Electronic Readout of Microchannel Resonators for Precision Mass Sensing in Solution

by

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Submitted to the Harvard-MIT Division of Health Sciences and Technology
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Abstract

Microfabricated transducers have enabled new approaches for detection of biomolecules and cells. Integration of electronics with these tools simplify systems and provide platforms for robust use outside of the laboratory setting. Suspended microchannel resonators (SMRs) are sensitive microfluidic platforms used to precisely measure the buoyant mass of single cells and monolayers of protein in fluid environments. Conventionally, microcantilever deflection is measured by the optical-lever technique, wherein a laser beam is reflected off the cantilever onto a position sensitive photodiode. This thesis introduces microchannel resonators with electronic readout, eliminating the use of external optical components for resolving the sensor’s resonant frequency.

Piezoresistors have been fabricated on SMRs through ion implantation integrated with the existing SMR fabrication process. We fabricated two designs: one with a cantilever length of 210 μm and resonant frequency of ~347 kHz, and the other with a cantilever length of 406 μm and resonant frequency of ~92 kHz. The work here builds upon knowledge of signal transduction from static and dynamic cantilever-based sensors because the piezoresistors are implemented on vacuum encapsulated devices containing fluid.

Electronic readout is shown to resolve the microchannel resonance frequency with an Allan variance of $5 \times 10^{-18}$ (210 μm) and $2 \times 10^{-17}$ (406 μm) using a 100ms gate time, corresponding to a mass resolution of 0.1 and 0.4 fg respectively. This mass resolution calculated from piezoresistive readout frequency stability, is approximately 3X better than optical readout for the 210 μm device and 1.3X for the 406 μm device using the same gate time. Resolution is expected to improve with further optimization of the system. To demonstrate the readout, histograms of the buoyant masses of a mixture of size standard polystyrene beads (with nominal diameters 1.6, 1.8, and 2.0 μm) and budding yeast cells were made.

Thesis Supervisor: Scott R. Manalis
Title: Associate Professor of Biological and Mechanical Engineering
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There is not much that can be done to make research easier - but excitement and passion are two key elements of success, and two of the many things I have learned from my advisor, Scott Manalis. It has been an awesome opportunity that I am especially thankful for, to work in nanoscale sensing with him.

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Chapter 1

Introduction

1.1 Background

Refined abilities in engineering today enable applications in a wide realm of disciplines. For example, tools developed through the sensitive electrical and mechanical design principles of silicon microfabrication can be used to probe biological systems. Using these tools, signals can be transduced from the biological realm to electrical, mechanical and other physical domains. This application of classical techniques to study new areas has enabled fields such as systems biology, which demand a more quantitative understanding of biological phenomenon, to blossom. In addition the application of these techniques from a different realm can increase versatility and performance in existing fields such as pharmaceutical research and clinical diagnostics. Micro-cantilevers, fabricated through today’s semiconductor fabrication techniques, are one such method amenable for performing this transduction. Micro-cantilevers, particularly when employed as resonant mass sensors, are amenable for measuring the mass of biological substrates due to their low (picolitre) sample volume requirements [7] and exceptional mass sensitivity (as low as sub-femtogram in a 1Hz bandwidth) in fluid [7]. These factors have enabled resonant mass sensors to be used for detection of single cells and viruses [28, 24, 49].

Cantilever sensors have traditionally been operated in either static or dynamic mode. In static mode, adsorption-induced surface stress changes cause variation in
the deflection of the cantilever. Sub-angstrom displacement (10 Hz bandwidth) [6],
and force sensitivities of 10 piconewton [42] have been demonstrated. In dynamic
mode, adsorption-induced mass or stiffness, or temporary effective mass changes, vary
the resonance frequency of the cantilever [11, 7]. Low frequency drift is a limitation
for static measurements. As well, the stress achieved on a surface will depend on prop-
erties of the target including steric hindrance, electronic charge and hydrophobicity.
For biological substrates, which are typically in fluid environments, static deflection
techniques have been routinely used because in dynamic mode, fluid encasing the
resonator precipitates a low quality factor because of hydrodynamic damping. Also,
for interactions that don’t alter surface stress or are heavy (protein-size molecules),
dynamic-mass sensing is advantageous.

Suspended microchannel resonators (SMRs) have enabled dynamic sensing of sub-
strates in fluid by containing the microfluidic channel within a resonator [8]. The SMR
translates mass changes into changes in resonance frequency. Fluid continuously flows
through the channel and delivers biomolecules, cells or synthetic particles. Extremely
sensitive mass resolution has been achieved by encapsulating the resonator in high
vacuum, reducing damping from the surrounding medium. Measurement of substrates
in fluid are by two means, a) binding, wherein species increase the effective mass of
the resonator by binding to a functionalized interior surface of the channel wall and
b) flow-through mode, wherein particles flow through the cantilever without binding
to the surface, and the observed real-time signal depends on the position of particles
along the channel as depicted in Figure 1-1. The exact mass excess of a particle can be
quantified by the peak frequency shift induced at the apex. Static sensors have been
analogously used for molecular surface binding measurement, although motion of the
sensor results from a stress mechanism instead of mass. Though, static sensors have
not typically been used for weighing cells and particles which are the applications
focused on here.
Figure 1-1: a) The general concept behind the SMR is that an increase in effective mass of the resonator results in a frequency shift, $\delta \omega$. b) Flow-through particle and cell weighing mode is used here: as a particle or cell flows through the microchannel, the frequency shifts as the particle goes from the base to the tip, where the mass sensitivity is greatest. If the fluid flow is controlled, induced frequency shift can be monitored in real-time.

1.2 Actuation and detection methods

Some noise sources that affect static sensors are common to resonant sensors as well. These include $1/f$ noise and environmental effects (e.g. from temperature or pressure) which can couple through parasitic capacitances. Implementation of resonant sensors also introduces issues that static sensing is immune from. These include dynamic noise sources such as AC environmental and parasitic noise sources and unwanted frequency modulation. All of these factors affect signal transduction to and from the resonator, but nonetheless groups have successfully built resonant mass sensors utilizing a variety of actuation and detection methods. In this section the common types of actuation and displacement detection methods used in resonant mass sensing are explored.
1.2.1 Actuation

Dynamic force on resonant sensors can be generated through a variety of mechanisms including thermomechanical [49], bimetallic thermal expansion [65], piezoelectric [45], electrostatic and magnetic or Lorentz force [31, 47]. Factors which determine the type of actuation method selected include the force distribution (point or localized), number of cantilever sensors to be actuated, frequency and bandwidth requirements, fabrication capabilities and geometric constraints.

1.2.2 Detection

Detection methods for measuring cantilever vibration frequency can be broadly classified into 1) optical detection and 2) electronic detection. The optical lever has been conventionally used for measuring deflection and frequency of Atomic Force Microscopy (AFM) tips in tapping and non-contact modes. This form of measurement therefore naturally translates to measuring the frequency of resonant cantilever sensors. Extremely sensitive sub-angstrom deflection has been measured through optical lever readout of cantilever deflection. Optical interferometry has also been translated to deflection and frequency measurements of cantilever sensors [27, 58] and provides angstrom resolution. Other methods which have also been used as detection methods for resonant sensors and require optics for imaging or focus of a laser spot, are photonic transduction and Laser Doppler Vibrometers (LDV) [49, 38]. Noise sources associated with lasers are introduced in these methods, however practically, laser alignment, focus and environmental disturbances are typically the main impedances to performance. In addition, all of these methods require the use of detectors and optical components separate from the sensor itself. The alignment and placement of these required components can limit the flexibility of sensor use. Integrated optical detection has been studied for arrays of static deflection mode sensing by the use of CCD, but currently is limited by a tradeoff between accuracy and number of cantilevers [41]. Prior to suspended microchannel resonators, turbidity of fluid and refractive index change also added complications to resonant mass sensing [34].
For the SMR, the optical-lever has been appropriate as readout in the current laboratory setting, since most applications require a single SMR operated at a time and alignment is not an issue in the laboratory setting. However, it is anticipated that the ability to operate arrays of SMR in portable, multiplexed configurations will result in new applications. Electronic methods of frequency detection also have the benefit of integration with and even during the fabrication of the resonator, robust batch fabrication methods, no external components required, and their performance may be better than optical methods in certain circumstances which will be explored more deeply in this thesis.

Electronic frequency detection methods which have been implemented on resonant mass sensors include capacitive [20, 35] and piezoresistive [31, 39, 56]. A major limitation of capacitive sensing are parasitic effects, however this method can work well when amplifier and other CMOS processing is integrated with the existing fabrication process [20]. We have considered harnessing the electrode, used for electrostatic actuation, also for capacitive detection of the resonance frequency [12]. In the current SMR geometry, parasitic capacitances in parallel with the electrode can be up to 1pF, from on-chip electrode and bond-wire capacitances, degrading the signal to be detected to a less than 0.01% capacitance change.

For nanoscale resonant mass sensors, a study of capacitive versus piezoresistive transduction schemes has shown that piezoresistive readout has fundamentally better overall performance (mass resolution) at frequencies greater than 10MHz [14]. Piezoresistors can be fabricated directly on cantilevers using robust fabrication methods. Also a variety of semiconductor and metal materials all have been used, and can be selected based on noise and frequency requirements. The piezoresistive effect in semiconductor materials [55] was first discovered in the 1950’s and first used on pressure transducers [64, 22]. Figure 1-2 illustrates key developments in piezoresistor technologies. Piezoresistance has been used in strain sensors, accelerometers and have been implemented on AFM cantilevers for force displacement detection [62], and used as strain sensors on static cantilever sensors for environmental [6], chemical [29] and biological [43] sensing.
Figure 1-2: Timeline of piezoresistance concept and applications. The discovery of the piezoresistance effect, as well as the timing of the first implementations of piezoresistors on certain applications, from strain gauges to micro and nanoscale resonant mass sensors.

[44, 64, 70, 22, 62, 29, 43, 31, 39]
<table>
<thead>
<tr>
<th>Group</th>
<th>Year</th>
<th>Piezoresistor Material</th>
<th>Mass Resolution (bandwidth)</th>
<th>Resonant Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hosaka</td>
<td>2004</td>
<td>doped silicon</td>
<td>5.5pg (1 kHz)</td>
<td>174 kHz</td>
</tr>
<tr>
<td>Yu</td>
<td>2005</td>
<td>doped silicon</td>
<td>29fg (1 Hz)</td>
<td>298 kHz</td>
</tr>
<tr>
<td>Roukes</td>
<td>2007</td>
<td>gold</td>
<td>&lt; 1ag (10 Hz)</td>
<td>127 MHz</td>
</tr>
</tbody>
</table>

Table 1.1: Previous implementations of piezoresistive resonant mass sensors and relevant specifications.

Resonant mass sensors have proven very sensitive for measurement of biological substrates. As well, some examples of resonant mass sensors have been integrated with piezoresistors for measurements in air. Table 1.2.2 lists the examples of this to date. Improved performance has been achieved by scaling down the sensors to nanoscale [39] or use of second-mode resonance with optimized excitation for the higher mode [31].

As of yet, mass measurements in liquid using resonant mass sensors have not been demonstrated using integrated electronic readout. In this thesis we implement piezoresistive readout on suspended microchannel resonators, for mass measurements of biological substrates without external optical components and compare the performance of the piezoresistive readout to existing optical lever readout.

1.3 Outline

The main contributions in this thesis are 1) development of piezoresistive readout for Suspended Microchannel Resonators, 2) mass measurements of particles and cells with piezoresistive readout, and characterization of the performance of two types of piezoresistive SMRs compared to our optical lever readout, 3) conception and implementation of the agglutination assay using mass measurements by the SMR.

Chapter 2 discusses the fabrication and considerations for two types of piezoresistive devices. The issues particular to implementation of piezoresistors on the SMR are highlighted.

Chapter 3 outlines characterization of the piezoresistors including the experimental setup and methods for sensitivity calibration of vacuum packaged devices.
Chapter 4 details mass measurements made using the piezoresistive devices and comparison of the frequency noise of each of the devices using optical and piezoresistive readout.

Chapter 5 presents the development and results of the agglutination assay by mass measurement in the SMR including analysis of how the number of measurements contributes to error.

Chapter 6 concludes with discussion of the future of piezoresistance and next steps for the work in this thesis.
Chapter 2

Fabrication and Considerations

Implementation of piezoresistors on suspended microchannel resonators present some similar and some unique design considerations in comparison to previous piezoresistor work. To date, piezoresistors have been fabricationed on many types of devices including microcantilevers used for biological sensing. However, piezoresistors have never before been fabricated on devices which are encapsulated with bonded pyrex lids, or on hollow micro-cantilevers which will contain biomolecules or cells. Finally, piezoresistor use on cantilever sensors has typically been on static-mode sensors with a few examples on resonant mass sensors thus far.

This section details the design of two types of piezoresistive SMR devices; one 210μm long and the other 406μm long. Design considerations for the piezoresistors, including fabrication materials and methods are detailed in this chapter. In addition, the expected signal magnitude for dynamic and static modes, and temperature effects of the optical lever and piezoresistor readout are analyzed for both types of devices based on the design.

2.1 Fabrication

Previously, piezoresistors have been implemented with semiconductor or thin metal film materials [62, 39]. Doped silicon and polysilicon are advantageous as the piezoresistive material on micron-scale cantilevers due to the band gap assisted gauge factor
which is much higher than the gauge factor of metals which exclusively relies on geometric effects. Furthermore, the gauge factor in doped semiconductor materials can be controlled via the doping conditions such as impurity concentration. In addition, silicon has a higher gauge factor than polysilicon. Common fabrication methods for integrating piezoresistors on silicon substrates are: ion implantation, epitaxy or diffusion. Ion implantation is the preferred method for doping silicon because it provides excellent control over doping concentration and dopant depth, and the implantation is straightforward to integrate with existing fabrication procedures. Previous generations of SMRs were fabricated in silicon nitride [9, 8]. At this time other electronic readout options were considered that did not involve changing or adding to the fabrication procedure [12]. Here we implement piezoresistors using ion implantation on a single crystal silicon-based SMR process.

2.1.1 Piezoresistor fabrication steps

Fabrication steps for the piezoresistor were added to the existing fabrication procedure for the SMRs [7]. The devices were fabricated at Innovative Micro Technology in Santa Barbara, CA, with a fusion-bonded silicon wafer containing integrated microchannels and two pyrex wafers for the top and bottom lids. Prior to release of the cantilevers, four ion implantation steps were added to create piezoresistors in the top silicon layer of the wafer stack. Cross-sections of the SMR, illustrating the point at which the piezoresistor fabrication steps are implemented in the process and the final device structure, are shown in Figure 2-1.

Ion implantation was performed at Innovion Corporation in Santa Barbara, CA. Background resistivity of the silicon wafers was approximately 1-20 Ω·cm (n-type). First, a pre-implantation oxidation was performed for 10 minutes at 850 °C. To ensure p-n junction isolation of the piezoresistors from the substrate, phosphorous was implanted in the device background, at an energy of 160 keV with a dose of $1.0 \times 10^{13}$ ions/cm². Wafers were then annealed at 1050 °C for 10 hours in a nitrogen environment. For the piezoresistive region, boron ion implantation was performed at 50 keV with a dose of $5.0 \times 10^{13}$ ions/cm². The substrate was also implanted with boron
Figure 2-1: a) Piezoresistors (red/top layer) are implanted on SOI wafers prior to release of the cantilever. b) A pyrex lid is anodically bonded to the cantilever surface after implantation. c) The final SMR cross section with pyrex lids on the top and bottom of the silicon wafer structure. A metal electrode (yellow/on bottom pyrex) is located below the cantilever for electrostatic actuation.
Figure 2-2: a) Top view of the resistor geometry. The piezoresistor area is in red (p-) and contact region (p+) is green. There is also a contact to the silicon substrate (n+) in light grey. b) Cross-section showing piezoresistor and contact implant areas. There is a diode formed between the piezoresistor and device layer of the SOI wafer which is reverse biased during measurement to prevent current leakage to the substrate. The blue line illustrates an oxide layer between the handle silicon and body silicon layers of the wafer.

at 120 keV with a dose of $5.0 \times 10^{15}$ ions/cm$^2$ for connections between the piezoresistors and the metal pads for wire-bonding. These connection areas create a surface which is suitable for a good quality bond between the pyrex lid and top silicon layer. Aluminum is selectively deposited on some of the connection area to decrease total resistance; however the top pyrex cannot be bonded directly on this metal layer. Finally phosphorous was implanted for contact to the background silicon ($5.0 \times 10^{15}$ ions/cm$^2$ at 120 keV). The implanted piezoresistors are parallel to the $<110>$ direction to maximize the gauge factor. After the piezoresistor and contact implants, the wafers underwent a second anneal in a nitrogen environment at 950°C for 1 hour. Figure 2-2 illustrates the resulting cross-section and top-view of the wafer after the implants.

Previous studies have shown that contamination by sodium from the pyrex layer occurs during anodic bonding [53]. To isolate the piezoresistor and background areas from contamination, we deposited a 5000 Å PECVD oxide layer at 350 °C before
anodic bonding of the top pyrex layer. The type of insulative material, deposition and thickness was determined empirically through a series of short loop tests. There were two types of tests performed to ensure that 1) the anodic bond of the top pyrex layer to the silicon is still high enough quality to maintain an ultra-high vacuum in the cavity surrounding the SMR, and 2) the insulator sufficiently protects the silicon from ionic contamination. For the first series, we used test wafers with etched membranes. On each wafer, prior to anodic bonding of the top pyrex lid, a different type of insulator material or thickness was deposited. Deflection of the membranes was then measured with an optical profiler. We found that with a 5000 Å PECVD oxide layer, membrane deflection matched expected results in vacuum environment, and the bond was consistent across the wafer. For the second test series, wafers with implant parameters identical to those used in our process, and with the same insulator film deposited before pyrex wafer bonding to the silicon, were used. Current-voltage characteristics were measured before and after the bonding (Figure 2-3). Reverse leakage current in the devices with 5000 Å PECVD oxide insulator is limited to less than 1μA, compared to approximately 100 μA at -0.6V bias with no protection, demonstrating successful insulation. The oxide was stripped from the cantilever surface before the anodic bonding. Subsequent fabrication steps follow as previously reported.

**Resistor Geometry**

SMRs of two different lengths (210 and 406 μm) were fabricated. Both are 28.5 μm wide, 12 μm thick, and each have two parallel microchannels with 8 μm × 8 μm cross-section. The resistor is approximately half the length of the 210 μm length devices, and a quarter length of the 406 μm devices. These two designs allow us to compare sensitivity of devices with different ratios of resistor length to cantilever length. Junction depths for the piezoresistive and contact areas are approximately 0.39 μm, and 1.3 μm, calculated using SUPREM simulation (see Appendix A). These values concur with junction depth measurements from Secondary Ion Mass Spectrometry (SIMS) results on wafers with implants of the same energy and dose. Both the SUPREM and SIMS results are illustrated in Figure 2-4. The junction depths are both less than the
Figure 2-3: Current-voltage characteristics between the piezoresistor (p-type) area and background (n-type). The substrate was held at ground (0V) and voltage on the p-region was varied from -0.9V to 0.8V.
Table 2.1: Summary of characterization results for the two different types of devices. Values are measured from one device, but representative of devices from an entire wafer.

top silicon layer thickness (2 µm), ensuring electrical isolation from the fluid which could be conductive.

Four-point measurements from our devices give a piezoresistor value of 52.5 kΩ when the substrate is reverse biased. This includes resistance of the piezoresistor, aluminum-silicon contacts, and aluminum connections to the piezoresistor. The metal is highly conductive, thus total resistance of the connections can be ignored. The piezoresistor resistance is estimated from geometry and resistivity measurements from SIMS by using a parallel resistor model. Contribution from the metal-silicon contacts is found by subtracting this estimated resistance from the four-point measurement. This gives a total contact resistance of approximately a few kΩ. The resistance value calculated from SIMS is from a previous batch of wafers. Consequently, the actual resistance value for the piezoresistors and contacts may vary due to small differences between the actual and specified implantation parameters.

Cantilever metrics

Metrics for the two types of devices fabricated are outlined in Table 2.1. Spring constants were determined through FEM simulation, by measuring the force required to deflect the cantilever a known amount. Resonance frequencies and quality factor were measured empirically through Lorentzian function fits of open loop frequency responses when the devices were filled with fluid. The electrical resistance was calculated using a parallel resistor model and resistivity measurements from SIMS.
Figure 2-4: Distribution of ions in the piezoresistor (a) and contact (b) areas as calculated through SUPREM, as well as results for piezoresistor areas from SIMS. See appendix for SUPREM code.
2.2 Expected resistance change

Piezoresistance is defined as a change in resistance due to an applied stress.

\[
\frac{\Delta R}{R} = GF\epsilon 
\]  

(2.1)

Resistance change is directly proportional to the longitudinal stress that the piezoresistor experiences. In equation 2.1, \(\Delta R/R\) is the normalized resistance change in the direction of the strain, \(\epsilon\). \(GF\) is the gauge factor, which relates these two, taking into account geometric and electrical effects. The geometric effect for silicon is approximately two orders of magnitude lower and thus can be ignored here [63]. Note that the actual normalized change in resistance should be multiplied by a factor \(\beta\) to account for the fact that the resistor penetrates the depth of the device, and is not localized to the surface [63]. For the device characteristics of the piezoresistive SMRs, \(\beta = 0.82\). In static-mode sensing, a point or distributed force causes stress on the cantilever and consequently a change in resistance of the piezoresistor [26, 63]. However for dynamic performance such as that of the SMR, the device is actuated in a multi-mode resonance, and the cantilever is stressed in its natural vibrational mode. In this section we examine expected resistance change using a conventional point-deflection static model and an analytical dynamic model. We also show static and dynamic finite element simulation results.

2.2.1 Motion of a vibrating beam

The classical solution for a vibrating lever is an accurate method of solving for all resonance frequencies and modes of vibration. We consider flexural resonant modes for a cantilever beam with moment of inertia given by:

\[
I = \int_A y^2dA 
\]  

(2.2)
The moment of inertia for the SMR with hollow interior channels (channel size \(\sim 8\mu m \times \sim 8\mu m\)), is approximately 12.5\% less than that of a solid beam of the same dimensions. Taking into account only the transverse linear acceleration of the beam and ignoring rotational inertia and shearing effects, and assuming that the moment of inertia and Young's modulus are constant along the length of the beam, the equation of motion for the beam is:

\[
EI \frac{\delta^4 z(y,t)}{\delta y^4} + \rho A \frac{\delta^2 z(y,t)}{\delta t^2} = f(y,t)
\]

(2.3)

where \(\rho\) is the density of silicon, \(A\) is the cross-section area of the cantilever, \(f(y,t)\) denotes an external-exerted transverse force per unit length, and \(z(y,t)\) describes the lateral position of each point on the lever along the neutral axis at time \(t\). Applying the correct boundary conditions and solving for the modal solution to this equation [52, 31], gives:

\[
z_n(y) = B(\cos(\kappa_n y) - \cosh(\kappa_n y)) + D(\sin(\kappa_n y) - \sinh(\kappa_n y))
\]

(2.4)

For \(n = 1\), the first resonance mode, the solution for \(\kappa_1 l = 1.875\). Using this, and we can calculate the moment of the vibrating lever at lateral position \(y\) can be calculated from the second derivative of the deflection. To compare stress between the two different SMR types, the average stress over the portion of the cantilever length covered by the resistor was calculated for each case. The results are illustrated in Figure 2-5. This calculation gives \(\Delta R/R = 5.4 \times 10^{-5}/nm\) for the 210 \(\mu m\) long device and \(1.8 \times 10^{-5}/nm\) for the 406 \(\mu m\) devices assuming \(GF = 100\).

2.2.2 Static cantilever bending

If a force, \(F\), is applied to the cantilever at the tip, where lateral position \(y = L\), the length of the cantilever, then the moment \(M\), can be calculated through the relation: \(M = F \cdot L = kx \cdot L\). Here we use finite element simulation to calculate the spring constant, \(k\), and a Laser Doppler Vibrometer (LDV) to measure tip deflection, \(x\). Then by invoking the relationship between radius of curvature, \(C\), and strain:
Figure 2-5: Average stress on a resistor, for resistor lengths of varying proportions of the cantilever length. For the devices fabricated, the resistor is approximately one-half the length of the 210\mu m long device, and one-quarter the length of the 406\mu m long device.
\[ \epsilon = \frac{t}{2} \cdot \frac{1}{L} = \frac{1}{2} \cdot \frac{M}{E_I} \] [52],

\[ \frac{\Delta R}{R} = GF \cdot \frac{t}{2} \cdot \frac{kx}{EI} \left[ \sum_y (L - y) \right] / y \] (2.5)

This result gives an estimated expected resistance change of $3.4 \times 10^{-5}/\text{nm}$ for 210\(\mu\text{m}\) devices and $1.0 \times 10^{-5}/\text{nm}$ for 406\(\mu\text{m}\).

### 2.2.3 Finite element calculation

Finite element analysis was used to calculate the stress experienced in the piezoresistive area of the cantilever under a point load which would correspond to the static-mode sensing case. For the dynamic case, eigenvalue analysis was first performed to calculate the resonance frequency of each device type. Then the dynamic response of the cantilever was calculated by applying a dynamic (point) loading at a matched frequency. The load was applied such that the tip of the cantilever would deflect a known distance. Stress on the piezoresistive area results in an expected normalized resistance of $3.2 \times 10^{-5}/\text{nm}$ for the 210\(\mu\text{m}\) devices and $1.0 \times 10^{-5}/\text{nm}$ for the 406\(\mu\text{m}\) devices. The analytical vibration and finite element calculations here assume the forcing function has one single frequency matched to the first resonant mode of the cantilever, however this should not alter the results by a significant amount because of the extremely high quality factor of these devices.

Expected resistance change from each of the methods is compared to previous measured resistance changes in Table 2.2. Traditionally the normalized resistance change measured from piezoresistors has been on the order of $\sim 1 \times 10^{-6}/\text{nm}$ and slightly increased using a stress concentrating design [33]. An order of magnitude larger was achieved in one of the designs in Tortonese’s work [63], predominantly due to the increased stiffness of that design ($\sim 10X$ over other devices in the same work). These values for SMR devices may be larger than typically reported values due to the relatively increased thickness ($t = 10\mu\text{m}$ compared to $1\mu\text{m}$) and increased spring constant ($k = 200N/m$ and $20N/m$ compared to $1N/m$) over traditional AFM cantilevers[45].
<table>
<thead>
<tr>
<th>Work/Author</th>
<th>Year</th>
<th>$\Delta R/R$ (per nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tortonese</td>
<td>1991</td>
<td>$3.5 \times 10^{-5}$</td>
</tr>
<tr>
<td>Thaysen</td>
<td>1999</td>
<td>$2.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Naeli</td>
<td>2005</td>
<td>$1.7 \times 10^{-6}$, 8.8 $\times 10^{-6}$ (stress concentrating design)</td>
</tr>
<tr>
<td>Lee</td>
<td>2008</td>
<td>$1.7 \times 10^{-6}$</td>
</tr>
<tr>
<td>SMR theory (vibrating lever)</td>
<td>2010</td>
<td>$4.3 \times 10^{-5}, 1.5 \times 10^{-5}$ (210(\mu m), 406(\mu m))</td>
</tr>
<tr>
<td>SMR theory (static deflection)</td>
<td>2010</td>
<td>$2.8 \times 10^{-5}, 0.8 \times 10^{-6}$</td>
</tr>
<tr>
<td>SMR Finite element analysis</td>
<td>2010</td>
<td>$2.6 \times 10^{-5}, 0.9 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

Table 2.2: Measured normalized resistance change for piezoresistive devices, and expected values for the two SMR designs based on the three methods used here. All values for the SMRs are adjusted by the factor $\beta$ to account for resistor depth.

### 2.3 Heat Transfer Analysis

Resonance frequency of the SMR is sensitive to temperature. Thus measurements can be affected by temperature fluctuations. Moreover, when it comes to biological cells or molecules, the importance of heat transfer analysis is augmented because heat may affect the viability of cells or molecules contained within the microfluidic channels. In addition, it is observed that measurements of resonance frequency by the optical lever differ from the resonance frequency measured with piezoresistive readout on the same SMR. This difference could be due in part to the different heating effects of the two types of readout. In this section we consider heat transfer from the two types of readout, and the implications for the two different SMR length devices.

#### 2.3.1 Heating sources

In piezoresistive readout, temperature increase in the cantilever is due to joule heating in contrast to heating from the optical lever which is by photothermal absorption.

Throughout the heat transfer analysis, we assume the microfluidic channel in the SMR is empty. Since fluid contained in the microchannel would increase thermal conductance, estimates based on a dry microchannel assumption result in more conservative temperature increases without losing generality. Heat transfer in the vacuum
packaged SMR can be approximated as a one-dimensional problem because there is a vacuum surrounding the device, so heat only flows in the direction of the base of the device. Finite element analysis was used to calculate the increase in temperature for both readout methods. The piezoresistor heating was calculated using power based on a Wheatstone bridge bias voltage of 5V, which was typically used during experiments. This voltage was selected to balance power consumption \( (V^2/R) \), with bridge output voltage signal, \( \Delta V \). Heating power from the laser depends on the intensity of the laser source (measured to be 4 mW in this case), and light transmission of the reflective layer on the cantilever. The reflective layer on the devices used is aluminum which has an absorptivity of \( \sim3\% \) at a wavelength \( \lambda=635\)nm. This should decrease heating using the optical lever compared to previous SMR devices which had a chromium layer on the cantilever surface, and a reflection coefficient at the same wavelength of \( \sim50\% \) [40]. For the piezoresistive readout, heat generation was modelled at the base of the device, and for the optical lever it was modelled at the tip. Finite element analysis was then used to calculate the resulting temperature distribution in equilibrium based on device material and geometric properties. However, the piezoresistive readout results in a lower maximum temperature. The maximum temperatures resulting for both device types and readout types are outlined in Table 2.3. Both the 210 and 406 \( \mu\)m long SMRs are designed with a fixed piezoresistor length located at the base of the cantilever. Since no heat flows out the tip of the cantilever, thermal conductance of the system is independent of the SMR length and identical steady state solutions are expected. In contrast, photothermal absorption always occurs near the tip of the SMR to achieve the best sensitivity. Thus thermal conductance \( (G_{th} \sim kA/L) \), where \( k \), \( A \), and \( L \) are the thermal conductivity, cross-sectional area, and length of the SMR, respectively) decreases with increasing length of the SMR. Consequently, the temperature estimate increases for optical readout linearly with the length of the SMR. Table 2.3 summarizes the results of the finite element calculation.
<table>
<thead>
<tr>
<th>Device length</th>
<th>Readout method</th>
<th>Heating power</th>
<th>$\Delta T_{max}$ (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>210 $\mu$m</td>
<td>Optical</td>
<td>120 $\mu$W</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3% of 4mW)</td>
<td></td>
</tr>
<tr>
<td>210 $\mu$m</td>
<td>Piezoresistive</td>
<td>125 $\mu$W</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.0V voltage bias on wheatstone bridge)</td>
<td></td>
</tr>
<tr>
<td>406 $\mu$m</td>
<td>Optical</td>
<td>120 $\mu$W</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3% of 4mW)</td>
<td></td>
</tr>
<tr>
<td>406 $\mu$m</td>
<td>Piezoresistive</td>
<td>125 $\mu$W</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.0V voltage bias on wheatstone bridge)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3: Maximum temperature on the two device lengths resulting from each readout method.

2.3.2 Temperature effects in resonant mass sensors

Resonance frequency of is a function of device geometry and material properties ($\omega_0 = \sqrt{K/m}$). Thus, to consider what effects temperature changes can have on resonant mass sensors, it is important to understand which aspects of the geometric and material properties have a temperature dependence, and how strong the dependence is.

Any object is subject to random temperature fluctuations, however out of plane deflection of a resonator is only expected for bimorph structures, unlike the SMR. The dominant sources of temperature change expected are the photothermal and joule heating sources described in section 2.3.1. Conventionally for resonant cantilevers, temperature dependent factors that affect resonance frequency are elastic modulus, density and geometry. For traditional crystalline silicon resonant cantilevers it has been shown that the effect of heating is dominant on the elastic modulus. Temperature effects resulting in geometric changes are negligible [25]. For the devices in this work, thermal effects from any structural thin films can additionally be discounted. Although oxide is known to have a positive effect on resonance frequency with increase in temperature [37], this effect can be neglected because native oxide on the cantilever surface is expected to be less than 100Å thick. The temperature coefficient of the elastic modulus has been shown empirically [67], and is negative. However for piezoresistive SMR devices ($8\mu$m x $8\mu$m channel size), it is observed
that the resonance frequency increases with increasing temperature. Density change
of the fluid contained in the SMR is also affected when temperature increases. For the
case of water, since the temperature coefficient of water density is negative (-0.256
\(\text{m}^3/\text{cm}^3/°C\)), the resonator will experience a decrease in total mass with an increase in
temperature, which has the opposite effect on resonance frequency as the variation in
elastic modulus. The contributions from the water density shift combined with the
device sensitivity of \(1.59 \times 10^5 \text{ppm/(g/cm}^3)\), yields a temperature coefficient of approximately 40ppm/°C. The temperature coefficient of Young’s modulus is typically
reported as approximately -60ppm/°C [30]. Linear combination of these coefficients
still yields a positive temperature coefficient, however there can be other mechanisms that affect the resonance frequency dependance on temperature. Since the
temperature coefficient of water density is well known, anything which changes the
temperature coefficient of the young’s modulus, such as a thicker oxide film, would
change the dependancy.

Temperature changes due to the optical lever or piezoresistive heating, however,
are not expected to cause changes directly at frequencies near the resonance of the
SMR devices. During experiments, when the device is in closed-loop operation, the
system is assumed to be at thermal equilibrium. The timescale over which temper-
ature fluctuations during an experiment would occur is expected to be slower than
the measurement bandwidth, however still relevant during experimental time scales.
Heat flow in the device is towards the base and thermal conductance of the device
in this direction (where \(\tau = \frac{C}{G}\), \(C\) is the heat capacity and \(G\) is the thermal con-
ductance; ignoring radiative effects), is approximately 0.5ms for the 210\(\mu\)m devices
and 1.8ms for the 406\(\mu\)m devices. This indicates that the effect of heat spreading
higher frequencies may affect the resonant frequency on time scales that matter for a
single particle passing through the resonator. Figure 2-6 illustrates the thermal time
constants in comparison to resonance frequencies of the devices.

In sum, it is not expected that temperature effects from SMR heating will be dom-
ninant at \(f_0\). Ambient temperature fluctuations that affect other parts of the system
(e.g. the downstream circuitry) could potentially cause changes at relevant frequen-
Figure 2-6: Heat will spread in the devices at time constants which are slower than the resonance frequencies, but still relevant during measurement. The heat spread is modelled here as a first-order system with pole at $\tau = C/G$. 
cies. An on-chip wheatstone bridge can more accurately serve to decrease thermal and other noise-effects through symmetric cancellation. However, good thermal isolation should still be used for all parts of the system.
Chapter 3

Device Characterization

For mass measurements using piezoresistive SMR devices, circuitry and components for additional system building blocks are required. This chapter describes the complete measurement setup. Design and calibration of the setup, inspired by traditional piezoresistor readout systems, is described here. Additionally, descriptions of practical aspects, in terms of device actuation and calibration, that are addressed specifically for the SMR are included. Sensitivity calibration of SMRs encapsulated in vacuum has never before been performed, either with the existing optical readout or with piezoresistive readout. This chapter includes description of three dynamic calibration methods which were performed on both 210\( \mu m \) and 406\( \mu m \) long piezoresistive SMR devices to understand sensitivity of the piezoresistive readouts. Furthermore, the sensitivity is compared to that of optical readout and to analytical calculations. Calibration through laser doppler vibrometer (LDV), deflection sensitivity of the piezoresistive readout is approximately three orders of magnitude less than the optical readout for 210 \( \mu m \) devices and two orders of magnitude less for the 406 \( \mu m \) devices.
3.1 Experimental Setup

3.1.1 System building blocks

The SMR chip is mounted on a printed circuit board (PCB), and wirebond connections are made to the piezoresistor, substrate, and electrostatic drive electrodes (Fig 3-1). The bond wires are protected by a UV epoxy coat. This insulative coating is particularly important for the piezoresistor connections in case of the presence of any fluid which could electrically short the connections. The chip is mounted in a Teflon manifold based on an earlier design [10], which holds 6 standard 1/32" OD Teflon tubes. The chip and the manifold are cradled in an aluminum clamp pictured in Figure 3-2. Fluidic connections are elaborated in section 4.1. The piezoresistor readout system consists of a Wheatstone bridge circuit used to convert the resistance change of the piezoresistor to a voltage signal, and a downstream amplifier. The Wheatstone bridge is built with the piezoresistor and three external resistors including a potentiometer to null out drift (Figure 3-3a). Wheatstone bridges have traditionally been used both in static and dynamic piezoresistor strain sensing [4, 63, 6, 56]. A half-bridge and AC coupling with subsequent amplification would suffice instead of the full bridge circuit because we are only interested in dynamic-mode sensing, and the full bridge would not be needed to cancel DC effects. Although the output noise of the Wheatstone bridge is limited by the Johnson noise of the piezoresistor, by using a half-bridge, the number of noise sources and places where environmental signals may couple in would be reduced. This should be explored in future implementations.

Differential output voltage from the wheatstone bridge, $\Delta V$, is directly proportional to both the normalized resistance change, $\Delta R/R$, and bias voltage, $V_b$:

$$\Delta V \approx V_b \frac{\Delta R}{4R}$$  \hspace{1cm} (3.1)

At lower operating frequencies, excessive noise is a limiting factor for the bias voltage since $1/f$ noise increases with bias voltage. Because we are operating at frequencies above the $1/f$ corner (Figure 4-5), Johnson noise, which does not depend on bias
Figure 3-1: Photograph of an SMR chip mounted on a PCB. The SMR chip dimensions are 8.5mm × 8.9mm. Most of the chip area is dominated by ports for fluidic connections.
Figure 3-2: The SMR chip, mounted on a PCB, resting in the clamp used when taking measurements. The same clamp is used for both piezoresistive and optical lever readouts. The piezoelectric crystal is centered below the chip, underneath a copper layer which the PCB sits on. A white teflon piece secures O-rings and tubing to the chip. The SMR chip is clamped down under the teflon piece and a supporting aluminum layer.
voltage, is the dominant noise source. The voltage bias, $V_b$, increases heating power and temperature in the device, thus must be chosen to limit power consumption and temperature but not sacrifice output voltage, $\Delta V$. The differential output voltage from the Wheatstone bridge is amplified by an off-the-shelf low-noise amplifier (Stanford Research Systems, SR560). For nanoscale devices at MHz resonant frequencies, intrinsically high resistance leads to frequency-dependent signal attenuation using direct-current biasing at $V_b$. Consequently, AC biasing has been implemented, where $V_b = V_b \sin(\omega t), \omega \ll \omega_0$, and piezoresistors are used as the signal down-mixers [5]. Factors contributing to signal attenuation for the piezoresistive SMRs are discussed in section 3.3. For optical-lever detection, a diode laser (635 nm) and a split photodiode are employed. Geometry of the optical lever, including optical focal lengths and component types, follows from a previous implementation [10].

Open-loop measurement of the amplified output from the Wheatstone bridge was used to find the resonance frequency and quality factor of the resonators. In this scenario the device is driven over a range of frequencies and differential output voltage versus frequency is recorded. The open-loop measurement results are discussed in section 3.2. For frequency measurements during experiments we use a closed-loop tracking system wherein positive feedback is used to create a free-running oscillator whose frequency matches the resonance frequency of the device, $f_0$. The output signal from either the Wheatstone bridge or the split-photodiode in the case of the optical lever, is amplified, phase-shifted, and fed back to the actuator (Figure 3-3). Thus, the SMR always resonates at its resonance frequency through this feedback. Idential downstream electronics can be used for both readout types. The electronics details have been elaborated previously [16]. Dynamic range of this type of measurement is enhanced beyond the bandwidth of the oscillator (here the 3-db bandwidth is approximately 40Hz for the 210$\mu$m devices and 20Hz for the 406$\mu$m). The resonance frequency, from the output of the low-noise amplifier in the piezoresistor readout, and from the transconductance amplifier when using the optical lever, are each heterodyne-mixed with a reference frequency from a function generator (33220A, Agilent). The reference frequency is chosen as approximately 1kHz from the resonance.
Figure 3-3: Both optical and piezoresistive readouts are interfaced with identical downstream electronics. Although we did not use the readouts simultaneously, they are both available concurrently. a) The SMR vibration results in an AC resistance change, which is translated to a voltage change using the Wheatstone bridge. b) The optical lever is typically realized using a laser source, which is reflected off of the surface of the resonant device. The position of the reflected laser spot is monitored on a photo-sensitive detector. This lever characteristic allows very fine motions of the resonator to be realized through the differential generated photocurrent from the detector. The dynamic displacement is measured either with laser and photo-detector or on-chip piezoresistor, amplified, phase-shifted, and fed back to the actuator.
frequency of the device in order to maintain a sufficient sampling rate for all measurements. The mixed signal is then low-pass filtered (with a cut-off frequency of ~5 kHz), converted to TTL levels, and measured with a frequency counter using a data acquisition card (NI PCI-6259, National Instruments).

3.1.2 Device actuation

Although many types of actuation have been realised with static cantilever sensors and resonant mass sensors, here we describe the two types of actuation that have been used with the SMR.

**Electrostatic actuation**

In electrostatic actuation, an electric field is created between two plates of a capacitor. For the SMR, the “plates” are a micropatterned electrode below the cantilever and the cantilever surface itself. A voltage which has an AC component superimposed on a DC voltage is applied to one plate of the capacitor. This field exerts a force which generates motion of the cantilever. One reason electrostatic actuation is appealing for resonant mass sensors is that it, amongst other actuation methods, allows direct displacement control. The force generated in electrostatic actuation is proportional to the square of the applied voltage, and the component of force which is at the resonance frequency is linear with the product of the DC and AC applied bias voltage components. Thus the force can be independently controlled via the DC and AC voltages. Electrostatic actuation is also appealing because it does not require any external components besides the electrical connections; the electrodes can be integrated directly on the device.

The dependancy of electrostatic force on voltage bias also means that stability is related to voltage bias. The exact dependance is elaborated in work by Burg [10]. Commercial power supplies have been found sufficiently stable for the measurements performed here. Another factor affected by the electrostatic force is the resonance frequency. The presence of a force gradient created through the electric field lowers
the effective spring constant and thus resonance frequency [10].

The total electrostatic force, $F_{es}$ can be approximated via a parallel-plate capacitive model:

$$F_{es} = -\frac{\delta W_e}{\delta x} = -\frac{1}{2} \frac{\delta C}{\delta x} V^2$$  \hspace{1cm} (3.2)

Where $W_e$ is the energy stored in the capacitor, $C$, and $x$ is the gap between the plates of the capacitor and $V_C$ is the applied potential. The capacitance is derived from the area of the electrode, $w_1 \cdot w_2$, the dielectric constant (here this is in low-pressure air) and the gap between the electrodes:

$$C(x) = \varepsilon_r \varepsilon_0 \frac{w_1 \cdot w_2}{x}$$  \hspace{1cm} (3.3)

The patterned electrode overlaps the entire length of the cantilever thus the electrostatic actuation can be thought of as a distributed load. Since the applied voltage has a DC component ($V_b$) and an AC component ($v_{ac} = V_{ac}\sin(\omega_0 t)$):

$$V_C = V_b + v_{ac}$$  \hspace{1cm} (3.4)

Substituting into equation 3.2, and because only the cross term is at $\omega_0$, the electrostatic force becomes:

$$|F_{es}| = \frac{C(x)}{x} |V_b| |v_{ac}|$$  \hspace{1cm} (3.5)

For typical drive conditions of $V_b = 60V$ and $v_{ac} = 20V_{pp}$, the force on the SMR devices is approximately 13nN. A requirement for electrostatic actuation is that the bias voltage must be well-defined relative to the substrate potential. Typically for SMR devices, to achieve this the silicon substrate is grounded. In cases where this is not possible (for example if the bulk silicon is required to float up to match the contained fluid potential [16]), actuation by piezoelectric crystal has also been employed.
Piezocrystal actuation

The dimensions of the piezocrystal used here (Model PL.022, Physik Instrumente GmbH & Co.) are $2\text{mm} \times 2\text{mm} \times 2\text{mm}$. The crystal is cradled between two copper layers in the clamp which the PCB connects to (Figure 3-2). Although the piezoelectric crystal is not integrated with the SMR device, this form of actuation can easily be applied to different fabricated devices as long as there is good force transfer between the chip and crystal.

Force from the crystal is transmitted through the entire clamp to the chip, thus there can be multiple frequency components generated, however the natural high quality factor of the SMR filters these extraneous frequency components. Laser doppler vibrometer (LDV) was used to measure the deflection of a $210\mu\text{m}$ device using piezocrystal actuation of $1\text{V}_{pp}$. Using the deflection at $f_0$, $x_{pc}$, the force from crystal actuation can be estimated through the relationship between force, $F_{pc}$, spring constant, $k$, and deflection:

$$|F_{pc}| = ma = kx_{pc}$$

(3.6)

As with electrostatic force, force from the piezoelectric crystal drive is also a distributed load; it is evenly distributed along the cantilever length. Using $k_d = \frac{8}{3}k$ to adjust for distributed loading conditions, and also since actuation is near the mechanical resonant frequency of the beam, the mechanical response of the beam is accounted for by the quality factor, $Q$, resulting in:

$$|F_{pc}| = \frac{k_d x}{Q}$$

(3.7)

With $1\text{V}_{pp}$ applied to the crystal, deflection at the resonance frequency for the $200\mu\text{m}$ devices is approximately $1\mu\text{m}$ (this will depend on the piezoelectric crystal and SMR clamping conditions). Although these were open loop measurements, the applied voltages were selected to match voltage drive conditions that result during closed-loop operation. The resulting force is: $F_{pc} \approx 63\text{nN}$. For experimental conditions, this force generated by piezocrystal actuation is approximately 1 order of magnitude...
larger than the electrostatic force. LDV open loop tip deflection measurement using electrostatic actuation is approximately 40nm for the 210μm devices. Using this deflection to calculate electrostatic force through the same relation in equation 3.7 gives $F_{es} \approx 2nN$, which is on the same order of magnitude as our calculation from equation 3.2.

Piezocrystal actuation has been used for all piezoresistive SMR measurements to date. The signal strength from piezoresistive readout, when electrostatic actuation is used, with drive parameters typically used when readout is by an optical lever, is not large enough for self-oscillation in closed-loop mode. This is likely due to coupling from the AC portion of the electrostatic signal (Equation 4-3) through parasitic capacitances between the drive electrodes and piezoresistor. The electrostatic force is proportional to both the DC and AC components of the applied signal. Thus the DC signal can be increased and AC decreased while still increasing the total drive force and decreasing the portion of the signal that can couple. Coupling of the drive signal can occur through the substrate or through other paths including air. Electrical isolation of the SMR chip from surrounding cables and signals should be optimised in order to prevent coupling through extraneous paths. In addition, the coupling through the substrate can be reduced with better isolation of the piezoresistor from the substrate. This can occur by shielding the piezoresistor from the substrate using physical or material isolation. This can occur through physically decreasing the area that the piezoresistor contacts the substrate through etching the substrate, or using an insulative layer between the two. Using metal as the piezoresistor may also evade signal transfer between the substrate and piezoresistor because there is no pn junction.

### 3.2 Open-loop response

Amplitude and phase response for the 210 and 406 μm long SMRs are obtained by open loop frequency sweep and lock-in detection, and examples of these are as shown in Figure 3-4. Each response shows curves that can be fit to a single harmonic oscil-
Figure 3-4: Resonance responses for 210 μm and 406 μm long resonators measured with optical and piezoresistive detection when each resonator is filled with de-ionized water. Parasitic coupling is observed (more visible in phase response), and is more severe for piezoresistive detection with the 210 μm long device.

The first generation of piezoresistive SMR devices (with no insulative oxide layer between the silicon and top pyrex lid) did not show such a clean phase shift or amplitude response. Although the response would improve with increased drive (using the piezoelectric crystal drive, this meant better contact or clamping for force transfer from the crystal to the chip), performance of different chips across a single wafer varied in terms of the amount of clamping required to increase the signal quality in open loop. Thus closed loop responses were not reliably obtained from these devices.
3.3 Measurement setup practicalities

Differences in resonance frequency measured through optical lever readout versus piezoresistive readout were discussed in section 2.3. However at frequencies away from the resonant frequency, the normalized amplitude and phase responses between the two designs also differ (Figure 3-4). There is a level of coupling between the drive and output signals which exists in both the piezoresistive and optical readout, however deviation from the simple harmonic oscillator (SHO) model is more pronounced in the spectrum from the piezoresistive readout than that from optical readout. This indicates that the piezoresistive readout experiences a higher level of coupling. The coupling experienced by piezoresistive readout can be largely attributed to parasitic capacitive paths between the drive and the readout circuitry, and can be decreased by isolation through low impedance grounds.

For example, there was found to be a large transfer of electrical signal from the drive signal on the piezoelectric crystal contacts directly to the SMR substrate and piezoresistor (Figure 3-5). This voltage coupling could be due to insufficient isolation by the grounded substrate. A grounded metal plane was inserted between the chip and crystal to further isolate the signals. This electrically shields the piezoresistor. Further shielding can be achieved with a thicker, lower impedance material, however the material used should not be so thick as to decrease vibration amplitude of the resonator by limiting the mechanical coupling between the piezoelectric crystal and the chip.

Further shielding of the entire measurement setup will reduce parasitic coupling in both readout methods. The first amplification stage for each readout, for the optical readout the transimpedance amplifier, and for the piezoresistive readout the wheatstone bridge stage, is particularly vulnerable because the signal is smallest at this stage. Although the 50-ohm coaxial cables used for connections are designed to function efficiently as RF transmission lines, a 1m length of cable presents approximately 100pF of capacitance. This capacitance in combination with the moderate impedance of the piezoresistors forms a lowpass filter with a cutoff frequency of ap-
Figure 3-5: Open-loop amplitude spectrum for a 210μm device with and without a grounded shielding plane inserted between the piezoelectric crystal and SMR chip. With no ground plane, there is an increased signal off resonance because the drive signal couples to the readout signal path.

approximately 20kHz, well below the resonance frequencies of the SMR devices. This means that output signals from the devices are attenuated. An amplifier placed as close as possible to the Wheatstone bridge should be used to counter these effects. Attenuation is also expected from the optical-lever readout system, but not to the same degree. The same level of cable capacitance and amplifier input capacitance are present, however the output impedance photodetector stage will be lower than that of the piezoresistor and thus the cutoff frequency will be higher.

3.4 Sensitivity calibration

3.4.1 Calibration methods

The atomic force microscopy (AFM) community has devised several methods for determining the spring constant of a cantilever, which is important to know for accurate measurement of forces in this type of microscopy. For cantilever sensors, determining
the deflection sensitivity is similarly important. Accurate measurement of substances being sensed requires the cantilever deflection to be calibrated. Many of the AFM spring constant calibration methods can be adapted for deflection calibration if the spring constant is known either through a previous measurement or calculation. These methods include:

- analytical calculation based on cantilever dimensions and material properties
- finite element analysis
- methods using the cantilever thermomechanical noise spectrum
- measurement of gravitational deflection due to addition of known masses
- measurement of deflection due to changes in viscosity of the medium surrounding the cantilever
- measurement of deflection due to an applied known force
- measurement of deflection with an optical profiler

Piezoresistor sensitivity to static cantilever deflection has commonly been calibrated by manual application of a known force and measurement of the resulting resistance change. This is often performed by bringing the cantilever into contact with a piezoelectric tube to apply a known displacement or force [62]. This method provides a very direct calibration which is accurate to the displacement capabilities of the piezoelectric tube. However, this method does not provide any insight to frequency resolution. Nor can this method be used if there is no access to the front or back of the cantilever. Finally the force applied must be located at a position on the cantilever surface that is precisely determined. For the SMR there is no access to the device in it’s final vacuum encapsulated packaging so external masses cannot be manually applied to the cantilever surface. And also uniquely for the SMR, any viscosity change would have to be in the fluid interior to the device. None of the static deflection calibration methods discussed here can be applied to the SMR, so other calibration methods, performed while the device is in motion, were employed.
Finite element analysis is often used to calibrate or confirm sensitivity measurements. As described in section 2.2.3, this can be done for static or dynamic cantilever operation modes. Often the greatest uncertainty with this type of analysis are geometric uncertainties, especially in the thickness of cantilever devices. For sensitivity of the SMR, the normalized resistance change calculated through FEM is converted to a voltage (equation 3.1) to compare with LDV and thermomechanical measurements.

Calibration via thermomechanical noise has been extensively used for optical lever readout systems. A benefit of this method is that no external input is required. The equipartition theorem relates the potential energy of the cantilever system: \( \frac{1}{2} k <x^2> \), where \( k \) is the cantilever spring constant and \( <x^2> \) is the mean-squared deflection of the cantilever caused by thermal vibrations, to the thermal energy: \( \frac{1}{2} k_b T \), in which \( k_b \) is the Boltzmann constant and \( T \) is temperature. In one implementation of thermomechanical noise calibration, the thermal noise spectrum of the cantilever is measured from the readout output (for optical lever systems from a photodetector), and the fundamental resonance mode is fit to the power response function of a simple harmonic oscillator [51]. This method accounts for a DC power response attributed to noise from the sensor or other white noise, and other unknown factors by a fitting procedure. Practically, this means that the background noise level does not have to be completely eliminated. Another calibration method using thermomechanical noise, determines the power contribution from the cantilever by subtracting the power off-resonance from on-resonance power [39]. This method assumes most of the power is close to the resonance frequency, which is still accurate when the resonator has a high quality factor.

In cases where a large spring constant precludes the measurement of the thermal noise spectrum due to low cantilever vibration amplitude (or if other noise sources in the readout are dominant), the cantilever can be driven at a range of frequencies near the fundamental mode resonance peak, and the same type of analysis as the thermomechanical calibration can be performed. The results will still be accurate for the quality factor and resonance frequency as those obtained from the thermal noise spectrum, provided the drive amplitude is not large enough to bring about
nonlinearities. This method has been extended to dynamic calibration by driving the cantilevers at their resonance frequency, and measuring output voltage via the piezoresistor and deflection at the tip via Laser Doppler Vibrometer simultaneously [50].

3.4.2 Calibration of pSMRs

Due to constraints of the experimental setup, it is not possible to measure from an LDV and the piezoresistor simultaneously. However, the piezoresistive SMRs were calibrated using variations on this theme. Each of the methods used were dynamic; they were made while the cantilever was moving.

Method 1: Calibration by LDV

Deflection of the 210µm and 406µm devices for a range of drive amplitudes to the piezoelectric crystal was measured using an LDV. A linear range of drive amplitudes was selected such that the output signal would not saturate either electrically or mechanically at the largest value. Figure 3-6 demonstrates linear and non-linear regimes for deflection of a cantilever via different voltages applied to the piezoelectric crystal.

The signal from the LDV was detected using a controller (Polytec OFV 511) and digitized through an analog to digital converter card. Measurements were performed without any extraneous pauses to avoid the effects of drift. Figures 3-7a and 3-8a show the measurement results for the 210µm (span 1 kHz, data point every 5 Hz) and 406µm (span 90Hz with a point every 0.5 Hz) devices.

The range of possible drive amplitudes (without reaching saturation) for the longer devices was 10X higher than those for the short devices. This can be attributed to a combination of effects; different crystal response at lower frequencies, and longer devices being more massive and consequently requiring greater actuation force. Since the transfer of force from the crystal to the chip depends highly on the clamping force used to secure the chip, the entire structure in figure 3-2 was carefully trans-
Figure 3-6: Deflection of a cantilever versus applied drive voltage to the piezoelectric crystal. Measurements were made using a 210μm length cantilever. Voltage was sourced directly from a function generator (HP 33120A). The exact deflection will depend on the clamping force on the SMR chip in the clamp. From 0 to approximately 3V the deflection is linear with the drive voltage, and above 3V the deflection saturates.
Figure 3-7: a) LDV amplitude measurements for a 210μm device along with optical lever and piezoresistive voltage outputs for the same range of drive conditions. b) Plot of deflection (from LDV at $f_0$) versus voltage (from each readout system at $f_0$). Since this is a linear range for device deflection, the slope of the line gives the sensitivity for each readout method (the sensitivity specified includes the gain of each system).
Figure 3-8: a) LDV amplitude measurements for a 406μm device along with optical lever and piezoresistive voltage outputs for the same range of drive conditions. b) Plot of deflection (from LDV at $f_0$) versus voltage (from each readout system at $f_0$). Since this is a linear range for device deflection, the slope of the line gives the sensitivity for each readout method (the sensitivity specified includes the gain of each system).
Table 3.1: Sensitivity (post-amplification) of the optical and piezoresistive readout methods for each of the device lengths. Optical lever readout is three orders of magnitude larger than the piezoresistive readout for 210µm devices and two orders of magnitude larger for the 406µm devices.

<table>
<thead>
<tr>
<th>Method</th>
<th>210µm</th>
<th>406µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical readout</td>
<td>43.3mV/nm</td>
<td>3.9mV/nm</td>
</tr>
<tr>
<td>Piezoresistive readout</td>
<td>68.5µV/nm</td>
<td>45.5µV/nm</td>
</tr>
</tbody>
</table>

ferred to the optical lever setup where optical lever and piezoresistor measurements were made in succession with the exact same drive conditions as used for the LDV measurements. The optical lever and piezoresistor measurements spanned the same bandwidth as the LDV measurements, but 1000 output voltage measurements were taken for both device types. The maximum output voltage measured (at \( f = f_0 \)) using the piezoresistor or optical lever can be correlated with the maximum deflection measurement from the LDV. For each of the drive voltages applied to the piezoelectric crystal, these maximums are plotted in figures 3-7b and 3-8b. The slope of the lines give the sensitivity of each method, which are tabulated (Table 3.1). The sensitivities listed include amplification that is commonly used for each readout system.

Method 2: Calibration by thermomechanical noise spectrum

The thermomechanical spectrum for the 210µm and 406µm devices was measured using optical lever readout. The cantilever was not driven by any external sources and the output voltage signal was obtained using a spectrum analyzer (Agilent 4395A) and averaged 500 times. The thermomechanical noise spectra were fit using a Lorentzian model and the spectrums and fitted curves are shown in Figure 3-9. Calibration was calculated using a previously outlined method, in which the voltage contribution from the cantilever’s thermomechanical motion is measured by the square root of the difference of the squares of the voltage noise spectral density on and off resonance [39]. This method assumes that the voltage noise density off resonance is reaching the displacement noise floor. Another method which fits the thermomechanical noise spectrum to the response function of a simple harmonic oscillator driven by thermal noise can be used, and does not assume a displacement noise floor-limited off reso-
Figure 3-9: Thermomechanical noise spectra for a) 210μm device and b) 406μm device using optical lever readout.
Table 3.2: Comparison of measured (LDV and thermomechanical) sensitivities and calculated (FEM and geometric) for the both device lengths using piezoresistive read-out.

<table>
<thead>
<tr>
<th></th>
<th>210µm</th>
<th>406µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Doppler Vibrometer</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Thermomechanical Noise</td>
<td>4.0</td>
<td>2.4</td>
</tr>
<tr>
<td>FEM simulation</td>
<td>39.4</td>
<td>12.9</td>
</tr>
<tr>
<td>Geometric analysis (section 2.2.2)</td>
<td>42.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Piezoresistor sensitivity was extrapolated from calibration of the optical lever by the ratio of optical lever to piezoresistor sensitivity determined through LDV measurements.

Table 3.2 summarizes these calibrations and also compares the obtained values to the sensitivities calculated through LDV, FEM and geometric analysis. The calculations concur with finite element analysis, however the measured sensitivities are less than expected by between approximately 20-26dB and 15-19dB for the 210µm and 406µm devices respectively. This is attributed to the low-pass effect created by the resistance of the piezoresistor and capacitances of connecting cables and input capacitance of amplifier. In the existing setup, the cable lengths and amplifier type contribute a total capacitance of approximately 166pF. With a piezoresistor impedance of 50kΩ, a first-order low-pass filter is created with a pole at approximately 25kHz. At the resonance frequencies for the 210µm device, this low-pass filtering effect contributes a factor of 24dB attenuation, and 14dB for the 406µm long device. Another contributing factor for this difference in sensitivity is the transverse component of piezoresistance. For p-type silicon, the transverse component is smaller than the longitudinal component, and has the opposite magnitude [32]. This would lower the effective gauge factor an amount comparable with the uncertainty in the gauge factor. Taking into account the magnitude of these effects, together the low-pass filtering and transverse piezoresistance are expected to cause the difference in measured and calculated values for sensitivity.
Chapter 4

Measurements

The first measurements of the buoyant mass of particles and cells using suspended microchannel resonators with piezoresistive readout are shown in this chapter. Comparable and better sensitivity than the optical lever is demonstrated using piezoresistive readout in certain cases.

Typically, the resolution of static-mode sensors is evaluated by way of the displacement noise. However for resonant sensors, frequency noise dictates mass resolution. Here, both forms of noise and the relationship between them are evaluated for the SMR, for each form of readout as well as device type.

Displacement noise analysis shows that the optical readout is limited by thermomechanical noise, while piezoresistive readout is limited by noise of the displacement sensor. Stability analysis performed via the Allan variance, shows that in the best case, piezoresistive readout achieves an order of magnitude better frequency stability and thus mass resolution compared to optical readout at the same gate time (210\(\mu\)m long devices, 0.1ms gate time).

Finally, the fundamental and practical factors interplaying in the displacement and frequency noise relationship are discussed here.
4.1 Particle Measurements

4.1.1 Sample preparation

Piezoresistive readout was demonstrated by measuring the buoyant mass of a mixture of polystyrene particles. National Institutes of Sciences and Technology (NIST) size standard polystyrene beads (4016A, 4018A, 4202A, Thermo Scientific) were diluted with de-ionized water and mixed to have similar final concentration of $1 \times 10^7$ particles/mL for each bead type. This concentration has been empirically determined to minimize clogging in the device channels, while still allowing for an acceptable throughput.

Measurement of the buoyant mass of biological substrates was demonstrated with budding yeast cells. Saccharomyces cerevisiae were grown overnight at 23°C in yeast extract plus peptone (YEP) media containing 2% glucose and 1 mg/mL adenine. The cultured yeast cells were then suspended and diluted with the same growth media to a concentration of approximately $1 \times 10^7$ cells/mL. Both the diluted particles and cells were sonicated for 1 minute to separate aggregates into individuals immediately prior to the measurement.

4.1.2 Fluidic measurements

The particles or biological cells of interest are introduced into and travel through the microchannel via pressure-driven flow. Upstream pressure on both bypasses and one downstream pressure (Figure 4-1) are all controlled with manual pressure regulators, and pressure on the downstream of the remaining bypass is fine-tuned with an electronic regulator. Detailed procedures and pressure settings regarding the pressure regulators and pneumatic valves used are adapted from previous SMR measurements [7]. For all the measurements here we used similar pressure gradients in both bypass channels.

The frequency transition as a single polystyrene bead (4.17 μm) passes through the resonator is plotted in Figure 4-2, for both readout types and device sizes. From
Figure 4-1: Fluid flow through the resonator is controlled via pressure regulators connected to fluid reservoir vials at the inlets and outlets. Pressure settings for sample loading are $P_1$ (15 psi) > $P_2$ (0 psi) and $P_3$ (15 psi) > $P_4$ (0 psi) and pressure settings for population measurements are $P_1$ (15 psi) = $P_2$ (15 psi) = $P_3$ (15 psi) and $P_4$ (variable, <15 psi).

In the experiment, the measured mass sensitivity is 5.42 Hz/pg (210µm) and 0.74 Hz/pg (406µm). Sensitivity can also be estimated from the mass of the device ($m_{eff}$) and measured resonance frequency ($f_0$). Assuming the spring constant, $k$, is constant:

$$\frac{df}{f_0} = \frac{1}{2k} \frac{1}{2\ m_{eff}} \approx \frac{1}{2\ m_{eff}}$$  \hspace{1cm} (4.1)

In Figure 4-2 it can be seen that frequency shift during a single particle transit is not always symmetric as a particle enters and leaves the device (most apparent for the 406µm device using piezoresistive readout). This asymmetry is due to particle acceleration and deceleration induced by vibration of the SMR. As a particle enters the cantilever and travels to the tip, the centrifugal force is in the same direction as the particle's velocity. However, as the particle turns around the tip, the velocity reverses direction. The centrifugal force remains in the same direction, and is now opposite to the particle velocity. Acceleration and deceleration from this centrifugal force increase with vibration amplitude of the SMR. If the forces are large enough, particles
Optical Detection | Piezoresistive Detection
---|---
210 μm |  
\[
\begin{array}{|c|c|}
\hline
\text{Time (ms)} & \text{Time (ms)} \\
0 & 0 \\
50 & 150 \\
100 & 250 \\
150 & 350 \\
200 & 0 \\
250 & 0 \\
300 & 0 \\
350 & 0 \\
400 & 0 \\
450 & 0 \\
500 & 0 \\
550 & 0 \\
600 & 0 \\
\hline
\end{array}
\]

406 μm |  
\[
\begin{array}{|c|c|}
\hline
\text{Time (ms)} & \text{Time (ms)} \\
0 & 0 \\
50 & 150 \\
100 & 250 \\
150 & 350 \\
200 & 0 \\
250 & 0 \\
300 & 0 \\
350 & 0 \\
400 & 0 \\
450 & 0 \\
500 & 0 \\
550 & 0 \\
600 & 0 \\
\hline
\end{array}
\]

Figure 4-2: Frequency shift during a single particle (4.17 ± 0.03 μm diameter polystyrene bead in DI water) transit through the 210 and 406 μm long resonators, each measured with optical and piezoresistive detection. Data from the 210 μm long resonator is sampled at 1 kHz and smoothed with a Savitzky-Golay filter (3rd order, n = 25) and data from the 406 μm pSMR data is sampled at 2 kHz and smoothed with a Savitzky-Golay filter (3rd order, n = 50).
or cells will stick to the microchannel near the tip. A longer SMR has higher vibration amplitude than a shorter one due to lower stiffness and higher quality factor. Thus the asymmetric peaks or particles sticking near the tip are more frequently observed with 406\(\mu\)m long SMR versus the 210\(\mu\)m SMR. Higher amplitudes are also expected with piezoelectric crystal drive compared to electrostatic drive (section 3.1.2.2). Consequently, higher pressure gradients are used in these cases to increase flow rate, since faster bulk flow rate is preferred to minimize particle sticking.

### 4.1.3 Histograms

Buoyant masses of the particle mixture measured using piezoresistive readout are tallied in figure 4-3. The distribution of particle masses for each particle size is Gaussian, as expected. As well, the mean particle volume derived from the histogram of measured masses and the particle material density is linear for this range of particle sizes (Figure 4-3b). Measurement from a mixture of particles demonstrates that different masses can be accurately resolved over the course of a single experiment.

Histograms of the budding yeast cell masses (Figure 4-4) show a wider distribution...
Figure 4-4: Histogram of the buoyant mass of yeast cells measured with piezoresistive readout from a 406μm resonator at room temperature.
than the NIST standard particle mass histograms due to natural variability in cell size. The distribution is log-normal as expected. Based on the length of time passed between initial culture of the cells and measurement, the yeast population should be either in a static growth phase or at the end of log growth phase.

4.2 Noise

For static-mode cantilever sensors, the minimum detectable amount of force or mass from a cell or molecular layer is determined by the minimum detectable deflection (MDD). The MDD is limited in two respects: 1) by changes in the static position of the cantilever due to means other than the object being measured, and 2) by limitations of the readout system sensitivity. However, for dynamic-mode sensing, added mass will not directly change the deflection, rather the resonance frequency of the device. Consequently, sources which can can obscure small values of the resonance frequency, changes in frequency \( \delta f \), are the relevant noise figures. Thus for resonant sensors, sensitivity is limited by the minimum measurable change in frequency, \( \delta f \), normalized to the resonant frequency of a particular mode (equation 4.1). This section contains description of the relevant noise sources for piezoresistive and optical readouts and how each may affect the frequency noise. In this section, description of the relevant noise sources is elaborated, and how they may affect frequency noise in the system.

4.2.1 Piezoresistive readout noise sources

The noise sources in a piezoresistive readout system are: resistor Johnson noise, resistor flicker \((1/f)\) noise, and noise from the downstream electronics. The total RMS output referred noise from the piezoresistive readout circuit (Figure 3-3) can be obtained by correlating all of the noise sources:

\[
V_n = G_a \sqrt[4]{\left(\frac{1}{4}(v_{n_1}^2 + v_{n_2}^2 + v_{n_3}^2 + v_{n_{PR}}^2)\right)} + v_{na}^2
\]  
(4.2)
where $G_a$ is the gain of the amplifier, $v_{na}$ is the input referred noise of the amplifier ($\sim 4nV/\sqrt{Hz}$ at frequencies of interest), $v_{nPR}$ is the RMS Johnson noise of the piezoresistor, and $v_{n1-3}$ are the RMS Johnson noise values for the other resistors in the Wheatstone bridge. Resistors matched to the magnitude of the piezoresistor are used for symmetry. Assuming the resistance values are perfectly matched ($R_1, R_2, R_3 = R_{PR} \approx 50k\Omega$), the total output referred noise is:

$$V_n = G\sqrt{4kT\Delta f R_{PR}} + v_{na}^2 \quad (4.3)$$

The amplifier was chosen specifically such that the piezoresistor Johnson noise, $v_{nPR}$ is dominant over $v_{na}$. Figure 4-5 shows the measured noise spectrum of the Wheatstone bridge. Above the $1/f$ corner the noise approaches the expected Johnson noise-limited floor from equation 4.3, of approximately $28.9nV/\sqrt{Hz}$.
At lower frequencies the blue and green curves in figure 4-5 collapse to lines with slope of -1, and the noise power spectrum \((S_f)\) follows the Hooge formula: \(S_f = \frac{\alpha V_b}{N_f}\) (where \(N\) is the number of electronic carriers, \(f\) is frequency, \(V_b\) is the bias voltage across the piezoresistor). The dimensionless parameter, \(\alpha\), can be found from its relationship with the diffusion length, \(\sqrt{D\tau}\), that has been shown in previous work for piezoresistors fabricated in similar materials as those here [26]. Then \(N\) can be found by fitting a line to the noise spectrum below the 1/f corner with slope = -1. This gives \(N \approx 3.4 \times 10^6\) carriers in the conduction band. Estimation of \(N\) from a simple calculation of the doping density times doped volume of the piezoresistor legs gives \(N \approx 2.0 \times 10^7\). Variation in the \(\alpha\) parameter is expected based on fabrication procedure, which is expected to allow for an order of magnitude difference in the value of \(N\).

In addition to the electronic noise sources, Thermomechanical noise, caused by coupling between the resonator and a dissipative environment, will affect any type of mechanical resonator. This results in damping of motion of the resonator when it is being driven at nonzero temperatures, and it induces spatial fluctuations in the resonator's position. The amplitude of these fluctuations peaks at the mechanical modes of vibration of the resonator, as outlined by the equipartition theorem. The power spectrum of a white noise force on a simple harmonic oscillator is:

\[
S_{ff} = \frac{4k_BTk}{Q\omega_0}
\]

Amplitude fluctuations from this thermomechanical displacement noise can be obtained by multiplying the power spectrum by the magnitude of the transfer function for a second order harmonic oscillator:

\[
H(\omega) = \frac{\omega_0^2}{k[(\omega_0^2 - \omega^2 + j\omega\omega_0)]}
\]

which results in:
The thermomechanical displacement noise, $x_{th}$, near the fundamental resonance has been measured for micro and nanomechanical resonators [39, 57]. Based on equation 4.6, the expected displacement from thermomechanical noise is $0.46 \text{ pm/Hz}$ for $210 \mu m$ and $3.5 \text{ pm/Hz}$ for $406 \mu m$. The displacement equivalent limits determined from the Johnson noise level and the thermomechanical calibration factor (found in section 3.4.2) are $7.2 \text{ pm/Hz}$ for the $210 \mu m$ SMR and $12.2 \text{ pm/Hz}$ for the $406 \mu m$ SMR at frequencies above the $1/f$ corner, which are larger than expected displacement from thermomechanical noise. Hence when the signal from an undriven cantilever is measured using piezoresistive readout, there is no measurable thermomechanical peak at the resonance frequency.

The fact that displacement sensor noise is dominant over thermal noise for piezoresistive readout is corroborated by the fact that the thermomechanical noise cannot be resolved by the piezoresistive readout. When the identical procedure for resolving thermomechanical noise using the optical lever (section 3.4.2b) is performed using piezoresistive readout, only the Johnson noise level (Fig 4-5) can be resolved. However, if the sensitivities were increased by a factor of approximately 10 times ($210 \mu m$) and 4 times ($406 \mu m$), the thermal noise spectrum could be resolved with piezoresistive readout. Although the voltage power spectral density of sensor noise would still be limited by Johnson noise (in $V/\sqrt{Hz}$), the corresponding displacement sensor noise ($S_{dd}$ in $m/Hz$) will decrease because of increased sensitivity. Frequency deviation ($\frac{d\omega}{\omega_0}$) decreases with the square root of $s_{dd}$, thus it is expected that displacement noise would decrease to a level wherein the thermal noise spectrum would become larger and could be resolved with piezoresistive readout.

### 4.2.2 Optical lever readout noise sources

Typical noise sources for optical detection methods are: laser intensity noise, laser phase noise, laser pointing noise, laser $1/f$ noise and laser shot noise. As in previous
optical-lever readout implementations, intensity and shot noise are expected to be
dominant [57]. In addition to the noise sources from the readout, fluctuations in the
resonator position from thermomechanical noise will also be present. Finally, noise
from electronics downstream of the photodetector, which include a transimpedance
amplifier and further amplification stages. The electronic schematics have been de-
scribed previously [16]. For optical readout, the noise other sources are not dominant
over the thermomechanical noise, illustrated by the fact that it is possible to elicit
the thermal spectrum shown in figure 3-9.

4.2.3 Displacement noise and frequency noise relationship

The limit of detection for dynamic, frequency-based sensors is the presence of noise
sources that obscure measurement of small values of \( \delta f \) (or \( \delta \omega \)). As discussed in
section 2.3.2, temperature changes can alter the resonance frequency. However, the
possible sources of temperature fluctuation likely will not to act in timescales which
are in the relevant measurement bandwidth. On the other hand, thermomechanical
noise will have an increased response at frequencies near the resonance (equation 4.6).
Noise from the displacement sensor will also be a dominant source in the relevant
bandwidth. By noting that the output referred noise, \( x(t) \), can be expressed in terms
of amplitude \( (A = A(t)) \) and phase \( (\phi = \phi(t)) \) modulation:

\[
x(t) = [A + \delta A(t)]e^{i\phi(t)}, \quad \phi(t) = \omega_0 t + \delta \phi(t)
\]

the power spectral density of the thermal noise and displacement sensor can be been
related to the normalized deviation in frequency \( (\delta \omega/\omega_0) \) [13]. Although generally all
readout types have some sort of non-linear element to limit the signal amplitude (here
this is set by amplifier rail limits in the feedback circuit), we can still use analysis
based on linear systems theory to provide some insight into the system behavior and
relationships between the signal and noise sources. Figure 4-6 shows how these noise
sources are represented in the system.

Normalized frequency deviation due to thermal noise is [10]:

\[
\delta \omega = \frac{\delta \omega}{\omega_0}
\]
Figure 4-6: Noise sources in the free-running oscillator. $S_{dd}$ is the power spectrum of noise from the displacement sensor and $S_{ff}$ is the power spectrum of a white thermal noise source. $H(\omega)$ is the transfer function for a second-order system, and $K$ is the gain of the feedback path.

\[
\frac{\delta \omega}{\omega_0} = \sqrt{\frac{(\omega_2 - \omega_1)k_B T}{2\pi\omega_0 kQ <x_{osc}^2>}}
\]  

(4.8)

This relationship is valid for a measurement bandwidth $(\omega_2 - \omega_1)$ limited to $\omega_2 \ll \omega_0$ and $\omega_1 \gg \omega_0/Q'$, where $Q'$ is the effective closed loop quality factor: $Q' = \left( \frac{1}{Q} - \frac{k}{k} \right)^{-1}$.

The frequency deviation resulting from displacement noise power $S_{dd}$ is derived similarly resulting in:

\[
\frac{\delta \omega}{\omega_0} = \sqrt{\frac{S_{dd}}{2\pi <x_{osc}^2> \left[ \frac{1}{3} \frac{\omega_2^3 - \omega_1^3}{\omega_0^3} + \frac{\omega_2 - \omega_1}{Q} \right]}}
\]  

(4.9)

Both of these results predict that frequency variation decreases at higher resonance frequencies and for greater oscillation amplitude. For devices with modest quality factor and resonance frequency, thermomechanical displacement noise is the larger of these two and is the limiting factor of frequency noise [57]. For other systems thermal noise has been reduced such that the system noise becomes dominant and is the limiting factor [18].

For the suspended microchannel resonator systems used here, with a measurement
bandwidth of 1kHz, equations 4.8 and 4.9 predict that displacement sensor frequency variation is dominant for piezoresistive readout, just as displacement sensor noise levels are dominant over the thermal noise. A sub-mHz stability due to thermal noise is predicted for both length devices (this is independent of readout method), and mHz-level stability due to the displacement sensor noise of the piezoresistors.

For optical readout, displacement sensor frequency stability from the optical readout is predicted to be better than the frequency stability due to thermal noise. The measured frequency noise values may be higher than the predicted levels because of parasitic coupling of AC sources which can increase frequency instability.

### 4.3 Allan variance frequency stability analysis

Differences in the frequency stability of each device and readout type predicted in section 4.1 is observed in closed-loop frequency measurements. For each of the SMR lengths and readouts, twenty seconds of acquired data is shown in figure 4-7, and for this acquisition time, the variance in frequency for each type of device and readout is compared in terms of parts-per-billion (ppb). However, frequency stability can manifest differently for different averaging times. For the same 20 second period, the data from the 406 pm device (optical readout) is shown in figure 4-8 for four different gate times. Clearly the minimum frequency shift due to a particle passing through the resonator during this time will vary depending on what gate time is used. For very short gate times at 0.01 seconds or below (pink and green lines), the frequency noise is much larger. On the other hand, at 1 second averaging time (red line), which is the slowest gate time here, the baseline would also presents variation over longer time periods. The 0.1 second gate time (blue line) has the most stability and minimum deviation for particle transit times of ~1 second, which is typically used.

Allan variance (AV), which is related to the fractional frequency variation, is a common measurement of stability in oscillators and can be used to investiagte these trends further. AV is defined as the variance over time in the measured frequency of a source, each measurement averaged over a certain time interval (gate time) $\tau_o$, with
Figure 4-7: Closed-loop frequency measurements for each of the readout types and device sizes. Data was acquired for twenty seconds using a heterodyne mixing scheme illustrated in chapter 3. No further filtering is applied to the data shown here.
Figure 4-8: A time-course representation of the closed loop frequency measurement for a 406μm device, using optical readout. Of the averaging times used, τ = 0.1s shows the best combination of stability and low frequency deviation for particles transiting in approximately 1 second. The frequency shift resolution is limited by the clock speed of the analog-to-digital converter.
Figure 4-9: The Allan variance for each device type and readout method. Variance was calculated for each of the gate times from the same one hour of data for each curve. Also plotted are the variances for one hour of data captured using a function generator in place of the SMR (at the resonance frequency of the 210μm and 406μm devices), and the thermal noise limits. Inverse of the gate time corresponds to sampling frequency.

no null-time between measurement intervals. Thus it is commonly used as a method to compare frequency standards in the time domain. The expression for AV is:

\[
\sigma_A^2(\tau_A) = \frac{1}{2f_0^2 N - 1} \sum_{m=2}^{N} (f_m - f_{m-1})^2 \tag{4.10}
\]

Where, \(f_m\) is the arithmetic mean frequency measured over the \(m\)th time interval. Each time interval is of length \(\tau_a\), \(N\) is the number of data points and \(f_0\) is the resonant frequency of the SMR, in this case.

All four curves in figure 4-9 have the best performance (lowest AV) at \(\tau_{AV_{min}} \approx 0.1s\). Variance increases at longer gate times in all four cases, and this could be due to slow drift effects, for example from temperature and pressure changes. At \(\tau_A = \tau_{AV_{min}}\) there is an order of magnitude difference between the variance for the short SMR devices measured by optical readout and the short devices measured using piezoresistive readout. The AV curves cross over for shorter gate times with
optical readout for 210μm outperform all of the others at gate time of 10^{-3}s. The SMR devices perform well compared to the AV of other resonant mass sensors [31, 71]. Previously groups have compared resonator frequency stability performance to a frequency standard such as a voltage controlled oscillator (VCO). Relevant limits in this case would be stability performance of the function generator used for mix-down, as well as the AV based on the thermal motion limit (both plotted in Figure 4-9). Function generator performance plotted in figure 4-9 was measured using a second function generator as the input signal instead of readout from the SMR. For calculation of the AV from thermal noise, the relationship between AV and phase noise density is used [13]:

\[ \sigma_A^2(\tau_a) = 2 \left( \frac{2}{\omega_0 \tau_a} \right)^2 \int_0^\infty S_{\phi\phi}(\omega) \sin^4 \left( \frac{\omega \tau_a}{2} \right) d\omega \]  

(4.11)

Since the phase noise PSD is related to the power spectral density of \( x(t) \), \( S_{xx} \) by:

\[ S_{\phi\phi}(\omega) = \frac{1}{2\pi} \int S_{xx}(\omega) d\omega = \frac{1}{2} \frac{S_{xx}(\omega)}{<x^2_{osc}>} \]  

(4.12)

And when the noise source is white thermal noise,

\[ S_{xx}(\omega) = S_{ff}|T(j\omega)|^2 \]  

(4.13)

where \( T(j\omega) \) is the closed loop transfer function:

\[ T(j\omega) = \frac{H(j\omega)}{1 - K_0 \phi H(j\omega)} \approx \frac{\omega_0^2}{4k^2(\omega_0 - \omega)^2} \]  

(4.14)

Combining equations 4.12, 4.13 and 4.14 into 4.11, gives:

\[ \sigma_A^2(\tau_a) \approx \frac{k_B T}{4m_{eff} \omega_0^3 <z_{th}^2> Q \tau_a} \]  

(4.15)

The thermal limit is plotted in Figure 4-9, and it can be seen that since the thermal AV curves are below the others, the frequency stability of the system is not thermally limited.

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4.3.1 Implications of Allan variance on measurement

Mass resolution

The detection of small masses is improved when fluctuations in the resonance frequency are decreased. These fluctuations are quantified by the Allan variance. The minimum detectable change is that which gives a fractional change in frequency equal to the Allan variance,

\[
\left(\frac{\delta f}{f_0}\right)^2 = \sigma_A^2
\]  

(4.16)

Assuming a mass increase corresponding to a particle positioned at the apex of the SMR, substituting the relationship between frequency shift \(\delta f\), and added mass \(\delta m\), which are given in equation 4.1, the minimum detectable mass is:

\[
\frac{\delta m}{m_{eff}} = \frac{2\delta f}{f_0} = 2\sigma_A
\]  

(4.17)

For each of the device types and readouts, this is plotted in Figure 4-10, along with the thermal limits.

Measurement type and timescale

Stability performance for different gate times can indicate how to optimize the type of measurement and bandwidth for each form of readout. Piezoresistive readout, which shows less variation over long time scales, may be conducive to measurements which require longer bandwidths, such as surface binding assays [66]. The optical lever, which has better performance (for the 210\(\mu\)m device) at small \(\tau_{AV}\), should be used with those measurements which are on shorter time scales such as particle flow-through measurements (1-100Hz bandwidth). To make measurements on a time scale which takes advantage of the bandwidth at which the Allan variance is minimum, two values are required: the gate time at which AV is minimum, \(\tau_{AV_{min}}\), and the number of points required for accurate resolution. It has been empirically determined that the particle transit time through the resonator should be adjusted to achieve \(N_{pt} \geq 1000\)
Figure 4-10: Mass resolution of the SMR for each device type and readout method at gate times from $10^{-3}$ to $10^2$ seconds. The resolution was calculated from the results in figure 3-9.

Given the minimum variance ($\tau_{\text{av,min}} \approx 100ms$) from figure 4-9, the transit time, $t_{\text{transit}}$, should then be calculated by:

$$t_{\text{transit}} \geq N_P \times \tau_{\text{av,min}}$$  \hspace{1cm} (4.18)

Here the transit time should be at least 10 seconds. Current flow rates result in particle transit every $\sim 1s$ or less. The data obtained for the histograms in figure 4-3 was at the rate of approximately 1000 particles/hour. There are no limits in the experimental setup for achieving lower flow rates, but measurement throughput would be drastically limited.
Chapter 5

Mass-based readout for agglutination assays

This chapter presents a new type of assay format for biomolecular measurement using the SMR. Pre-functionalized microsphere are used as the reaction surface instead of pre-functionalized SMR channels. A model system of streptavidin functionalized microspheres and biotinylated IgG-antibody as the analyte is used to demonstrate the assay, and this type of assay presents a useful method for protein detection in general. Although agglutination is currently used in many commercial and clinical applications, detection of agglutinates by mass in the SMR provides a new way to monitor early stage agglutination as opposed to population ensemble techniques that are typically used. A dose-response curve over a concentration range of 0.63 - 630 nM is achieved, comparable to what has been previously reached by image analysis and conventional flow cytometry.

Agglutination presents a simple measurement technique conducive to use when the precision and reagents required for surface functionalization are not readily available. Piezoresistive readout reduces the need for external components for focus and alignment of the optical lever. Together, these two concepts can further simplify use of the SMR for outside the laboratory applications.
5.1 Agglutination background

Agglutination assays based on nanometer and micrometer sized particles were originally inspired by natural agglutination of cells [54]. By coating the beads with antibodies, coagulation, or agglutination, occurs in the presence of an antigen, resulting in a turbid precipitation of aggregated beads. Agglutination is useful for identification and quantification of proteins or other biomolecules for clinical diagnostics, in drug discovery or proteomics research and in food-industry quality controls. In addition, there are several commercial examples of agglutination assays used for clinical diagnostic applications [2]. Agglutination has long been popular in point-of-care tests for diagnostic purposes due to its simplicity, low cost and speed [17].

In its simplest format, the agglutination test utilizes a sample placed on a test slide and the presence of agglutination is observed visually as a qualitative “yes/no” indication. An extension to this is based on the measurement of scattered or absorbed light. Techniques for measuring agglutination include turbidimetry [15], dynamic light scattering [3], and UV-vis spectroscopy [46]. In some cases, particle counting techniques such as flow cytometry and image analysis can improve sensitivity by quantifying small aggregates that are produced during the initial stages of aggregation [69], allowing for a reduction of the required incubation times. Additionally, particle counting enables more specific information about the agglutination distribution in a population, rather than average agglutination information typically obtained by ensemble measurement techniques. Furthermore, microfluidic approaches for particle counting can reduce the required sample volume from milliliters to microliters and enable integration with sample treatment steps [48].

The SMR provides a non-optical alternative for particle counting where early stage aggregation is quantified by measuring mass with the SMR. In SMR detection [7], each aggregate is weighed in real time by measuring transient changes in resonant frequency as it flows through the vibrating microchannel.
5.2 Methods and materials

To enable measurement with the SMR as well as visualization by optical microscopy, 0.97 μm spherical streptavidin-labeled polystyrene microspheres were used. The biotin-streptavidin model system takes advantage of a high binding affinity reaction, as well allows comparison to previously demonstrated detection techniques [69] for agglutination. The analyte consisted of anti-FLAG M5 monoclonal antibody biotinylated with approximately six biotins per antibody (Sigma Aldrich, St. Louis, MO). The microspheres were centrifuged, washed and resuspended in buffer [phosphate-buffered saline (PBS), pH 7.2 with 0.1% bovine serum albumin and 1% Tween 20] at a concentration of 2 × 10¹⁰ particles/ml. To minimize nonspecific aggregation, the microspheres were then ultrasonicated for 3 minutes (15s on, 10s off). The biotinylated antibody analyte was added to give final concentrations of 0, 0.63, 6.3, 63, and 630 nM, and the mixtures were incubated for 30 minutes at 25 °C with gentle mixing at 15 and 25 minutes. After incubation, the sample was diluted with PBS to quench the reaction and create a final particle concentration of approximately 5 × 10⁸ particles/ml, which has previously been determined to optimize throughput while decreasing the probability that two or more aggregates simultaneously pass through the SMR channel [7]. The SMR used had a channel size of ~ 3 μm × 8 μm. Electrostatic actuation and optical lever were used, as devices with piezoresistors had not been fabricated yet.

Due to the short incubation time, the early stages of the agglutination reaction could be observed when the solutions consisted mostly of monomer and dimer structures, with any larger structures grouped as “multimers.” For visualization by optical microscopy, solutions were prepared as above and left for 10 minutes on glass slides before viewing to allow structures to settle into a single layer and be counted (Figure 5-1b). For detection with the SMR, structures were classified by flowing the solution through the device and weighing aggregates one by one (Figure 5-1d).

In order to obtain a dose-response curve using the SMR, a mass histogram from approximately 2000 aggregates was acquired at each analyte concentration. As an
Figure 5-1: a) Streptavidin (SA)-coated microspheres are incubated with the target protein (SA is illustrated by diamonds). The analyte biotinylated antibody creates agglutination by binding to two different SA receptors located on separate microspheres. b) Optical micrograph of monomer, dimer, and multimer structures which are formed by agglutination of 0.97 SA microspheres with 0.63 nM of analyte. c) Schematic of the suspended microchannel resonator SMR for counting aggregates by weighing them one at a time. The resonance frequency of the SMR is sensitive to the presence of particles whose mass density differs from that of the solution in the microfluidic channel. d) The SMR is used to classify a monomer right and a dimer left and anything larger as a multimer not shown. The transient frequency shift from each structure is converted to a mass by using a calibration factor (see Reference [7]) and accounting for the buffer density.
example, figure 5-2 shows the mass histogram for an analyte concentration of 6.3 nM. Here, two clearly distinguishable distributions reveal the monomer and dimer proportions in the sample. The relative area under each of these distributions changes as the analyte concentration is varied. The monomer and dimer distributions of figure 5-2 overlap due to the polydispersity of the polystyrene particles (5%-10%) [1], which contributes to some uncertainty in the measured aggregate fraction. Moreover, differently sized particles within the nominal distribution may have different binding capacities which could also contribute to uncertainty. A value of 95 mHz was chosen as the threshold between monomer and dimer populations for all experiments, and all peaks above 190 mHz were classified as multimers. Frequency shifts can be converted to buoyant mass by using a calibration factor (see Reference [7]). Absolute mass can be calculated if the buffer density is known.

5.3 Suspended microchannel resonator agglutination measurements

The percentage of each structure type is plotted versus analyte concentration in the dose response curve shown in figure 5-3a. In order to confirm the trend of the dose response curve, optical microscopy was used to classify approximately 1500 structures that were agglutinated at each of the same analyte concentrations as measured by the SMR (Figure 5-3b). For both optical and SMR based readout, the proportion of dimers and multiples in the sample increases as the analyte protein concentration is increased from zero up to 6.3 nM. Beyond this concentration, the number of dimmers and multiples decreases as the number of monomers increases due to saturation of the particles by the biotinylated antibody. This hook effect [19], whereby the slope of the dose response curve changes sign past the “equivalence point,” is visible after the data point at 6.3 nM. Negative control experiments (antibody without biotin) do not demonstrate this phenomenon (Figure 5-3).

As outlined by Wiklund et al. [69], the detection limit of an agglutination assay
Figure 5-2: Mass histogram from aggregates weighed by the SMR. There are primarily two types of structures monomers and dimers that resulted from an analyte concentration of 6.3 nM and a particle concentration of 5108 particles/ml. In each experiment approximately 2000 peaks were counted in 2 hours. Particle types were classified by integrating the number of agglomerates between 0 and 95 mHz for monomers, 95 and 190 mHz for dimers, and 190 mHz and above for multimers thresholds marked by dashed lines.
Figure 5-3: a) Mass-based dose response curve obtained by weighing aggregates with the SMR. Each data point represents the mean obtained from three experiments, and at 0.63 nM, five experiments. The dependence of structure proportion is shown vs analyte concentration (biotinylated antibody in solid lines and pure antibody in dashed lines). Each data point represents the mean percentage of structures obtained from three separate experiments at each concentration. In each experiment approximately 2000 aggregates were weighed, and error bars represent the standard deviation from the mean. b) A dose response curve was also obtained by optically imaging approximately 1500 aggregates per analyte concentration.
scales linearly with the particle concentration and binding capacity provided that the capacity is greater than the binding affinity constant. The dynamic range of agglutination assays is typically limited to the range between the minimum detectable concentration and the equivalence point. For the data shown in figure 5-3a, the dynamic range is approximately tenfold, which is comparable to what was achieved by optical inspection (Figure 5-3b), and in prior work, by flow cytometry and image analysis [69]. While the optical and SMR dose response curves in figure 5-3 show similar trends for monomers, a relatively larger percentage of multimer aggregates are measured optically. This is likely attributed to larger structures either not flowing into the suspended microchannel due to size constraints (the fluidic channel inside the cantilever is 3 μm tall by 8 μm wide), structures breaking because of shear forces in the microchannel, or settling of the larger aggregates. The SMR's tendency to undercount aggregates of three or more particles could be reduced by using smaller particles.

Limited sample size (total number of aggregates counted in an experiment) may contribute to uncertainty in the proportions of structures at each analyte concentration. However, the sample size along with the particle counting rate determines the total measurement time for each assay. In order to determine the extent to which sample size contributes to overall error between experiments, the standard deviation from three experiments was calculated over a range of sample sizes. At the analyte concentration of 0.63 nM, the mean proportion of monomers was calculated by using the first 1000 counts, the first 1500, and up to a sample size of 3500. The resulting standard deviation is plotted in figure 5-4 and is normalized to the standard deviation from 2000 counts in order to enable a comparison to error bars shown in figure 5-3a. The standard deviation continues to decrease with sample sizes larger than 2000 which implies that the uncertainty in figure 5-3 could be reduced still by measuring more aggregates.
Figure 5-4: Normalized standard deviation (SD) of the mean percentage of monomers versus the number of aggregates counted normalized to the SD at 2000 counts. Aggregates were counted for 4 hours in each of three experiments using the same concentration analyte 0.63 nM biotinylated protein. The data point at 2000 counts includes data from two earlier experiments, representing five experiments total. The number of monomers was examined for the first 1000 counts and up to 3500 counts in increments of 500.
5.4 Conclusion

The sensitivity and dynamic range achieved by agglutination assays is considerably less than what can be achieved with a surface-based assay inside the SMR [66]. Agglutination tests in general suffer from low sensitivity with detection limits in the nanomolar range [68]. Limiting factors are mainly the relatively high bead concentrations needed for sufficiently high particle collision rates, and nonspecific agglutination of beads. In addition, the hook effect constrains the dynamic range, preventing the possibility of measuring a full binding curve. Some work has been done by groups to counter these effects including using ultrasonic standing wave enhancement [68] to increase sensitivity via the rate of particle collisions, increasing binding capacity of each bead to increase the concentration at which saturation occurs, consequently increasing the dynamic range (this has achieved up to 3 orders of magnitude increase in dynamic range) [23]. Also a heterogeneous sandwich immunoassay has been developed wherein the decrease of signal at high antigen concentrations is avoided by still using one antibody immobilized to the beads, but incorporating the use of two different “tracer” antibodies having different affinities and different specificities to the antigen. The antibody with lower affinity will have a more significant contribution only at high antigen concentrations, thus forestalling the hook effect.

However, benefits of mass readout for agglutination assays using the SMR are that functionalization of the SMR channel walls is not necessary, and assays can be developed and prepared independently of the device. Furthermore, particles with a wide range of functionalities and sizes are readily available, enabling assays with varying reaction rates and aggregate sizes. Moreover, multiple analytes may be detected simultaneously within the same mixture by multiplexing particles with differing masses.

In terms of readout, in a 1Hz bandwidth, the SMR has mass resolution of minimum 0.4fg (refer to mass resolution figure in chapter 4, piezoresistive readout with 200\mu m long 8\mu m \times 8\mu m channel devices), which enables structures equivalent to 80 nm gold, 200 nm silica, or 500 nm polystyrene particles to be detected in water with a signal-
to-noise ratio of 10. Reduction in device size will ultimately enable the measurement of even smaller particles. In order to achieve the current resolution, the counting rate is limited to approximately 1 particle/s. However, based on the work in Chapters 2-4, piezoresistive integration with SMRs enables the possibility of parallel detection which can increase throughput.
Chapter 6

Conclusion

This thesis has introduced two new methods for biological sensing. First, two types of suspended microchannel resonators were fabricated with piezoresistors. Heat transfer in the devices from the optical lever and piezoresistive readout schemes was analyzed and as well sensitivity of the devices was calibrated using FEM, LDV and thermo-mechanical noise. The piezoresistive readout was demonstrated by measuring the buoyant mass of populations of polystyrene particles as well as yeast cells. Piezoresistive readout shows mass resolution down to 0.1 fg in a gate time of 100 ms, which outperforms the optical lever system currently used in our laboratory. Electronic readout shows benefits in sensitivity and provides a more robust system platform for use outside of the laboratory setting, as well as introduces the possibility of scaling up to arrays of devices on a single chip. This is the first demonstration of piezoresistors on resonant mass sensors used for measuring in fluid environments; particularly useful for biological substrates.

Second, the agglutination assay was adapted for the SMR. Measurement of early stage agglutination of cells or particles by mass allows more specific information about the agglutination process than ensemble measurement techniques. The SMR agglutination assay is demonstrated using a model system of streptavidin functionalized microspheres and biotinylated antibody as the analyte. A dose-response curve over a concentration range of 0.63-630 nM is achieved, comparable to what has been previously achieved by image analysis and conventional flow cytometry. Despite limited
sensitivity, this assay has provided a new method for molecular sensing using the SMR, while avoiding functionalization of the resonator surface.

In this chapter, future applications and implementations of these technologies are elaborated, as well as recommendations for subsequent designs and system improvements.

6.1 System and packaging

Incorporation of electronic readout evades the alignment and equipment necessities of optical lever readout, and consequently makes the readout conducive for use outside of laboratory settings. For use in laboratory settings, piezoresistive readout can also expand SMR measurement capabilities. The optical lever system requires access to one side of the device for the laser, either the top or bottom. Currently optical imaging can be implemented on the side of the device opposite to the optical lever. This is used to monitor particle transit into and out of the device (Figure 6-1). Without this requirement there is optical access to the device on both sides. Thus further optical techniques, such as fluorescence imaging, could be incorporated in lieu of the optical lever, expanding the number of metrology methods that can be used simultaneously. For example, an assay which tags particular cells or molecules fluorescently can be monitored to measure mass correlated with fluorescence. This would require a microscope with a fluorescence filter and access to one side of the device. Access to the opposite side of the device would still be available for optical imaging to monitor flow in and out of the channels.

Elimination of optical components also makes available the possibility of bringing the device outside of the laboratory setting, to less robust environments where laser alignment and air currents would otherwise impede use of the device. SMR detection of particular cells, molecules and disease markers would be useful in the home setting for personal care use or in the field, away from larger laboratory facilities. However, in these point-of-care use cases a portable vacuum or pressure source for fluid control will still be required.
Figure 6-1: Access for the optical lever laser and microscope for optical imaging require use of both sides of the device. Integration of electronic readout eliminates need for the laser, allowing further metrology techniques to be implemented for simultaneous use.
Agglutination assays are an example of a technique which is conducive to portability; they do not require the SMR to be pre-functionalized and assay reagents can be prepared and stored separately from the SMR. One SMR device could be used for a variety of measurements given that beads functionalized appropriately for the desired antigens are available.

A packaged SMR with integrated electronic detection also allows for types of measurement which are not possible when external components must be included and require precise alignment. Rotation of the device and experiments in non-standard position can be performed in succession very rapidly because electronics, opposed to optical components, can be moved and do not need to be secured during the measurement process. This may, for example, allow for study of inertial effects simultaneously with mass measurements.

### 6.2 High-mode sensing

All measurements in this work were made using first flexure mode resonance. Compared to first mode, higher modes have been shown to improve mass sensitivity in resonant sensors \[31\]. When using higher modes, detection sensitivity can also be optimized by piezoresistor placement. For first mode sensing of the SMR, the piezoresistor is placed near the clamped end of the resonator. This is optimal for static modes as well as all flexural resonance modes; the clamped edge bears the maximum induced stress because the radius of curvature (section 2.2.2) is always minimal at the clamped edge as a result of applied boundary conditions. However in resonance at a select higher mode, the objective is to enhance detection of an overtone. Then, the piezoresistor location should be altered. For each flexural mode of a resonator, there is a location where the induced stress is locally maximized for each particular overtone. By placing the piezoresistors on this location the detection selectivity for that particular mode can be increased. These locations can easily be determined by investigating the points of maximum longitudinal stress on the device surface in different flexural modes. The longitudinal stress, \(\sigma\), is proportional to the strain, \(\epsilon\),
at any point a distance $t$ from the neutral axis:

$$\sigma = E\epsilon = \frac{Et}{C}$$

(6.1)

Where $C$ is the radius of curvature ($= \frac{1}{\sqrt{E}t}$) as described in section 2.2.2. The radius of curvature is also proportional to the moment ($\frac{d^3}{dt^3}$) [52]. Thus a plot of the moment versus position on the lever will indicate areas of local stress-maxima, as has been done in previous work by Naeli [33].

### 6.3 Arrays and measurement throughput

Alignment of multiple lasers is a complex problem for micron and smaller scale devices. This alignment issue along with constraints on the amount of chip area required for fluidic port areas are the main factors that currently preclude scaling of the number of SMR devices on a single chip. Integration of electronic frequency detection eliminates the alignment constraint and consequently can contribute to increasing measurement throughput. The first device with four SMR devices has been fabricated, and it incorporates piezoresistive readout (Figure 6-2).

A variety of readout methods have been harnessed in both static and dynamic modes when addressing multiple sensors per chip [38, 72]. For simultaneous measurements, parallel actuation or detection methods such as optical imaging (a CCD captures reflected light from all cantilevers simultaneously), or photonic transduction (all cantilevers are actuated by the same broadband light source, and one photodetector receives the light simultaneously from multiple devices for open-loop measurement), have been employed. The same actuation or detection methods could be integrated with the SMR. In the methods currently employed (piezoelectric crystal or electrostatic actuation, piezoresistive detection) each device is individually addressed. Upstream and downstream multiplexing can be employed to address a large number of devices without requiring an equally large set of downstream electronics. Speed of the multiplexer and demultiplexer will have to be adequate enough to access each of the sensing devices at their resonance frequencies, for enough time to gather a
Figure 6-2: SMR design with four cantilevers in parallel. Each of the inlet and each of the outlet channels lead to the same bypasses.
sufficient number of frequency samples. In addition, when scaling up the number of devices, the resonance frequencies of each resonator on a single chip should be adequately spaced to avoid coupling of signals which will interfere with accurate signal acquisition.

Given the current fluidic system of the SMR, throughput will increase linearly with the number of devices. However increasing the number of devices on a single chip also allows for new types of measurements on single or low numbers of particles and cells. For example, with an array of devices, a single cell could be measured in serial with different devices or in a multitude of different buffer environments all in one package.

6.4 Electrostatic Actuation

As described in chapter 3, to-date, all measurements made by piezoresistive readout from SMRs have used piezocrystal actuation. Electrostatic actuation is desirable because it provides direct force control based on the applied voltage. In addition, it can be used to individually address resonators on a single chip.

Anticipated limitations to the DC voltage are the pull-in and breakdown voltages for the SMR structure. The breakdown voltage of an insulator is the minimum voltage that causes a portion of an insulator to become electrically conductive. Paschen’s law has been used to find the breakdown voltage of parallel plates in a gas as a function of pressure and gap distance. From the work of Torres and Dharwal [61], results in vacuum show that the electrode material does not influence to any great extent the breakdown voltage. Also from this work, assuming millitorr pressure levels in the SMR cavity, a breakdown voltage in the range of $1 \times 10^3 - 1 \times 10^4 V$ is predicted.

For electrostatic MEMS devices, a voltage applied between parallel plates of a capacitor (electrostatic drive voltage in this case) will reduce the plate separation. At small drive voltages the electrostatic force, $F_{es} = -\frac{1}{2} \frac{dC}{dx} V^2$, is countered by the spring force, $F_k = kx$. However as the voltage increases, the plates will eventually snap together. This is when the stiffness of the system becomes unstable. To find the...
bias voltage at which this occurs, first note that equilibrium occurs when:

\[ kx = \frac{1}{2} \frac{\varepsilon A}{(d - x)^2} V_C^2 \]  

(6.2)

where \( d \) is the original distance between the plates, \( x \) is the distance the plates are pulled together, \( A \) is the plate area, \( \varepsilon \) is the dielectric constant of the surrounding media and \( V_C \) the voltage applied across the plates. Total restoring force in the system is found by differentiating with respect to \( x \), \( (\delta F/\delta x) \). Solving for the plate distance at the equilibrium point when restoring force is zero (unstable):

\[ \frac{\delta F}{\delta x} \bigg|_{F_k=F_k} = \frac{2kx}{(d - x)} - k \]  

(6.3)

gives \( x = \frac{1}{3} d \). Substituting this value for \( x \) into equation 6.2 allows solving for \( V_C = V_p \), the pull-in voltage:

\[ V_p = \sqrt{\frac{8 k d^3}{27 \varepsilon A}} \]  

(6.4)

Which for the SMR system is approximately \( 3.1 \times 10^4 \) V. Thus the pull-in and breakdown voltages must both be carefully considered for each SMR design, however immediate limitation is set by the breakdown voltage.

### 6.5 Future of piezoresistance

Transduction of signals from the mechanical domain to the electrical domain will continue to be an indispensable requirement of gathering and processing new forms of data from the physical world. Piezoresistance is an inherent property of particular materials that is conducive for this purpose. Traditionally, as described in chapter 2, piezoresistors in semiconductor technologies are made of polysilicon, doped single crystal silicon or metal. The fabrication process and device geometry usually dictate the type of material used. More recently, other materials are being explored which can support novel applications. Both carbon nanowires and nanotubes have been
shown to exhibit a piezoresistive effect and have dimensions that are conducive to use on devices down to the nanoscale regime. The piezoresistive coefficient for carbon nanowires has been comparable to that of p-type silicon [60]. Carbon nanotubes (CNTs) have been shown to exhibit a pressure-resistance relationship which is not as temperature dependant as polysilicon, and fabrication does not require high temperature environments. CNTs are also suitable for integration with polymer MEMS devices, such as microfluidics, which can be flexible and made for low-cost. Other organic and polymeric materials, which themselves demonstrate piezoresistive properties, and would be suitable for these applications have also been investigated. An indium-tin-oxide poly [2-methoxy-5-(2'-ethylhexyloxy)-1,4-phenylenevinylene] structure has been shown to have a longitudinal piezoresistive coefficient three to five orders of magnitude larger than p-type silicon piezoresistors on silicon devices [59]. However, the range of tensile stresses under which the polymeric piezoresistors are functional is limited due to physical damage of the polymer-electrode interfaces. Graphite filled polymide has low permittivity thus can be employed in applications requiring insulative properties [21]. Organic films have also been shown to have piezoresistive effects with gauge factors an order of magnitude smaller than n-type silicon and can be integrated with flexible and transparent substrates [36].

Over time, the types of materials and geometry of the suspended microchannel resonators may change, to incorporate new technologies which may provide benefits in terms of packaging, processing or sensitivity. Given the current ion-implantation process, performance can be enhanced in many ways. For example, work is underway to fabricate nanoscale SMR devices which will have increased sensitivity from the devices here due to their decreased size. Piezoresistor thickness can be decreased using a rapid thermal anneal or arsenic dopants, which have a lower diffusivity than boron. Geometric optimization for the current process parameters can still be performed for both the nanoscale and microscale resonators. Given the current resistor size and biased voltage used, power dissipation is limited to approximately 100μW. It has been determined experimentally that this can be increased to at least 2mW without inflicting any device damage. Assuming the temperature increase associated
with this power (~3K assuming linear relationship with temperature increase and power dissipation) is tolerable for the interior substrates (temperature through the cross-section is approximately uniform even at high flow rate [10]), the resistor area and length can be decreased in order to increase the stress on the resistor. Figure 2-4 shows how much the expected change in resistance is for resistors different proportions of the total cantilever length. A change from the current length (approximately one-half the length of the 210\(\mu\)m devices and one-quarter the length of the 406\(\mu\)m devices) can increase the \(\Delta R/R\) by a factor of 46% (210) and 19% (406). Decreasing the resistor depth would function to increase the stress felt by the resistor and \(\beta\) would increase, to a maximum of 1 (~25% increase in \(\Delta R/R\)). However, a shallower resistor also would serve to give a higher total resistance which increases the noise level, so the depth must be selected to balance the these two effects. Once the noise and power limits are decided on for a given type of device, these geometric parameters can be further optimized.

The work in this thesis should be used as a starting point for integration of piezoresistive readout with suspended resonators. The first implementation of piezoresistors on suspended microchannel resonators have shown comparable or better mass sensitivity, derived from frequency noise measurements, than the optical lever for certain measurement conditions. As well, piezoresistors provide benefits over the use of an optical lever in terms of packaging and system design. There are many types of measurements of biological systems in fluid environments by mass that will be enabled by integrating electronic readout with the SMR.

Performance of both the piezoresistive readout and the optical lever used here can be further optimized to increase frequency stability. The piezoresistor geometry can be optimized by increasing the average stress that the piezoresistor experiences, while managing other parameters such as the voltage bias to keep power dissipation limited to an appropriate range. Electrical and thermal noise can also be decreased by better shielding for the device and downstream electronics.

In addition, the agglutination assay demonstrates a novel way to measure biomolecules
without surface functionalization. Other assays using particles as the measurement or reaction surface should be investigated. These techniques add to the functionality of the SMR and the total body of knowledge on biological sensing.
Appendix A

TSUPREM-4 code

$ Simulation of implant conditions for pSMR
$ Rumi Chunara

$ vertical specifies a 1-D oxidation model and pd.full is the most accurate model of dopant diffusion
METHOD VERTICAL PD.FULL

$ setup grid
LINE X LOC=0 SPAC=.2 tag=left
LINE X LOC=40 SPAC=.2 tag=right
LINE Y LOC=0 SPAC=.0005 tag=topsi1
LINE Y LOC=2 SPAC=.1 tag=topox1
LINE Y LOC=3 SPAC=.1 tag=topsi2

$ start with <100> p-type wafer region oxide1 ylo=topox1 yhi=topsi2
xlo=left xhi=right

$ background resitivity of wafers is 1-20 ohmcm, meaning dose can be between 2.3e14 - 5e15
INITIALIZE <100> IMPURITY = PHOSPHORUS I.RESISTIVITY=20
$ oxidize before implanting $ thin oxide - want 200A oxide
DIFFUSION TEMP=850 TIME=10 DRYO2

$n$- implant (for background)
IMPLANT PHORPOROUS DOSE=1e13 ENERGY=160

$d$ dopant anneal
DIFFUSION TEMP=1050 TIME=600 NITROGEN

$p$++ implant (for conducting contact - not simultaneous with $p$- implant)
$IMPLANT BORON DOSE=1e16 ENERGY=120

$n$+ implant (for background contact)
$IMPLANT PHOSPHORUS DOSE=5e15 ENERGY=130 TILT=7

$p$- implant
IMPLANT BORON DOSE=5e13 ENERGY=50 TILT = 7

$d$ dopant anneal
DIFFUSION TEMP=950 TIME=60 NITROGEN

$ plotting and saving the results
OPTION device=ps-c file.sav=n-.ps
SELECT Z=LOG10(BORON)
PLOT.1D LINE.TYP=1 COLOR=4
SELECT Z=LOG10(PHOSPHORUS)
PLOT.1D COLOR=2 ^AXES ^CLEAR

SELECT Z=DOPING
EXTRACT SILICON X.VAL=0 VALUE=0 D.EXTRAC ASSIGN NAME=DJ

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SELECT Z=BORON PRINT.1D X.VALUE=0 OUT.FILE=boron.dat
SELECT Z=PHOSPHORUS PRINT.1D X.VALUE=0 OUT.FILE=phos.dat
Bibliography


