**20.309 / 2.673 / MAS.402**
**Biological Instrumentation and Measurement, Fall 2008**
*Department of Biological Engineering*
*Massachusetts Institute of Technology*

## Lab Assignment #2 *(version 2)*

Due: Tuesday, October 24

Introduction and Motivation

Force sensors such as the optical tweezers and atomic force microscope (AFM) provide a unique means for investigating single biomolecules. Examples include the real-time monitoring of enzymatic activity with the optical tweezers and the direct measurement of forces required to unfold individual protein domains with the AFM. At the heart of these force sensors is an ultrasensitive displacement detector that resolves the position of compliant structure (i.e. microcantilever or optically trapped mircobead) with nanometer, or in some cases, sub-nanometer resolution. The performance of the force sensor is determined by the mechanical properties of the structure (spring constant, resonant frequency, damping, etc) and the resolution of the displacement detector. When designing a force sensor for a particular application, it generally desirable to achieve a performance metric that is thermally limited. In this limit, the noise level of the sensor is governed by $k_bT$.

The goal of this section of the course is to i) understand how the thermal limit of detection is related to the mechanical properties of the force sensor, and ii) experimentally determine if a force sensor is thermally limited. The lectures and homework will focus on the first goal while the lab module will focus on the second one.

As we will see from lectures, the power spectral density (PSD) is a useful function to calculate and measure for a given sensor. Since we will model the mechanical properties of the force sensor a simple harmonic oscillator with spring constant $k$ and RMS displacement $\langle x^2 \rangle$, the Equipartition Theorem,

$$\frac{1}{2}k_bT = \frac{1}{2}k\left\langle x^2 \right\rangle$$

tells us that the integral of the position PSD, $S_x(f)$, is directly related to Boltzmann's constant by

$$\left\langle x^2 \right\rangle = \int_0^\infty S_x(f)df = \frac{T}{k}k_b$$

Thus, by measuring the PSD, one can essentially "measure" $k_b$ and thereby determine if a force sensor is thermally limited by comparing it to the known value of $1.38 \times 10^{-23}$ J/K. This assumes that the temperature and spring constant are known. Conversely, if a force sensor is known to be thermally limited, one can determine its mechanical properties by measuring the thermal noise.

Lab module requirements

1. Measure the biased z-mod response for the 275 μm and 350 μm cantilevers. Plot the output voltage of the photodiode as a function as the voltage to the z-mod scanner. Make sure to normalize your data for any gain applied to your signal downstream of the photodiode output. Include the appropriate units on your plot.

2. Using the diffraction equation in Section 3.1.2 and accounting for the correction factor described in Section 3.2.6, plot your data with the abscissa in nanometers (instead of the z-mod scanner voltage). What is the distance of adjacent minima and maxima? How does this compare to the wavelength of the illumination?

3. Measure the thermally excited motion for the 275 μm and 350 μm cantilevers. Following the description in Sections 6.2 and 6.3, plot the Power Spectral Density (PSD) of each cantilever on the same graph using a log-log scale and units of Angstroms per √Hz for the ordinate. From this plot, estimate the Quality factor $Q$ and resonant frequency $f_o$ of each cantilever.

4. Estimate the spring constant $k$ for each cantilever as described in item 3 of Section 6.1. Using these values, calculate the theoretical low-frequency limit of the thermomechanical motion of each cantilever. This limit is calculated from $\delta^2$ in Section 6.1 which is equivalent to $S_x(\omega=0)$ in the October 9 lecture notes. Sketch this limit in your PSD from Part 3. How well does the theoretical calculation compare to your measurement? If your measurement differs by more than an order of magnitude, suggest what factors could give rise to such a difference, making rough estimates if necessary. Note that it is common to see '1/f' noise at low frequencies (typically below a few hundred Hertz for our system) so you will want to make the comparison of $S_x$ (or $\delta^2$) at a frequency above this corner point yet below the resonant frequency.

5. (OPTIONAL) Following the guidelines in Section 6.4, fit the analytical expression of the thermomechanical noise (without the low frequency approximation) in order to determine $Q$, $f_o$, and $k$.