pulses, or when

$$a \approx \frac{2k_B T (n_s^2 - n_m^2)}{3\lambda^2 \nu \eta}, \quad (7.2)$$

where  $a$  is the particle radius,  $k_B$  is the Boltzmann constant,  $n_s$  is the sensor refractive index,  $n_m$  is the surrounding medium refractive index,  $\nu$  is the frequency of scans (*not* the optical frequency),  $\lambda$  is the laser wavelength, and  $\eta$  is the viscosity of the medium through which the particle is moving. Using low coupled power and small scan scan frequencies ( $\nu$ ) assures that the heat may dissipate between scans and the effects

can be attributed to the forces.

## Improving Specificity Through Multi-Functional Sensors

Another interesting line of research would aim to improve the specificity of all immunoassay-based biosensors, including the WGM biosensor, by abandoning the paradigm of one antibody binding to one analyte. As noted in Chapter 2, antibodies are a very convenient targeting species because they are raised against a single target and can have  $K_D$  values as low as 10 pM. In neither commercial biochemical production methods nor in natural biology are antibodies typically selected for the ability to reject interactions with more than a few structural analogs of the analyte. An organism benefits from investing its energy into making antibodies that are sufficiently non-specific that they may be effectively useful more than one target. Additionally, even variation within a batch of a monoclonal antibody produced by a biochemical company will lead to a distribution of binding affinities for the intended antigen. This issue can be overcome to a small degree when a pharmaceutical company invests a great deal of research and care in the quality control of antibody-based therapeutic production, as required by FDA regulations. This level of care far surpasses that commonly found in non-therapeutic grade antibody, however.

It would be foolish to turn our backs on this convenient targeting species platform unless a simpler or less expensive one were already available. While there has been progress raising polymers capable of specific binding as well as designing hybrid architectures for combining targeting species with other materials to improve stability or affinity, these methods remain inadequate alternatives to the antibody-antigen specific sensing platform. Perhaps the most promising avenue for improving sensor specificity lies in the seemingly counterintuitive method of depositing a collection of targeting species, all with different affinities for any given analyte [158]. An array of sensors may then each present a different mixture (or at least mixtures with different constituent ratios) and much more information is available to identify the analyte or analytes present.

Such a mixture of targeting agents would present a signal identification challenge. No longer would the resonance shift of a given sensor be sufficient to determine the species, but rather the relative signal

magnitudes from each sensor in the array. In essence, an analyte would interact with several different types of targeting species, but would generate a particular pattern of responses in the array that can serve as its fingerprint. This type of measurement is common for "artificial nose" sensors which often have far more limited specificity than the current immunoassay methods employed in WGM sensing. Of course, careful calibration must be done before any data can be extracted, and it may take a great deal of work to establish a reliable platform based on a mixture of antibodies.

One way this surface-functionalization strategy might be simplified involves the use of hybridized, multivalent targeting species. It is well established that multivalency is a route to increasing the specificity of an interaction [158], as demonstrated by the frequency with which it occurs naturally on the cellular scale. Cholera toxin B, for example, is a hexamer where each of the five active subunits binds to an individual GM1 ganglioside in the cell membrane. The affinity of each of the five sequential binding interactions exceeds that of the last, such that the only way this protein can remain attached to the cell membrane long enough to undergo endocytosis (its goal) is if all subunits are bound. While an all-at-once, pentadentate binding event is exceedingly unlikely, it is assisted in part due to the freedom with which the gangliosides may diffuse in the cell membrane. The presence of "lipid rafts," or regions within the fluid cell membrane where mobility is locally decreased due to elevated concentrations of molecules like cholesterol, have been hypothesized to exist aid in multivalent interactions like this one by "herding" the receptors while still enabling them to diffuse locally.

The rational design of hybridized, multivalent targeting species as therapeutics has already been demonstrated [159, 160] and continues to be refined [161]. It remains to be seen whether one could create a multivalent molecule with different targeting species (of a controlled ratio) at each position, although such a molecule could serve as a precalibrated building block where using a mixture of different blocks could help tune specificity. Creating this heterogeneous multivalent targeting species requires a great deal of knowledge about the chemistry involved, but analysis of the data is well suited for a chemical engineer. This is especially true if the relative timescales of the various interactions between an analyte and the range of targeting species are dissimilar.

The concept of integrating an array of sensors, each with its own composition of targeting species, relies on two additional challenges being addressed. The first is that a multiplexed sensor must be fabricated, which has been demonstrated previously [162, 163, 164]. The current form of the WGM biosensor apparatus, in particular the use of a single, delicate tapered optical fiber waveguide for each resonator, precludes this kind of parallel scale-up. Devices with integrated waveguides must be developed for robustness and simplicity, and light used to excite WGMs must be split from a single laser source to make data processing more manageable.

Additionally, these devices must facilitate the second challenge, which is to fabricate a single microscale device with different regions (i.e., sensors) presenting different functionalities reliably. Unless identical distributions of targeting species can be deposited from one device to the next, prohibitively extensive calibration must be done for each. It would seem that laminar flow in a microfluidic channel (one channel for each device) may be used to deposit different surface chemistries in a suitable precise fashion (see Fig. 7.3), although the deposition of microdroplets of solution on individual sensors may also prove useful (assuming such a technique is possible through careful control of surface wettability)

Using flow or another mechanism to control the position of a surface reaction, characterizing that surface reaction as well as the one that occurs during specific sensing of biomolecules, and the rational design of reagents to enable parallel scale-up of a unit operation (sensor) represent challenges through which a chemical engineer may make significant contributions to the field of WGM sensing as well as sensing in general. I believe this work should begin with the two relatively simple steps of (i) functionalizing a chip, absent any sensors, with different targeting species in different regions and (ii) covalently attaching a collection of antibodies raised against similar antigens in varying ratios to a WGM sensor to explore how the endpoint resonance shift may be used to surpass current specificity-related limitations.

## **Bridging Vapor- and Liquid-Phase Sensing Methods**

The suggestions for future work I have already discussed add up to several doctoral theses worth of work, but one remains as a fascinating idea for the Flagan Lab, in particular, and merits inclusion here. Not only have WGM biosensors been demonstrated as tools useful for both liquid- and vapor-phase detection, but also

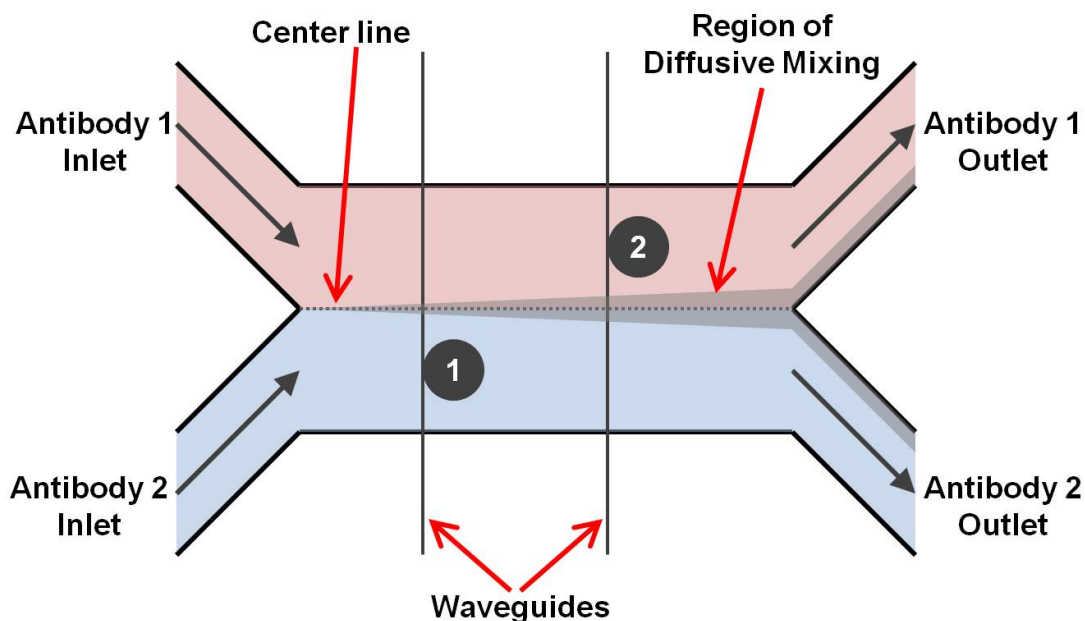


Figure 7.3: This microfluidic device diagram demonstrates how the laminar flow in such a device may be used to deposit different targeting species (referred to as *Antibody 1* and *Antibody 2*) on different sensors (labeled *1* and *2*) simultaneously. For sufficiently short channels, diffusive mixing between the adjacent flow paths will be limited to the small area indicated.

for aerosol sampling [96]. It is in this capacity that I believe WGM biosensors still have untapped potential. For the most part, aerosol particles are quite dilute in air and require filtration or impaction to capture and concentrate these particles for analysis. These techniques add additional steps to the process of isolating the aerosol species, which bring new opportunities for contamination. It would be preferable to just introduce the aerosol to the analytical tool directly, but it is difficult to get any information out of such a measurement due to the absence of a targeting species to isolate analytes of interest.

A new avenue of research into exacerbation of asthma by pollen has been to analyze the fragments of pollen that are present in the atmosphere that go unidentified using traditional pollen-count methods. It is believed that these species, much smaller than the whole pollen spores commonly considered, can make their way farther into the respiratory tract and, thus, interact with a part of the lungs that has been overlooked to date. Collaborators in Austria have successfully raised antibodies against some of these fragments and hope to apply these molecules to highly sensitive bioassays to quantify the level of pollen fragments in the



air so that this data may be correlated to the instance of asthma attack. These fragments are present at sufficiently low concentrations that capturing them is only a part of the challenge; they must also be concentrated. However, WGM biosensors could be used, in conjunction with antibodies for specificity, to avoid these inefficient concentration steps if the fragments could be transferred from the air into liquid.

Other members of the Flagan Lab are already working on efficient means of vapor-liquid transfer for small aerosol particles, but a simple solution exists if only a single challenge could be overcome. One could imagine that a jet of ambient air impinging on a reservoir of liquid, within which resides a WGM biosensor, could transfer some of these particles directly into the effective "flow cell" surrounding the device. In order to minimize reservoir volume (increasing analyte concentration) and avoid evaporation of the liquid, measures must be taken to trap the liquid in the cell. I believe this may be accomplished by coating the devices with a hydrogel, a polymer structure often used as a biocompatible matrix known to serve this very purpose in tissue and biomolecular engineering applications.

A hydrogel is usually cross-linked, with either a chemical or ultraviolet light serving as the activator. A monomer may be spin-coated onto a chip of sensors, possibly functionalized as discussed above, and then cross-linked. The film thickness can be controlled through the spin-coating process and its chemical nature controlled through careful choice of monomer. In this way there will be a structure in place to keep a liquid layer surrounding the sensors even in the presence of impinging air, and this layer should still allow diffusion of analyte species now captured in liquid to diffuse to the sensors. An extra, sub-hydrogel reservoir of water or buffer may be necessary to ensure against drying, but this architecture would allow for a WGM biosensors strengths (specificity and sensitivity) to be exploited for the purpose of analyzing aerosol composition and concentration.

There also remains an issue to resolve concerning the observed kinetics and binding affinities between analyte and targeting molecule observed experimentally. As noted elsewhere, there exists an optical gradient force that acts to drive material toward regions of high electric field intensity [106]. This may be opposed or aided by Coulombic forces between surface charges on the resonator-bound targeting species, the resonator material itself, or because the analyte itself carries a charge in solution. This force has thus far only been

observed in WGM sensors to affect large ( $d_p > 100$  nm) nanoparticles, but its significance for protein-sized analytes ( $d_p < 10$  nm) must still be determined. These forces can drastically affect the binding kinetics, as is clear in the limiting case where a purely attractive force on a protein both draws it toward the sensor more efficiently than diffusion and also keeps it from escaping if it dissociates from the analyte-target complex.

I consider these ideas as best suited for the engineering community to address, which is only beginning to work with WGM sensors. It is an entirely different (and exciting) game to imagine the purely scientific uses these tools may have. For example, I discuss during Chapter 5 how biophysical considerations may play a part in accurately describing heat transfer in the WGM sensing system. One could imagine looking at the role of hydrophobicity, both on the part of the analyte and the resonator surface, in creating a water environment that promotes or complicates energy transfer. Such an experiment could employ nanoparticles of varying surface chemistries or the use of established silane vapor deposition methods to control the sensor surface.

Additionally, one could explore the concept of molecular "heat" transfer by controlling the linkage between a nanoparticle tethered to the sensor surface. By varying the nature of the covalent linkage that tethers a nanoparticle to the resonator, the absorption coefficient of the nanoparticle material, and the WGM driving power, one may consider how vibrational energy is transmitted through different chemical structures. Along with the hydrophobicity experiments discussed above, this work would allow measurement of physical properties on a single-molecule level. Such quantities are masked during bulk, ensemble-averaged measurements, where distributions and variations in the property are lost.

Ultimately, my work contributes to an ongoing debate about the behavior of WGM sensors. Though the debate was, at times, less civilized than I would have imagined or hoped, I have enjoyed playing. There are many clever researchers already using WGM sensors, and I hope my work makes them more accessible and interesting to new ones.