20.309 Schedule for Fall 2008

LAB

	Lecture 1: **Course Overview**		*Thurs, September 4*
	Electronics		
	Lecture 2: **Voltage dividers and electrical impedance**		*Fri, September 5*
	Reading: H&H p. 3-24		
0. Intro to	Lecture 3: **Capacitors and RC circuits**		*Tues, September 9*
Electronics	Reading: H&H p. 32-35 and 6.002 notes p. 703-718, 993-1004		
|	Lecture 4: **Transfer Functions and RC filters**		*Thurs, September 11*
|	Reading: H&H p. 37-40, 46-53 6.002 notes p. 1004-1012, 1030-1054		
|	Lecture 5: **Thermodynamics of DNA melting**		*Fri, September 12*
|	Reading: SantaLucia, p. 1460-1462		
1. DNA	Lecture 6: **Feedback and Amplifiers I**	**Quiz #1**	*Tues, September 16*
Melting	Reading: H&H p. 163-176 and 6.002 notes p. 1185-1191		**HW #1 Due**
|	Lecture 7: **Feedback and Amplifiers II**		*Thurs, September 18*
|	Reading: 6.002 notes p. 1191-1220		
|	Lecture 8: **The Reality of Amplifiers**		*Fri, September 19*
|	Reading: TBA		
|	*Signals and Systems*		
|	Lecture 9: **Intro to Fourier Analysis**	**Quiz #2**	*Tues, September 23*
|	Reading: Strang p. 263-275, 309-315		**HW #2 Due**
|	Lecture 10: **Power Spectral Density, Noise and Bandwidth**		*Thurs, September 25*
|	Reading: Press p. 496-500 (on PSD)		
|	Lecture 11: **Sampling and Discrete Analysis**		*Fri, September 26*
|	Reading: Tutorial and Press p. 500-504 and		
|	Lecture 12: **Convolution**		*Tues, September 30*
|	Reading: Seung notes (except Sections 3,5,7,8)		**HW #3 Due**
|	Lecture 13: **Application of Convolution Theorem**		*Thurs, October 2*
|	Reading: none		
|	DEMO: **Thermal Measurement Laboratory**		*Fri, October 3*
|	Reading: Lab Module #2		
2. Thermal	Lecture 14: **Mechanical Systems**	**Quiz #3**	*Tues, October 7*
Measurement	Reading: Strang p. 316-320		**HW #4 Due**
|	Lecture 15: **Ultimate limits of force and position detection**		*Thurs, October 9*
|	Reading: none		
|	**Student presentations 1**	**Lab #1 Due**	*Fri, October 10*
|	**Laser Safety, Student presentation 2**		*Tue, October 14*
|			**HW #5 Due**
|	Lecture 16: **Optics and Microscopy I**		*Thurs, October 16*
|	Reading: Hecht 2.1-2.9, 4.1-4.5, 5.1-5.3		
|	Lecture 17: **Optics and Microscopy II**		*Fri, October 17*
|	Reading: Hecht 7.1, 7.3, 9.1, 9.3		
3.	Lecture 18: **Optics and Microscopy III**		*Tues, October 21*
Fluorescence	Reading: Hecht 10.1, 10.2.1, 10.2.5, 10.2.6		
Microscopy			**HW #6 Due**
|	Lecture 19: **Optoelectronics I**	**Quiz #4**	*Thurs, October 23*
|	Reading: Hecht 13.1-13.1.4		
|	**Optical Construction; Student presentation 3**	**Lab #2 Due**	*Fri, October 24*
|	Lecture 20: **Optoelectronics II**		*Tue, October 28*
|	Reading: Masters & So 12.1-12.5.7		**HW #7 Due**

	<u>Lecture 21:</u> **Image Processing I**	**Quiz #5**	*Thurs, October 30*
	<u>Reading</u>: Gonzalas & Wood 4.1-4.4, 8.4.1-8.4.2		
	<u>Lecture 22:</u> **Image Processing II**		*Fri, October 31*
	<u>Reading</u>: Gonzalas & Wood 7		
	<u>Lecture 23:</u> **Fluorescence spectroscopy I**	**Quiz #6**	*Tue, November 4*
	<u>Reading</u>: Cantor & Schimel 8.2, p.433-444, Lakowicz, 1.1-1.6		
			HW #8 Due
	<u>Lecture 24:</u> **Fluorescence spectroscopy II**		*Thur, November 6*
	<u>Reading</u>: Cantor & Schimel 8.2, p.444-465		
	Final project presentation		*Fri, November 7*
4. Project	<u>Lecture 26:</u> **Optical trap & Biomechanics**	**Quiz #7**	*Thur, November 13*
Labs	<u>Reading</u>:		**HW #9 Due**
	Student Presentation 4	**Lab #3 Due**	*Fri, November 14*
	<u>Lecture 27:</u> **Advanced Fluroescence Microscopy I: Resolution**		*Tue, November 18*
	<u>Reading</u>: Hell, N.Bio, 2003, Rust, N.. Meth. 2006		**HW #10 Due**
	Student Presentation 5		*Thur, November 20*
	<u>Lecture 28:</u> **Advanced Fluroescence Microscopy II: Biochemistry**		*Fri, November 21*
	<u>Reading</u>: Kim, Nat. Meth. 2007, Jares-Erijman, Nat. Biotech., 2003		
	Student Presentation 6		*Fri, November 25*
	<u>Lecture 30:</u> **3D Microscopy I: Confocal**	**Quiz #8**	*Tue, December 2*
	<u>Reading</u>: Pawley, 1		
	<u>Lecture 31:</u> **3D Microscopy II: Multiphoton**		*Thurs, December 4*
	<u>Reading</u>: So, Ann Rev 2000, p.400-410, 414-418		
	<u>Lecture 32:</u> **3D Microscopy III: Demo**		*Fri, December 5*
	Student Presentation 7		*Tue, December 9*
		Lab #4 Due	*Fri, December 19*

Problem Set #1   <u>Due</u>: Tuesday, September 16

(see Wiki version on the 20.309 website to access on-line links)
1. **Wheatstone Bridge** The resistor network shown below is known as a Wheatstone bridge. This is a common circuit used to measure an unknown resistance. R_x is the component being measured, and R_3 is a variable resistor (often called a <u>potentiometer</u> or just a <u>pot</u> for no sensible reason).
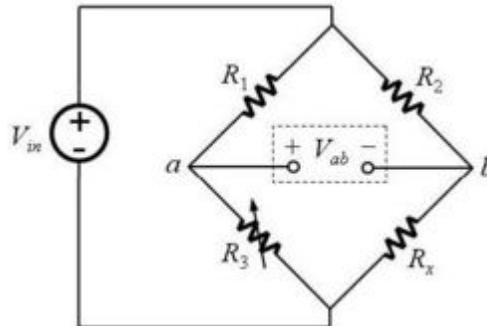
a) The bridge is balanced when V_{ab} is zero. Assuming R_3 is set so the bridge is balanced, derive an expression for R_x in terms of R_1, R_2 and R_3.

b) Now let R_3 also be a fixed resistor. Suppose that R_x varies in a way that makes V_{ab} nonzero. Derive an expression for the current that would flow if you connected an <u>ammeter</u> from a to b. Assume the ammeter has zero internal resistance.

2. **Measuring Physical Quantities with a Wheatstone Bridge** A thermistor is a resistor whose value varies with temperature. Thermistors are specified by a zero power resistance, R_0, at a given temperature and a temperature coefficient, α. As shown below, a small person inside the thermistor observes the temperature on a thermometer and adjusts a variable resistor so that $R=R_0+\alpha T$, where T is the temperature.
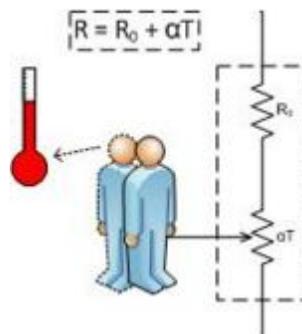
Mister Thermistor (with apologies to <u>Horowitz and Hill</u>)

Now imagine a Wheatstone bridge made out of four identical thermistors, as shown in below. One of the thermistors (R_4) is attached to an odd-looking blue apparatus that varies in temperature. The other three are maintained at a constant 20°C.
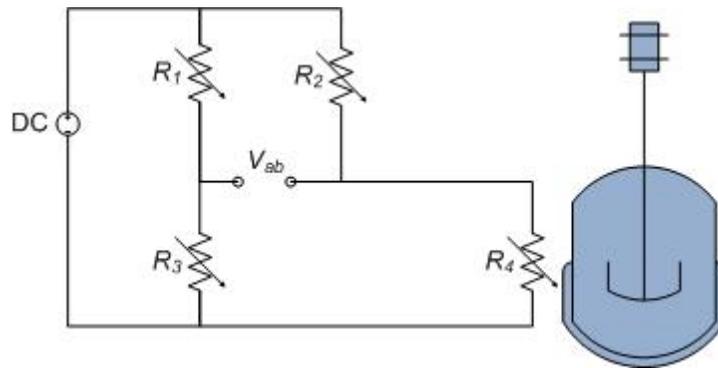
Wheatstone Bridge consisting of four thermistors

a) Derive an expression for V_{ab} as a function of temperature.

b) What if both R_1 and R_4 are attached to the apparatus? Which configuration is more sensitive to temperature variations?

3. **Photodiode I-V Characteristics** Using the data that you collected in the lab for the photodiode, generate three to four *i-v* curves for a photodiode at different light levels (including in darkness). Plot these on the same graph to see how incident light affects diode *i-v* characteristics. Give a brief (qualitative) explanation for why photodiodes are best used in reverse bias?

4. **Unknown transfer functions** For the black boxes that you measured in the lab, determine what kind of circuit/filter each one is (two of them will look similar, but have an important difference - what is it?). Determine a transfer function that can model the circuit, and fit the model to the data to see whether the model makes sense. Of the four boxes, "D" is required, and you should choose one of either "A" or "C". You can fit "B" for bonus credit.

5. **Power in a voltage divider** Referring to the circuit shown below, what value of R_L (in terms of R_1 and R_2) will result in the maximum power being dissipated in the load?

Hint: this is much easier to do if you first remove the load, and calculate the equivalent Thevenin output resistance R_T of the divider looking into the node labeled V_{out}. Then express R_L for maximal power transfer in terms of R_T.
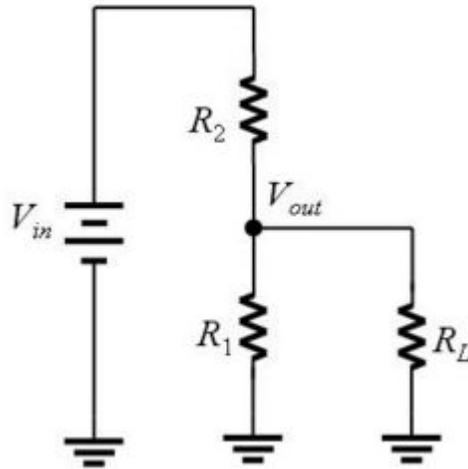
A voltage divider formed by R_1 and R_2 driving a resistive load R_L.

Problem Set #2   <u>Due</u>: Tuesday, September 23

1. **Simulation of DNA Melting Lab** Refer to instructions located at the class wiki:

http://scripts.mit.edu/~20.309/wiki/index.php?title=Matlab:Simulating_DNA_melting

2. **Transimpedence Amplifier** Lab Module 0 introduced this op-amp circuit:
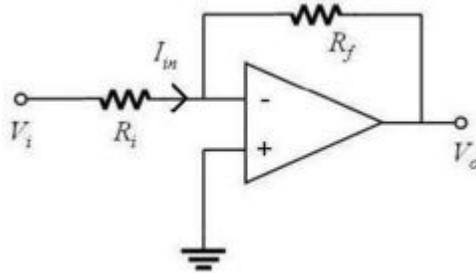
Inverting Voltage Amplifier

(a) Calculate the gain of this circuit, V_o/V_i in terms of the input voltage and the two resistor values.

(b) In the DNA melting lab, fluorescence intensity will be determined by measuring the output current of a photodiode. The schematic below shows a circuit called a transimpedance amplifier that converts a current to a voltage.
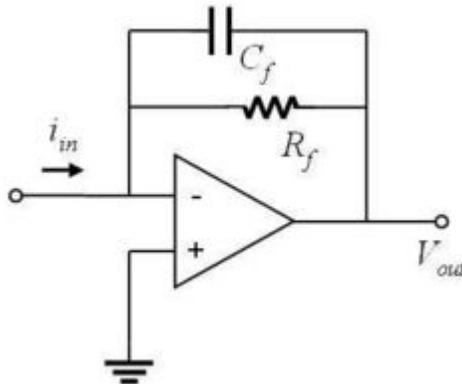
Transimpedence Amplifier

Derive an expression for the output voltage of the circuit produced by a DC current input at i_{in}. (At DC, you can ignore the affect of the capacitor.) Express your answer in the form of a transfer function, V_{out}/I_{in}.

(c) What is the high frequency gain of the transimpedence amplifier? Remember that a capacitor acts like an open circuit at low frequencies and a short circuit at high frequencies.

(d) A transimpedence amplifier with a gain of approximately 10^8 V/A will be required for the DNA lab. What value of resistor in the above circuit would achieve this gain?

(e) It is undesirable to use the large value of resistor you computed in part (d). Shown below is another possible implementation of the transimpedence amplifier. Derive an expression for the output voltage of this circuit in terms of the input current and the three resistor values.
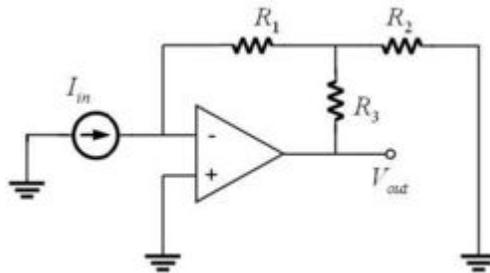
High gain transimpedance amplifier

(f) In part (c), you determined the effect of putting a capacitor across the feedback resistor in a transimpedence amplifier. High gain amplifiers are susceptible to noise coupling from a variety of sources. Since high frequencies are not of interest in the DNA melting lab, it is beneficial to insert a capacitor to reduce the noise. In the above circuit, where would you connect the capacitor and how would you choose its size?

(g) Now write down the expression for this new circuit's output with respect to the current input for AC signals.

Problem Set #3   Due: Tuesday, September 30

(adapted version of problems from Prof Seung, MIT)

1. **Fourier series for a sawtooth** Define the sawtooth function by $s(t) = t / \pi$ for $-\pi < t < \pi$

   a) In the Fourier series for the sawtooth,

   $$f(t) = a_o + \sum_{n=1}^{\infty}(a_n \cos \omega_n t + b_n \sin \omega_n t)$$

   What values does ω_n take on? Find the coefficients a_o, a_n, and b_n by computing the integrals

   $$a_o = \frac{1}{T}\int_{-T/2}^{T/2} dt.f(t)$$

   $$a_n = \frac{2}{T}\int_{-T/2}^{T/2} dt.f(t)\cos \omega_n t$$

   $$b_n = \frac{2}{T}\int_{-T/2}^{T/2} dt.f(t)\sin \omega_n t$$

   where $T = 2\pi$ is the length of the interval $(-\pi, \pi)$.

   b) Write `MATLAB` code to sum the first 50 terms of the Fourier series and graph the result in order to verify that you did the integrals correctly.

2. **The Fourier modes as an orthonormal basis** Define the inner product of two functions f and g on the interval $[-T/2, T/2]$ as

$$\langle f, g \rangle = \frac{2}{T}\int_{-T/2}^{T/2} dt.f(t)g(t)$$

Show that the Fourier modes $1/\sqrt{2}$, $\cos \omega_n t$, and $\sin \omega_n t$ for $\omega_n = 2\pi n / T$ constitute an orthonormal set of functions, where n ranges over all integers from $-\infty$ to ∞. In other words, verify that the inner product of each function with itself is unity, and that the inner product of each function with a different function vanishes.

This is why the Fourier modes can be regard as a set of perpendicular axes in the infinite-dimensional space of functions on the interval $[-T/2, T/2]$. The formulas for the Fourier coefficients are just projections onto the Fourier modes (projection is just the term for inner product).

3. **Chirp** Generate a five second long chirp at a frequency that decays exponentially from an initial value of 1000 Hz with a time constant of $\tau = 5$ seconds,

$$v(t) = 1000e^{-t/\tau}$$

The chirp itself is given by

$$x(t) = \cos(2\pi v(t)t)$$

Use a sampling frequency of 22,050 Hz. Listen to the chirp for fun. Type `specgram(x,8192,22050)` to display the spectrogram. If you generated the chirp right, you should see the exponential decay of the frequency starting from 1000 Hz.

4. **Power Spectral Density (PSD)** Here we will look at power spectral density in MATLAB using the command `pwelch`. To begin, enter `help pwelch` and become familiar with its parameters. Next, generate a signal composed of two pure tones with frequencies $v_1 = 1000$ Hz and $v_2 = 1100$ Hz:

$$f(t) = \cos(2\pi v_1 t) + \cos(2\pi v_2 t)$$

using a sampling frequency of fs $= 22050$ and an interval of 100 μs.

(a) Graph $f(t)$ versus time and then run the commands:

```
[Pff, freq] = pwelch(f,[],[],[],22050);
loglog(freq,Pff)
```
Why is there only a single peak?

(b) Play around with the parameters `nfft` and `window` until you find a way to make the peaks at 1000 Hz and 1100 Hz visible. Note that [] denotes the default value.

(c) Now display the spectrum of the square of the signal, f^2. Use the same parameters in the `pwelch` command so that all the peaks are visible. You should see that f^2 contains frequencies that were not present in the original signal. This a mark of a nonlinear system and is called harmonic and intermodulation distortion by audiophiles. They avoid nonlinearity like the plague.

(d) Find a formula that expresses f^2 as a sum of sinusoids. You can think about it as a Fourier series, or just use trig identities. Use your formula to explain the peaks in the PSD of f^2.

(e) In lecture, we used Parseval's Theorem to show that the mean square average, $\langle f(t)^2 \rangle$ is related to the PSD by

$$\left\langle f(t)^2 \right\rangle = \int_0^\infty S_f(\omega) d\omega$$

where $S_f(\omega)$ is the PSD of $f(t)$. Verify that this is the case for $f(t)$ given in this problem. MATLAB's `var` and `sum` functions will be useful here. Remember that you're effectively computing the area beneath the PSD plot so make sure the units match up (`pwelch` give us you the PSD in units^2/Hz).

Problem Set #4   <u>Due</u>: Tuesday, October 7

1. **Discrete convolution** Compute the discrete convolution of the two sequences g_n and h_n given by

```
g_i = [1 1 1 1] (i = 0..3), g_i = 0 (else)
h_i = [1 1 1 1] (i = 0..3), h_i = 0 (else)
```

a) Do this in `MATLAB` by shifting, multiplying and adding the appropriate vectors. You may also use the graphical procedure outlined at the end of this problem set.

b) Check your result using the `conv` function. Consult the online help (`help conv`) to find out how to use this function. Make a stem plot of your result (using `stem(…)`), and label the x-axis with the appropriate indices.

2. **Convolution theorem applied to NMR** This problem uses Nuclear Magnetic Resonance (NMR) signals as an example to explore some frequently used techniques in measurement and noise analysis.

In NMR one measures the precession of atomic nuclei around and externally applied magnetic field. In equilibrium, the nuclear spins orient themselves parallel and anti-parallel to the field lines, with a slight preference towards the parallel orientation. At the beginning of an experiment, an electromagnetic impulse flips the spins away from their equilibrium orientation, so that their precession induces a time-varying voltage in an adjacent receiver coil:
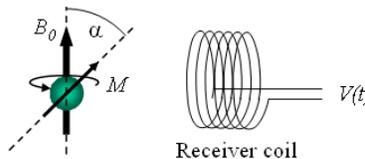
The resulting signal has the form

$$V(t) = V_0 \cos(\omega_p t) \cdot e^{-t/T^*} u(t) \text{ where } u(t) = \begin{cases} 1 & (t \geq 0) \\ 0 & (t < 0) \end{cases}$$

where $\omega_p = \gamma B_0$ is called the Larmor frequency (γ = 42 MHz / T for hydrogen). The exponential term accounts for the fact that the precessing spins rapidly loose their coherence, so that the net signal decays with a characteristic time constant $T^* \sim$ 100ms.

a) Sketch the magnitude of the Fourier transform of *V(t)* (only qualitatively).

   <u>Hint</u>: Use the convolution theorem and the Fourier transforms:
   $$\cos(\omega_p t) \rightarrow \pi \cdot (\delta(\omega + \omega_p) + \delta(\omega - \omega_p))$$
   $$e^{-t/T^*} u(t) \rightarrow (i\omega + 1/T^*)^{-1}$$

   You should be able to do this without calculations. Indicate the approximate width of the peaks.

b) The amplitude of the NMR signal is measured by lock-in detection. This means that the rapidly varying voltage from the receiver coil is multiplied by a frequency and phase matched reference signal $V_r(t)$ and then low-pass filtered:

Reference: $V_r(t)=\cos(\omega_r t)$

Low-pass filter (cutoff $\omega_{LP}<2\omega_p$)
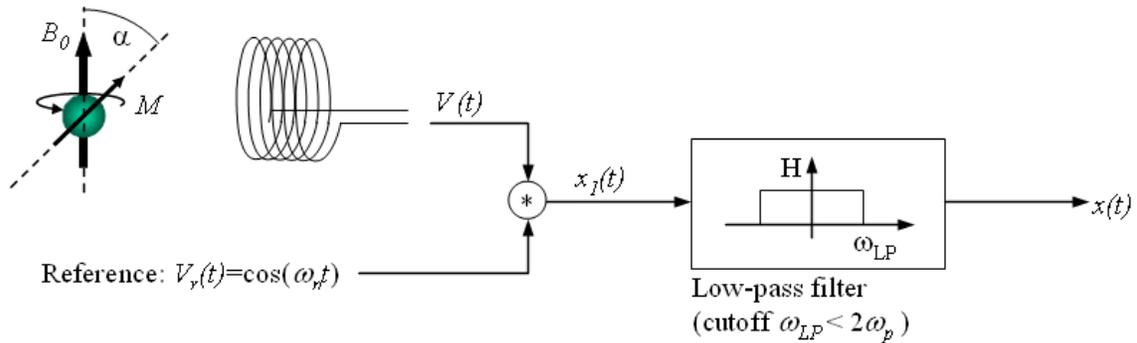
Sketch the magnitude of the Fourier transform of the intermediate signal x_1 and the output signal x. (Use graphical convolution or trigonometric identities. There should be three separate peaks in the spectrum). Copy the transfer function of the low-pass filter shown above to your sketch. What is the purpose of the low-pass filter?

c) Now we will consider the influence of various noise sources on the measurement. The electrical resistance R of the receiver coil generates white thermal noise S_R. The amplifier contributes some 1/f noise S_A:
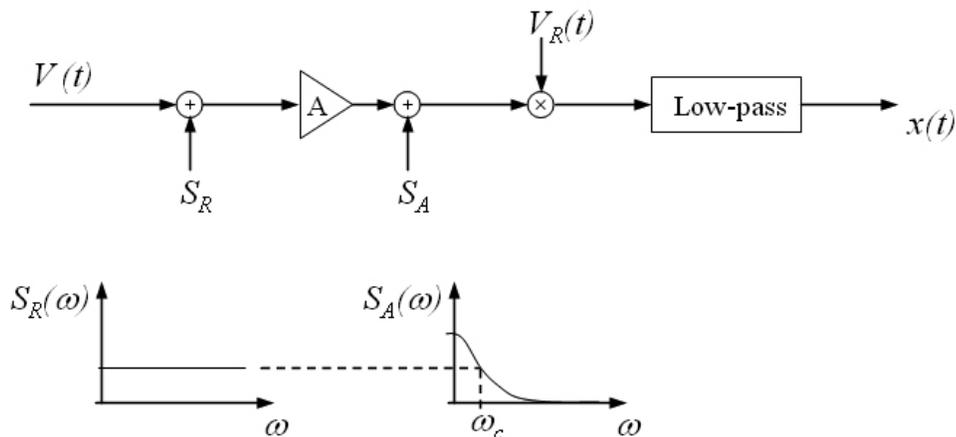
Sketch the power spectrum of the output x qualitatively. Assume a cutoff frequency $\omega_{LP} = \omega_p/10$ for the low-pass filter. Would the 1/f noise S_A be a concern if $\omega_C \sim 1$ kHz and $B_0 = 10$ T? Why?

Hint: Since the system is linear, you can first sketch the contributions of $V(t)$, S_R, and S_A to the power spectrum of x individually and then add them together.

Graphical approach for taking the convolution of two vectors

The discrete time convolution of the two sequences g_n and h_n can be found "by inspection" using the following method:

Draw a stem plot of the two sequences. The x-axis represents the index:
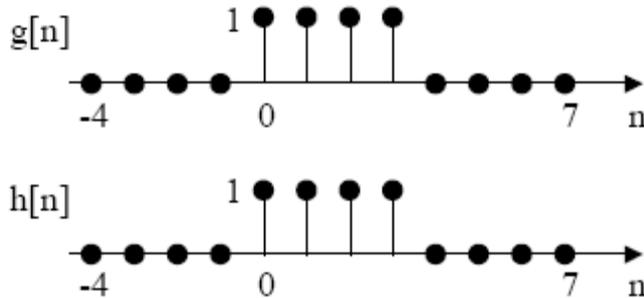
To find the kth point of the convolution $(g*h)_k$ (Always leave g_n fixed):

1. Mirror the sequence h_n about $n = 0$

2. Shift the mirrored h_n to the right by k points e.g. for
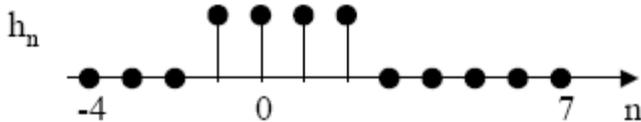
3. Multiply the original g_n with the modified h_n from step 2 point by point, and add the results. For k=2, this would give you $(g*h)_2 = 3$
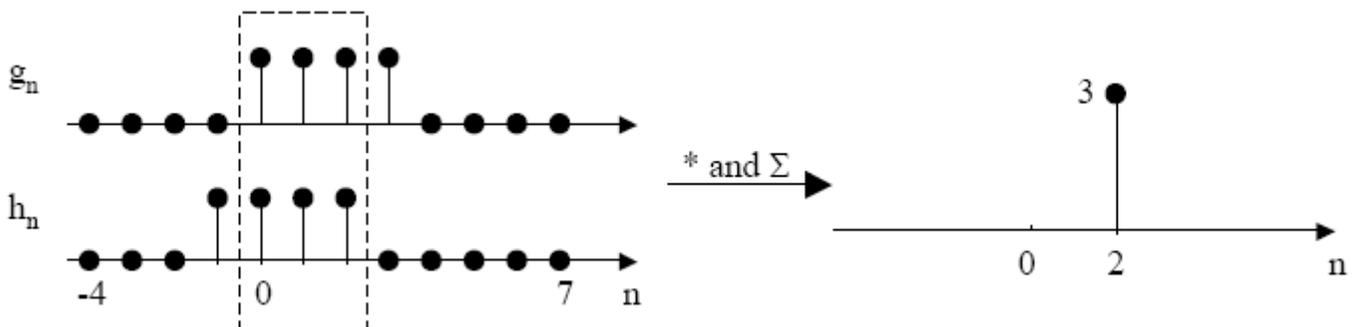
Problem Set #5   Due: Tuesday, October 14

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 $<x^2>$, the Equipartition Theorem,

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

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

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

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 1.38×10^{-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.

Problem
   (a) As discussed in the lecture, a harmonic oscillator model for a cantilever can be described by the differential equation

$$\ddot{x} + 2\gamma\dot{x} + \omega_0^2 x = 0.$$

   Solve for x(t) by assuming the solution is of the form

$$x(t) = Ae^{s_1 t} + Be^{s_2 t}$$

   where s_1 and s_2 are roots to the polynomial

$$s^2 + 2\gamma s + \omega_0^2 = 0$$

   The initial conditions are $x(0) = 0$ and $dx/dt(0) = v_o$.

(b) The initial conditions can be realized experimentally by accelerating the end of the cantilever with a very short impulse, $v_o \cdot \delta(t)$:

$$\ddot{h} + 2\gamma\dot{h} + \omega_0^2 h = v_o \cdot \delta(t)$$

where $h = h(t)$ is the impulse response. What is $h(t)$?

(c) Find the frequency response of the system, $\hat{h}(\omega)$, by taking the Fourier transform of the differential equation for $h(t)$.

For the remainder of this problem, assume that the cantilever has the following mechanical properties:

Resonant frequency, f_o: 10 kHz
Quality factor, Q: 20
Spring constant, k: 0.04 N/m

(d) Plot the magnitude of the frequency response $\hat{h}(\omega)$ on a log-log scale for $v_o = 0.01$ m/s. Make sure to label your axes with the correct units.

(e) Plot the impulse response, $h(t)$ for a time interval of 10 μs, sampled at a rate of 100 kHz. Again, label the axes with the appropriate units.

(f) Now assume that the cantilever has an effective mass of $m = 10^{-9}$ kg and is being driven by a Gaussian distributed random force $F(t)$ of zero mean and standard deviation $<F(t)^2>^{1/2}$. Use MATLAB to generate a 25,000 point random vector that represents the acceleration, $F(t) / m$:

```
m = 1e-9;
F = randn(25000,1)*1e-6;
g = F/m;
```

Plot $F(t)$ versus over a time interval of 10 μs, sampled at a rate of 100 kHz.

Verify that $F(t)$ is a white noise signal by plotting the square root of its power spectral density:

```
fs=1e5;
[PSD,freq] = pwelch(F,[],[],[],fs);
loglog(freq, sqrt(PSD));
xlabel ('Frequency [Hz]');
ylabel ('Power Spectral Density [N / sqrt(Hz)]');
```

(g) Find the cantilever response, $x(t)$, by convolving the random noise vector g with the impulse response h. You may use the conv command to calculate at the convolution integral. Since each discrete point represents a time interval of 1/fs, you will need to multiply the result of the conv command by 10^{-5} to get the correct scaling. Truncate x to the length of the original vector g. Plot the resulting vector x[t] and the square root of its power spectral density. Use a log-log scale for the PSD.

(h) Now assume the cantilever is thermally driven (ambient temperature is 300 K). Write down the expression for the PSD of $x(t)$ and plot it on a log-log scale with units Å/√Hz .

(i) Write down an approximate expression for the PSD of $x(t)$ by assuming your measurement bandwidth, B, is much less than the resonant frequency, f_o. Using an ideal position detector (no noise), $x(t)$ is measured for 1 second and then averaged. What is the smallest change in deflection that could measured over this interval? What is the minimum detectable force? Give some examples of biological forces that could be measured with this resultion.

(j) Perhaps the two most useful expressions for the mechanical of beam are the spring constant,
$$k = \frac{Ewt^3}{4L^3}$$
where E is the Young's modulus, t thickness, w width, and L length, and the resonant frequency,
$$f_o = \frac{t}{2\pi L^2} \sqrt{\frac{E}{\rho}}$$
where ρ is the mass density. Using these expressions, determine how the minimum detectable force depends on the microcantilever geometry. This expression is used for designing ultrasensitive force sensors.

(k) Carefully read Module 2: Atomic Force Microscope with the exception of sections on imaging and elastic modulus (Sections 3.3.2, 3.3.4, 4, and 5).

Lab Assignment #1   Due: Tuesday, October 24

Data Analysis

Use `Matlab` to convert your raw data to fraction hybridized and temperature. Filter the data to remove noise. This can involve smoothing the data from individual experimental runs as well as combining data from multiple runs. Plot relative fluorescence versus temperature comparing:

- 20 bp oligos in solutions of varying ionic strength
- Perfect match, single mismatch, and complete mismatch (unknown) 20 bp oligos
- 40 bp versus 20 bp perfect match oligos

In some cases, it may be helpful to include multiple melting curves on the same plot. Make a table of the melting temperature T_m for each of these experiments. To accomplish this, you may process the data how you wish, however a useful command in Matlab is `resample`. This function can not only resample data, as the name implies, but will also apply a *low*-pass filter (decreasing the *high*-frequency noise). A larger vector of filter coefficients or number of samples on each side of the current sample will smooth the data more. Using this command, pay attention to the resulting length of your new data, as well as any inaccuracies at the ends (consider what resample assumes for the data points before and after your data). Derivatives may require filtering as well.

Model vs. reality

In class, we derived an expression that relates the melting temperature to the enthalpy change $\Delta H°$ and entropy change $\Delta S°$ of the hybridization reaction:

$$T(f) = \frac{\Delta H°}{\Delta S° - R\ln(2f/C_T(1-f)^2)}.$$

Here, f is the fraction of DNA strands hybridized (dimerized) at a particular temperature (at T_m, this is 1/2), and C_T is the total concentration of single-strand oligonucleotides (or 2X the dsDNA concentration when all strands are hybridized). Choose one of the perfect-match sequences that you measured, and use `Matlab` to fit the model to your measured data, which will allow you to extract the $\Delta H°$ and $\Delta S°$ parameters. To perform the fit, you will need a `Matlab` function that will evaluate $T(f)$ given an input `const` for the $\Delta H°$ and $\Delta S°$ parameters. The function will be something like this:

```
function Tf = melt(const, f)
R=8.3;
C_T=33e-6;
dH = const(1);
dS = const(2);
Tf = dH./(dS - R*log(2*f./(C_T*(1-f).^2)));
```

You can then invoke matlab's `lsqcurvefit` routine to do the fit, which will return the best values for $\Delta H°$ and $\Delta S°$.

```
FitVals = lsqcurvefit(@melt, [dH_guess, dS_guess], frac_vector, temp_vector)
```

Short questions *(please do not use more than two pages for this section)*
- What practical problems did you run into?
- Briefly describe (in recipe format) how you processed the raw data. Include the expressions that you use to make this conversion.
- Do the shapes of your melting curves make sense? (It may be helpful to compare your results to others in the class)
- Can you identify the unknown samples?
- What factors affect the DNA melting temperature and the sharpness of the melting transition?

Bonus (optional):
Calculate $\Delta H°$ and $\Delta S°$ for one of the perfet match sequences using the nearest-neighbor model from class. Compare the calculated values to the best fit parameters. What might explain the differences? What factors affect $\Delta H°$ and $\Delta S°$?

External references *(see wiki for links)*

- [National Instruments data acquisition card user manual](#)
- [SYBR Green I datasheet](#)
- [LM317 datasheet](#)

Lab Assignment #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 $k_b T$.

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 $<x^2>$, the Equipartition Theorem,

$$\frac{1}{2} k_b T = \frac{1}{2} k \langle x^2 \rangle$$

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

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

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 1.38×10^{-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. As described in Section 6.1, estimate the spring constant k and the low frequency limit of the thermomechanical noise, δ, by using the known value of 1.38 x 10^{-23} J/K for k_b. Sketch the magnitude of δ on your PSD from Part 3 for each cantilever. Does your measurement agree with the low frequency limit δ? 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 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.

6. For each cantilever, estimate the spring constant (as described in item 3 of Section 6.1) and use your measured low frequency limit of the PSD (above the 1/f corner and below the resonant frequency) with the expression of δ to estimate k_b.

Module 0: Introduction to Electronics

Contents

1   Objectives and Learning Goals

1. Familiarize yourself with standard lab electronics and some common circuit elements: linear and nonlinear, passive and active.

2. Understand fundamental electronics concepts, including:

   - current/voltage dividers
   - impedance & load
   - frequency response & transfer functions

3. Analyze and build several circuits, which will be of use later.

    This module is an extended lab orientation of sorts. Take this time to explore electronic circuits and really get to know all the features of our lab's instrumentation. It will pay off later. What you learn and do this week will underpin many of the experiments during the rest of the semester.

    Since you are mainly concerned with understanding your toolset and learning its uses and limitations, no formal write-up is necessary. However, many parts of this module work hand-in-hand with questions on Problem Set 1, so you will still want to record data for completing them.

2   Roadmap and Milestones

1. Practice making DC and AC signal measurements.

2. Measure circuit input and output impedances and observe loading effects.

3. Study the nonlinear characteristics of a diode, and learn how a photodiode responds to incident light.

4. Test several unknown circuits to determine their transfer functions

3   Lab Procedures

3.1   DC measurements

The simplest type of circuit is the direct current (DC) situation. This means simply that the applied voltage does not vary in time. Alternating current (AC) is discussed in Sec. 3.4. Most often, we separate complex signals into their DC and AC components (e.g. a sine wave with a constant offset) to make analysis simpler.

To get started, wire the circuit shown in Figure 1 on your breadboard, where R_1=330Ω and R_2=150Ω. Using the power supply, set V_{in} to 5V. We'll make a few measurements of the behavior of R_2 in the circuit. We want to know the voltage across it and the current through it.
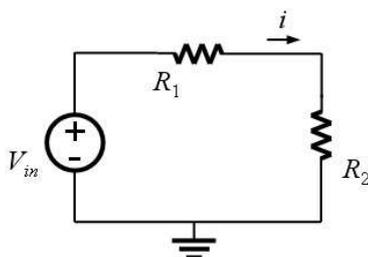
Figure 1: Resistive voltage divider circuit.

Measuring Voltage with the DMM:

1. First switch the DMM to voltage mode. *Note: Make sure that the DMM test leads are plugged into the right connections. Remember, the correct configurations for current and for voltage/resistance measurements are different. See Sec. B.1*

2. Place the two leads across the terminals of R_2 so that it is in parallel as shown in Figure 2.

3. In voltage mode, the DMM has a very large equivalent resistance (ideally infinite) so that when placed in parallel with the circuit you are measuring, it will have minimum effect on the circuit under test.

    To prove this:

    (a) Assume first that the effective resistance of the DMM is small, such as 100Ω. What is the combined resistance of the parallel combination of R_2 and the DMM?

    (b) Now assume the DMM's resistance is something very large, like 10MΩ. Now what is the resistance of the parallel combination of R_2 and the DMM?

2
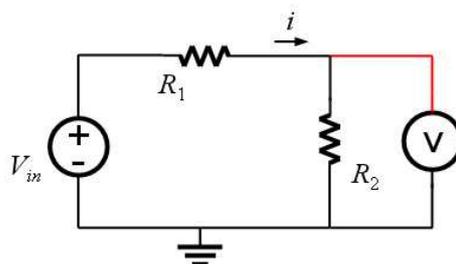
Figure 2: Measuring voltage across R_2.

Why would a DMM in voltage mode with low input resistance be poor for voltage measurements? Hint: think about how it affects the voltage divider circuit in this case.

Measuring current with the DMM:

1. Switch the DMM to current mode.

2. Place the leads of the DMM in series with a device in the path that you want to measure, shown in Figure 3. For this type of measurement you actually need to break the circuit and insert the DMM.
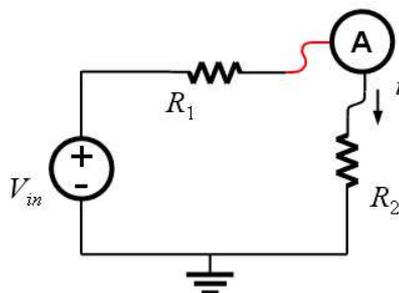
Figure 3: Measuring current through R_2.

3. What would you expect to happen if you reverse the leads of the DMM? Reverse the leads and see if you were correct.

4. The input resistance of the DMM in current mode is very small, ideally zero. Why is it important for the effective resistance of the DMM to be small in current mode? Again think about the effect on the circuit under test.

Calculate the resistance of R_2 using Ohm's law and the current and voltage you measured. Also determine the error in the nominal resistance value:

$$error = 100 \times \frac{R_{exp} - R_{meas}}{R_{exp}} \ .$$

Is this within the tolerance value indicated by the color bands on the resistor?

Measuring resistance with the DMM:

1. Turn off power to the circuit, and disconnect the resistor you want to measure. This is important both in order to protect the DMM and because other parts of the circuit will affect the resistance you measure for one particular branch.

2. Switch the DMM to resistance mode.

3. Place the leads in parallel with the resistor of interest (in this case R_2), as you did for the voltage measurement in Figure 2. Does this match your calculated resistance?

Resistor *i-v* characteristics

The current-voltage (*i-v*) curve of a circuit element is simply a plot of the current through it as a function of applied voltage. In your lab notebook, sketch the *i-v* curve of the resistor you measured. What is the slope of this curve? (Ohm's law should make this very easy).

3.2   Impedance and load

From the previous section you already have a sense of the importance of considering the equivalent impedances of your instruments – when making voltage or current measurements (or connecting any two circuits together) we must always keep in mind the relative *output and input impedance* of these components.

An easily observable example: Suppose you have a 5V power source, and need to drive an LED with approximately 2 volts. A voltage divider may seem straightforward to use for this purpose, but one must be careful when designing the circuit. To see why, wire up the circuit in Fig. 4, first using relatively small resistors (50-500Ω range), then do it using resistors that are 100× larger.

- Why does the brightness of the LED change so drastically?

- Measure the voltage at the + node of the LED, before hooking it up, and after. Also, measure the current through the LED in each case. Does this help you understand what's going on?

- Calculate the output impedance of the driving voltage dividers in the two varieties you built.
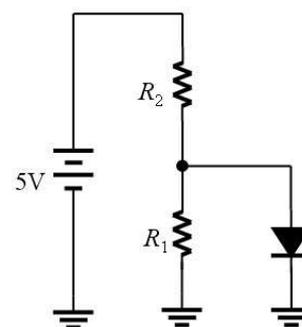
Figure 4: A voltage divider driving an LED.

A brief discussion:

Impedance is a generalization of resistance (including capacitance and inductance), and we use the terms somewhat interchangeably here, but you should know that they are not strictly speaking the same.

A ***load*** is a general way of referring to any part of a circuit that has a signal delivered to it, such as a measurement device, or a particular component. What is considered the "load" depends entirely on how the parts of the circuit are being considered. In the case of the circuit in Fig. 4 the LED is the load for the voltage divider.

The output impedance of a circuit or device is seen "looking into" the output *port* of a circuit (i.e. between the output signal node and ground). Likewise, the input impedance of a device/circuit is the impedance seen between the input node and ground. The agent doing the "seeing" is whatever connects to the circuit in question – e.g. if an oscilloscope is hooked up to a circuit to do a

measurement, that circuit "sees" the input impedance of the oscilloscope. Here, the voltage divider being used to drive the LED "sees" the LED's input impedance, and the LED "sees" the output impedance of the driving circuit.

3.3 Photodiode *i-v* characteristics

In this section, our aim is to measure and plot the current-voltage relationship for a diode in the transition region from non-conducting to conducting. After that, we also want to measure the behavior of photodiodes (see Sec. A.3, which we'll use a number of times in the course as light detectors.

Start by covering the window of an FDS100 photodiode with black tape — with no light coming in, it is just a regular diode. Then we'll illuminate it to see its photodiode action.

(A) Construct the circuit shown in Fig. 5. You have at your disposal your DC power supply, and a variable resistance R (we recommend you use values of 100kΩ, 27kΩ, 8.2kΩ, 4.7kΩ, 2.2kΩ, and 820Ω — this is more straightforward than using a pot and measuring its value every time you turn the knob).
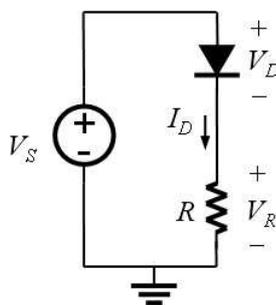
Figure 5: Circuit for diode *i-v* measurements.

(B) Given this circuit, come up with a scheme to measure the diode's *i-v* curve. Think about these questions to help guide you:

- Is current or voltage easier to measure?
- For a given setting of V_S and R in Figure 5, how can you calculate the current and voltage through the diode by making a single measurement?
- What should you do differently for the forward and reverse bias regions of the curve? From what you know about diodes, how does their impedance in forward bias compare to that in reverse bias?

You'll want to generate a set of I_D and V_D values in your notebook to be used for creating the *i-v* plot. Then put a plot together using the program of your choice (MS Excel is fine).

(C) For photodiode behavior, uncover the window of the device, and aim a Fiber-Lite illuminator at it. You should repeat the measurements you made at two or three levels of light intensity.

You can now combine your data to produce four *i-v* curves for this diode at different light levels. Plot these on the same graph to see how incident light affects diode *i-v* characteristics. You'll need this data for your first Homework Set.

3.4   Time-varying signals and AC measurements

Generally, we refer to signals that vary with time as AC signals (alternating current, as opposed to DC - direct current). When we leave DC behind, the DMM we've used so far is no longer enough to observe what is happening. At this point, you'll need to get acquainted with the function generator (Sec. B.3) and the oscilloscope (Sec. B.4), to generate and record AC signals, respectively. We'll also start making extensive use of BNC cables and connectors.

First, let's look at how the resistive voltage divider with which you're already familiar behaves with AC signals. Build the divider circuit as you did in Sec. 3.1, but use the function generator in place of V_{in}, and the oscilloscope in place of the DMM (Figure 6).
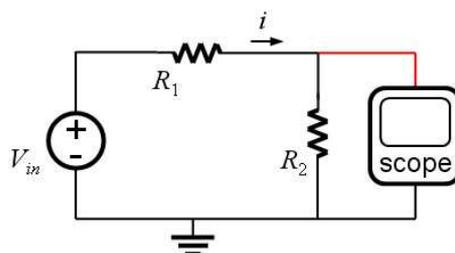
Figure 6: The familiar divider circuit, with a voltage measurement across R_2.

1. Set the FG503 frequency to 5kHz, and the waveform to sinusoid with no offset.

2. Set the voltage to 3V peak-to-peak (often written as $3V_{pp}$). Verify that the voltage is set as you intend with the scope, since the FG503 has no markings on its knob.

3. Connect the waveform to your circuit.

4. Use the other channel of the scope to measure V_{pp} across R_2. Also measure the frequency across R_2 on the scope. You can display both the input and output waveforms at the same time by using the scope's DUAL mode. Does this resistive voltage divider behave any differently at AC than it did at DC? What's the relationship between the output and input waveforms?

Now replace R_2 with a capacitor in the 0.05-0.1 μF range. Again use dual mode on the scope to see both the input and output waveforms. Qualitatively observe what happens to the output as you change the frequency of the input. What kind of circuit is this?

3.5   "Black-box" transfer functions

For this part, you'll find prepared for you several metal boxes with "mystery" circuits wired up inside, labelled "A" through "D." Your goal is to determine their transfer functions. To streamline the process, we've provided a program that will output a frequency-sweep function, which you can feed into the circuit. The program will then record the amplitude of the output, and plot the data for you. Ask your lab instructor how to use it.

Appendices

A   Circuit Components

A.1   Resistors

The important things to know about resistors are: (1) value, (2) tolerance, and (3) power rating. The power rating indicates the maximum amount of power a resistor can withstand, e.g. 1/4 watt, 1/2 W, etc. The value and the tolerance of the resistor is printed on the package in the form of a color-band code (see below).

Figure 7:   Two ways potentiometers are shown on schematics.

   Potentiometers (or "pots") are variable resistors with three leads. The top half of Figure 7 shows a full schematic, but the lower picture is usually used for compactness. The arm at lead #2 is called the wiper, and turning the potentiometer knob controls its position. While the resistance between #1 and #3 always remains the same, the knob varies the resistance between leads #1 and #2 (and between #2 and #3). Another way to think of this is as a variable voltage divider. The value between either #1 and #2 or #2 and #3 can be varied from zero to the pot's full value.

Reading the Resistor Color Code

We provide this table here for your convenience, but you can always easily look this information up on the web. For instance, `http://www.elexp.com/tips/clr_code.gif` contains a good table of 4-band and 5-band resistor color codes, while this URL does it in an interactive clickable Java applet: `http://samengstrom.com/elec/resistor/index.html`.

1. Orient the resistor so that the band that is most separated from the rest is on the right (typically this is gold or silver).

2. On a four-band resistor, form the number from the first and second band by placing them as the tens and ones place respectively (e.g. from the left a blue band then a green band means 65).

Color	First or Second Band (digit)	Third Band (multiplier)	Fourth Band (tolerance)
Black	0	1	
Brown	1	10^1	1%
Red	2	10^2	2%
Orange	3	10^3	
Yellow	4	10^4	
Green	5	10^5	0.5%
Blue	6	10^6	0.25%
Violet	7	10^7	0.10%
Gray	8	10^8	0.05%
White	9	10^9	
Gold		0.1	5%
Silver		0.01	10%

Table 1: Table of 4-band resistor colors. For a five-band resistor, the first band becomes the 100s digit, the second band is the tens the third the ones, the fourth is the multiplier, and the fifth the tolerance.

3. Multiply the resulting number by the multiplier from the third band (e.g. blue-green-red = $65 \times 100 = 6.5\text{k}\Omega$).

4. The most common types of resistors are 5% and 1%, so for quick designation the bodies of these resistors are often color coded (brown – 5% and blue – 1%).

A.2   Capacitors

Capacitors immediately make for much more interesting types of circuits than simple resistive networks, because (1) they can store energy and (2) their behavior is time-dependent.

An intuitive way to think about capacitor behavior is that they are reservoirs for electrical charge, which take time to fill up or empty out. The size of the reservoir (the capacitance C) is one of the factors that determines how quickly or slowly. Because of this, circuits with capacitors in them have time-dependent and frequency-dependent behavior. Capacitors act like open circuits at DC or very low frequencies, and like short circuits at very high frequencies.

A.3   Diodes

This is one of the simplest non-linear electronic devices, and is remarkably versatile. It can function as an electronic "valve", as a light-emitter (LED) or a light-detector (photodiode). Fig. 8 shows how they appear on schematics.

A diode as an electrical "valve." In the simplest model, we can imagine a diode as a one-way electrical valve - it behaves almost as a short circuit (wire) when a positive voltage is applied across it (called *forward bias* – shown in Fig. 8) and as an open circuit with a negative voltage (*reverse bias*). As you might guess, this is not the whole story, and is only true for relatively large voltages. You will explore diode behavior in more detail, especially around the critical transition region near 0 volts.

Figure 8: The diode symbol on a schematic.

Photodiodes are optimized to work as a light detector by capturing photons and converting them to electrical signals. This happens when photons absorbed in the semiconductor generate electron-hole pairs. Run in reverse bias, the current out of the photodiode is linearly proportional to the light power striking the device.

Light-emitting diodes (LEDs) are designed to output light when current passes through them. In this case, we have recombination of electron-hole pairs producing photons in the semiconductor. Light is emitted in forward bias, and power output depends on the current flowing through the device (what's the relationship? linear? quadratic?).

A.4   Breadboards

Breadboards are a platform that allows for easily building prototype circuits without the need for soldering. Their advantage is that parts can be added, swapped in and out, and different configurations tested very easily and quickly. However, breadboards are bulky, pick up significant excess noise from the environment, have large parasitic capacitance, as well as other limitations. Once a circuit design is finalized, it's typically made in a printed circuit board (PCB).

Figure 9 is an example of a breadboard, where each square represents a hole in which a wire lead can be placed. The lines drawn over the board represent the basic connectivity. The two outermost lines on each side represent power "buses" that are connected across all rows. In the very center of the board is a divider that separates columns A-E and F-J. Each row is connected: i.e. 1A-E are all connected to each other, as are 1F-J. However, A-E are electrically isolated from

8
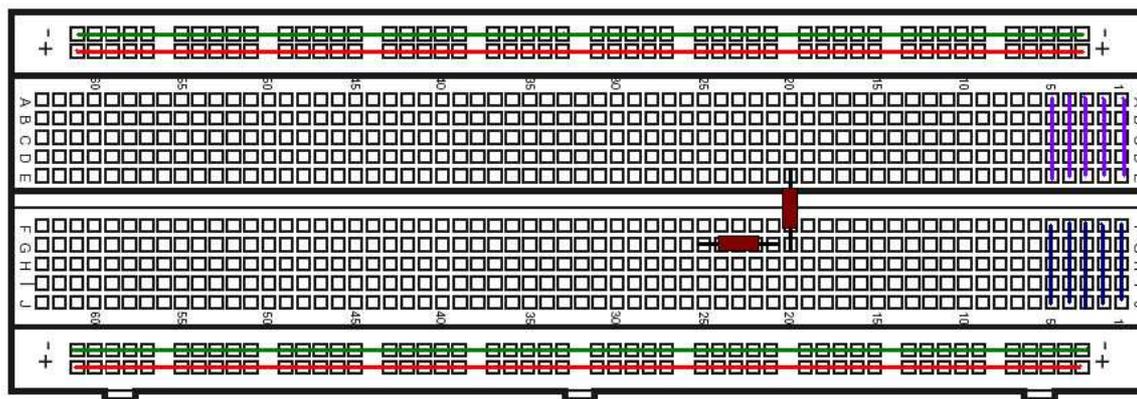
Figure 9: A typical breadboard layout. Some breadboards have several panels such as these adjacent to each other, with banana cable jacks for power and ground connections.

F-J. Finally, rows that are not in the power rail are also electrically isolated (these connections are explicitly shown only in the first five rows).

Of course, if connectivity is unclear, you can use the multimeter to test for electrical continuity between two points on the board. Multimeter leads often don't fit in the holes directly, so you can use a wire as a connector between the meter and the board.

An example of how to place a component in the breadboard is shown in the figure. A resistor is depicted as a red box with two metal leads. There are many ways to place this resistor, and the figure shows two of these ways.

Breadboard Tips and Techniques

1. Choose the right length of wire and clip leads to keep components and wires close to the board. This has two benefits: (1) It makes debugging a circuit easier if you can easily see all the connections and (2) It prevents pick-up of additional noise from the environment, since big loops of conducting material make for good antennas.

2. Utilize the power rails, e.g. use one each for the positive supply voltage and negative supply voltage (referred to as $+V_{cc}$ and $-V_{cc}$), and one for ground.

3. Create a common ground. If you use a power supply for DC power, a function generator for an AC supply, and measure using the oscilloscope, then you will have four independent grounds that may not be at the same potential unless connected together (the four grounds are: circuit ground, FG ground, PS ground, and scope ground).

4. Always turn off power when building and making any changes to the circuit. Also, when measuring resistance, power off the circuit and disconnect the resistor being measured.

5. As a rule of thumb, always connect the ground lead of an instrument to the circuit first before the live lead.

6. In future labs, we will work with Integrated Circuit (IC) packages. Static electricity can destroy ICs. To prevent damage, ground yourself before handling them by touching a metal object, e.g. a metal case or metal bench top.

9

A.5   Operational Amplifiers

In the upcoming lab module we will start using integrated circuits (ICs) known as operational amplifiers, or op-amps. They are an enormously versatile circuit component, and come in hundreds of special varieties, built to have particular characteristics and trade-offs. We will use some very common general-purpose op-amp, of which a typical example is the LM741.

Every op-amp manufacturer provides a *datasheet* for every IC they make, and you should always familiarize yourself with it. It provides information on everything from pin and signal connections, to special features, limitations, or applications of a particular IC. We have copies of the datasheets available in the lab for the op-amps we are using, and you'll want to refer to them regularly as you work.

As you'll see in lecture, a typical op-amp circuit looks something like Fig. 10. This is called the *inverting configuration*, because the input is connected to the inverting (−) input. As you might guess, the output signal is the negative of the input, times a gain factor set by the circuit.

Figure 10: A basic inverting op-amp circuit.

The LM741 package of course does not look like the triangle drawn above. Instead it looks more like Figure 11. Plugged into a breadboard, the two rows of pins straddle a trough.
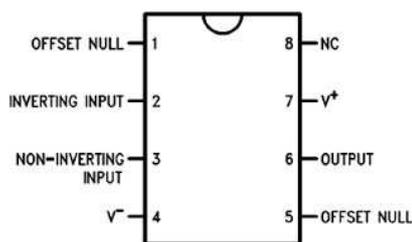
Figure 11: The pin assignments of the LM741 in a DIP-8 package.

Besides the (−) and (+) (*inverting and non-inverting*) inputs, an op-amp needs DC power connections, which is what enables it to be an active circuit element. These power connections are usually omitted on a schematic (as in Fig. 10), but always shown on the datasheet (in Fig. 11 they are pins 4 and 7). Typically ±15 volts is used, but you should check the datasheet to be sure.

Every IC has a marking on the package to indicate Pin 1, and the datasheet shows the relative positions of the other pins. On your LM741 there is a dot near Pin 1 (or a semi-circle on one end of the chip, as in the figure to the right). NC on the datasheet stands for No Connection.

Important: ICs are sensitive to static electricity discharges. Your body can easily store enough charge to damage an IC, especially on a dry winter day. To prevent this, always make sure to touch the grounded metal case of an instrument to dissipate the charge. Use caution when handling the chips.

B   Instruments

The brief descriptions in this section will give you an introduction to each instrument. You can always refer to the manuals available in the lab for more details.

B.1   Digital Multimeter (DMM)

A very versatile tool, the multimeter serves as a voltmeter, ammeter, ohmmeter, and has a number of other functions as well (see Figure 12(b)). Modern DMMs, such as our Fluke 111, are highly intuitive to use: select the function you want with the central mode dial, plug the leads into the appropriate connectors, and measure. The black (negative) lead always plugs into COM while the red (positive) lead is adjusted depending on the function. The voltage and current measurement modes of the DMM are very different (you'll see why), so don't forget to reconnect the leads.

In alternating current (AC) mode, the multimeter gives root-mean-square (RMS) measurements, which are useful when you know what the waveforms are. We'll discuss RMS later in the course.

B.2   DC Power Supply

The power supply we will use is a Tektronix PS503, shown in Figure 12(a). It has one fixed 5V output and two adjustable ones. The (+) and (−) outputs have adjustable CURRENT LIMITS and voltages up to ±20V can be set either independently, or together (using the DUAL TRACKING knob). The white OUTPUT button on the upper right allows power to flow to the outputs: **always remember to turn this off or disconnect it when rewiring your circuits**.

Note that the black COMMON voltage connector is "floating," i.e. not directly connected to ground, and can't be assumed to have zero voltage. You'll want to connect it to the white connector of the fixed 5V supply, which is tied to ground.
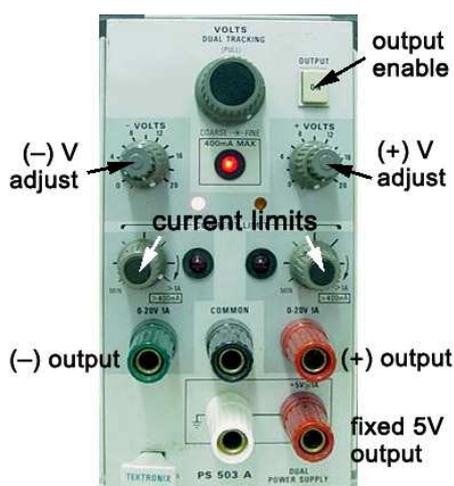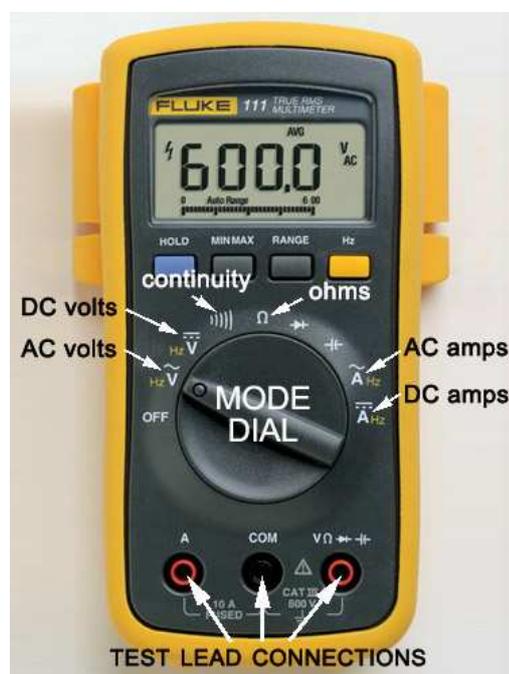
(a) Tektronix PS503A.   (b) Fluke 111.

Figure 12: (a) The DC power supply and (b) the digital multimeter used in our lab

B.3   Function Generator

A function generator does what its name says — generates signal waveforms for standard functions: sinusoids, triangles, square waves. The FG503 is very basic. Its frequency range is about 0.1Hz to 300kHz, and amplitude range from about ±0.35V to ±10.0V. It can output waveforms with and without offset. The large central dial in Figure 13 is the main frequency knob, which sets the output frequency together with the multiplier knob (labeled). Be warned that the large dial's markings can be fairly inaccurate – always verify the actual output frequency with an oscilloscope or spectrum analyzer.

Note that higher-end function generators, such as the DS-345 from Stanford Research Systems, have much more precise controls for waveform frequency, amplitude, and offset, and may have other advanced features like sweeping frequency, generating nonstandard or arbitrary waveforms, and modulation.
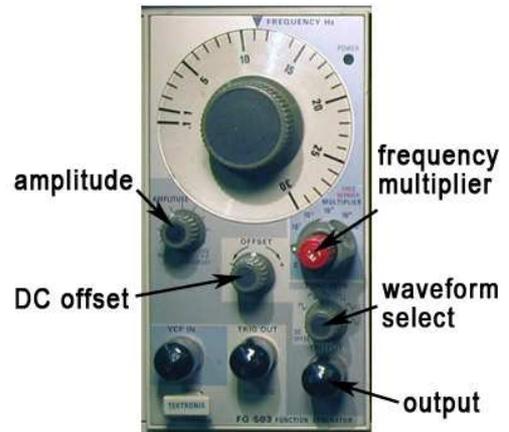
Figure 13: An FG503 function generator. (In this image, the BNC signal connectors are capped.)

B.4   Oscilloscope

An oscilloscope is designed for observing signal waveforms that change faster than can be usefully seen on a DMM. Most often, the signals observed are periodic, and the scope is effectively a "time magnifier" letting you stretch and compress the timebase (as well as the waveform magnitude) for convenient viewing.

Ours is an OS-5020 model made by EZ Digital, the front panel of which is shown in Figure 14. It's about as basic as a two-channel analog scope can be. Below is a brief description of the most important controls:
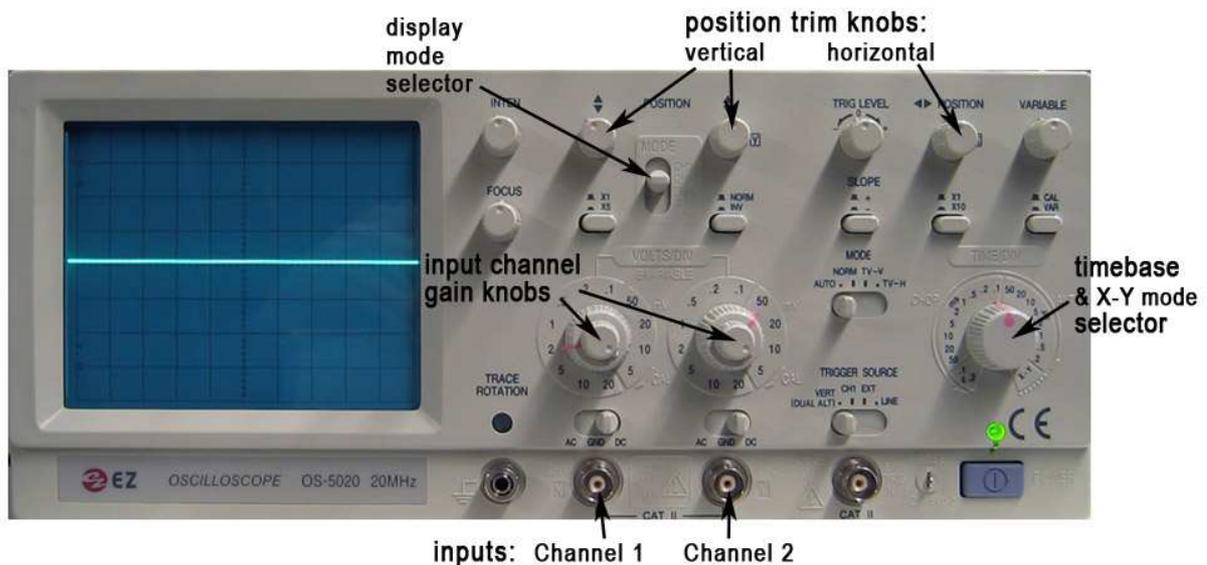
Figure 14: The OS-5020 oscilloscope.

CH1, CH2: channel inputs (2) - Signals connect to these via BNC cables. Above each input is a three-position *input coupling* switch (AC - GND - DC). Understanding the three settings is crucial to knowing how the scope is measuring incoming signals.

VOLTS/DIV: channel gain knobs (2) - Set the "magnification" of the waveform in the vertical axis. The scale around the knob tells you how many volts each square of the grid represents at a given magnification.

MODE select - Choose whether the scope is displaying the signal on Ch. 1, Ch. 2, both simultaneously (DUAL), or their sum (ADD).

POSITION knobs (2 vert., 1 horiz.) - Set the zero-position of the trace for each channel, and the time-trace to enable accurate measurements.

TIME/DIV: timebase selector - Like channel gain, but for the horizontal (time) axis, this sets how much time each square of the grid represents. In its rightmost position, it selects "X-Y mode", which plots the two input channels one vs. the other, with no time dependence.

Most of the other controls deal with triggering, which refers to synchronizing the scan of the display with the input waveform. You will get a feel for these as you use the instrument in lab.

You'll notice that the scope *only* measures voltages – there are no modes for directly measuring current or resistance. It's also important to remember that *scope measurements are always referenced to ground*. The shield (black lead when using grabber wires) of the BNC connector is hard-wired to ground. This means you can't use just one channel of a scope to measure the voltage between two non-ground nodes in a circuit.

B.5   LabVIEW and the data acquisition (DAQ) system

LabVIEW is a software and hardware system designed to perform the functions of many standard bench-top measurement devices. The hardware provides signal input and output for the PC (see Fig. 16, while the software runs what are called "virtual instruments" (VIs), resulting in a very general-purpose data collection and processing platform. In addition to collecting data, LabVIEW can be used to control instruments, for example via a GPIB interface.

You will use a number of different VIs throughout the course that have been written for you. While they will all perform different functions, they all have common run/stop controls in the upper left hand corner, as shown in Fig. 15. The arrow at the left is used to start each VI, and the red "stop" button will halt it once it is running.

Figure 15:

The great advantage of LabVIEW is its breadth and flexibility. The major disadvantage is that when performance is important (high speed, low noise, high precision, etc.) dedicated instruments quickly outperform it.

Figure 16: The data acquisition (DAQ) connection board, used for signal input and output with LabVIEW. The analog input channels are in two rows, labeled ACH0 through ACH7, and ACH8 through ACH15. The two output channels are to their right, labeled DAC0OUT and DAC1OUT, one above the other.

20.309:Measuring DNA Melting Curves

From OpenWetWare

Contents

DNA Melting Apparatus

Introduction

Example DNA melting curves showing the effect of varying ionic strength. The data has been filtered to reduce noise.
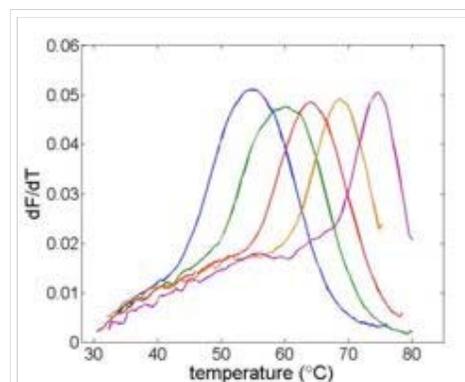
Differentiating the melting curve simplifie finding T_m.

In this lab, you will measure the melting temperature of several DNA samples to determine the effect of sequence length, ionic strength and complementarity. A common application of this technique exploits the length dependence o DNA melting temperatures to examine PCR products in order to determine whether a desired sequence was successfully amplified.

The measurement technique utilizes a fluorescent dye that binds preferentially to double stranded DNA (dsDNA). Thi characteristic of the dye allows the relative concentration of dsDNA to be determined by measuring the intensity of fluorescent light given off by an excited sample.

The DNA melting apparatus you will construct consists of four major subsystems: excitation, fluorescence measurement, temperature sensing, and data acquisition. You will build these subsystems out of an LED, a photodiod a resistance temperature detector (RTD), and a PC data acquisition system.

The goal of your time in the lab will be to measure fluorescence intensity versus temperature for each of the samples over a range of about 90°C to room temperature. This will provide a basis for estimating the melting temperature, T_m of each sample. (T_m is defined as the temperature where half of the DNA in the sample remains hybridized.)

Three of the samples will be unknown. All the unknowns will have the same length, but different degrees of complementarity: complete match, single mismatch, and complete mismatch. Using the data you gather, you will attempt to identify these three samples.

Overview of the apparatus

In most DNA melting apparatuses, the temperature of the sample is ramped up at a controlled rate and the concentration of dsDNA recorded. In our homebrew setup, however, we will first heat up the sample in a bath. That way, natural cooling will provide the range of temperature conditions needed. As the sample cools, a PC data acquisition card will record the photodiode and RTD outputs over time. During data analysis, you will convert these voltages to temperature and relative dsDNA concentration. The melting temperature, T_m can be estimated from a graph of this data or its derivative.

SYBR Green I (http://www.sigmaaldrich.com/sigma-aldrich/datasheet/s9430dat.pdf) is most sensitive to blue light with a wavelength of 498 nm. The dye emits green light with a wavelength of 522 nm. You can easily observe this – a room-temperature sample of dsDNA and SYBR green looks yellow from the combination of blue excitation and green fluorescence. At higher temperatures, when there is no dsDNA to bind to, the sample will appear blue or clear.

As shown in the diagram, an aluminum heating block holds a cuvette containing the sample under test. The sample is a combination of DNA and a fluorescent dye called SYBR Green. In addition to being a convenient holder, the block gives the setup enough thermal inertia to facilitate a measurement from natural cooling. (Without the block, the sample would cool too quickly.)

Blue light from an LED illuminates one side of the cuvette. An optical filter shapes the output spectrum of the LED so that only the desired wavelengths of light fall on the sample.

A photodiode placed at 90 degrees to the LED source detects the green light emitted by bound SYBR Green. The photodiode is placed behind an optical filter to ensure that only the fluorescent light given off by the sample is detected.

The DNA melting apparatus includes excitation, fluorescence measurement, temperature sensing, and data acquisition functions.

Since photodiodes produce only a very small amount of current, it will be necessary to build a very high gain transimpedence amplifier to produce a signal that is measurable by the PC data acquisition cards. Photodiode amplifiers are particularly challenging because many of the non-ideal characteristics of op amps become apparent at high gain.

An RTD attached to the heating block and wired to a voltage divider provides an indication of temperature. The temperature of the heating block will be a proxy for the sample temperature. Unfortunately, the block cools faster when it is hot than when it is near room temperature. You will have to get the heating block set up in your apparatus quickly after you remove it from the heating block.

A PC data acquisition card digitizes the amplified photodiode and RTD signals. A LabVIEW virtual instrument (VI) records the signals over time. Data from the DNA melting VI can be saved to a file. The file can be loaded into Matlab for analysis.

Objectives and learning goals
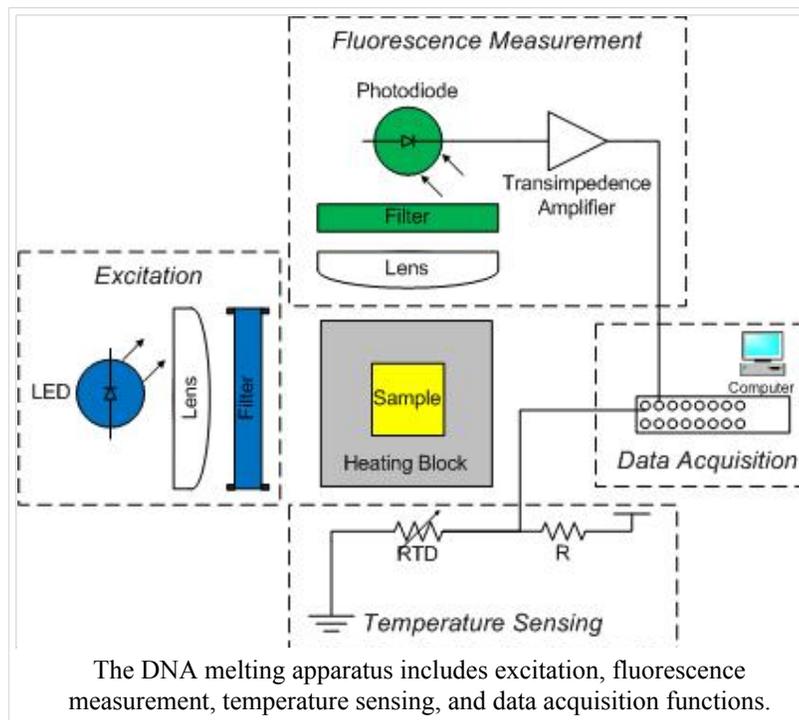
- Measure temperature with an RTD.
- Implement a high gain transimpedence amplifier for photodiode current multiplication.
- Measure light intensity with a photodiode.
- Build an optical system for exciting the sample with blue light and gathering the fluorescence output on the photodiode.
- Record dsDNA concentration versus temperature curves for several samples.
- Estimate T_m from your data.
- Compare the measured curves with theoretical models.
- Identify unknown DNA samples.

Lab procedure

Roadmap

1. Build an optical system containing the LED, heating block, sample, photodiode, filters, and lenses.
2. Hook up a three terminal voltage regulator to create an electrical power supply for the LED.
3. Build, test, and calibrate the temperature-sensing circuit.
4. Build an amplification/offset circuit for the DNA fluorescence signal.
5. Troubleshoot and optimize your system.
6. Heat a samples of DNA with SYBR Green dye and record DNA melting curves as the samples cool.
7. Analyze the data. Identify the three unknown samples. Compare your observations to theoretical models.

Optical system

DNA Melting Optical System Diagram
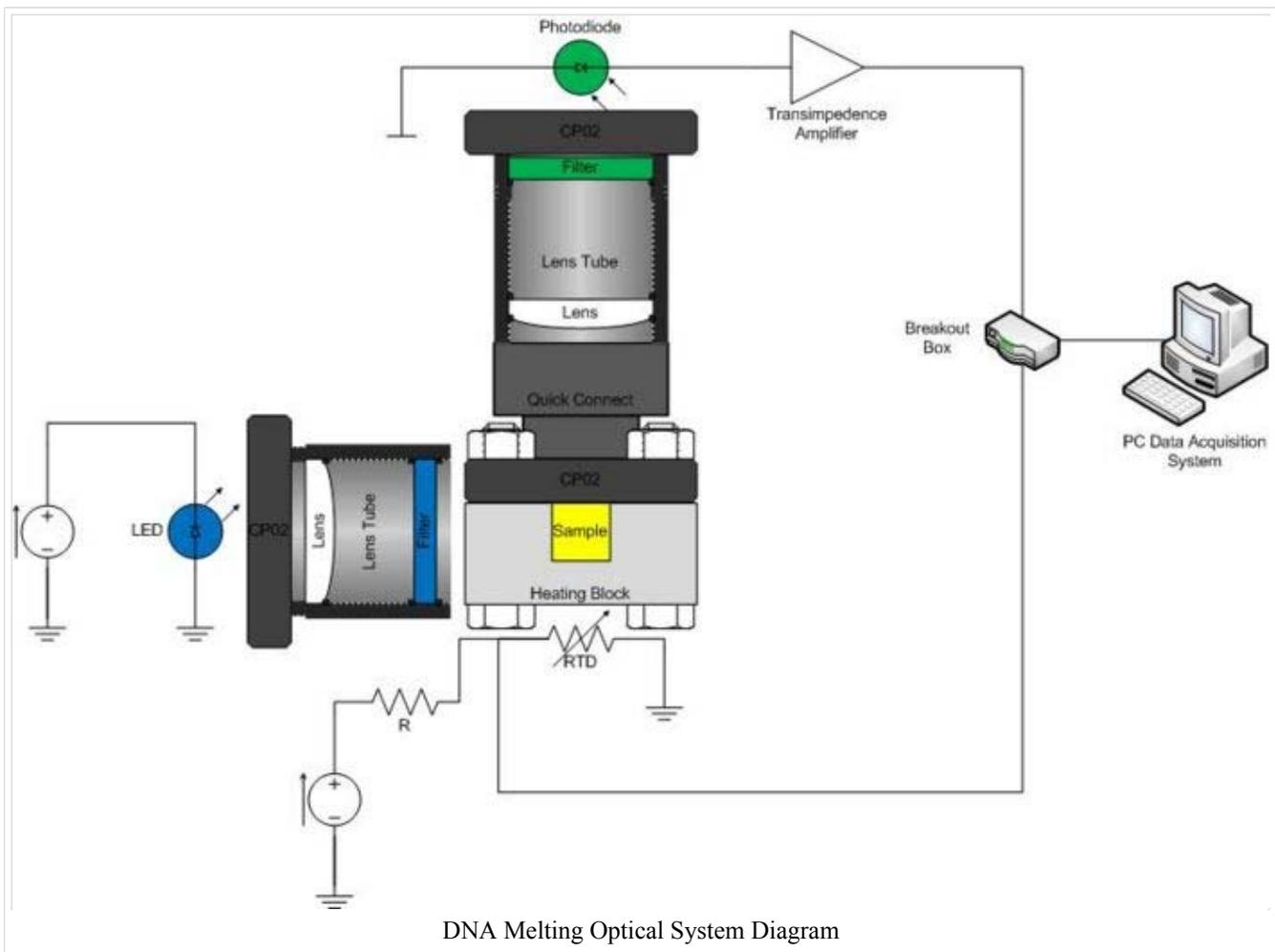
The optical system consists of an LED, excitation filter, sample cuvette, heating block, emission filter, photodiode, optional lenses, and associated mounting hardware. Construct your system on an optical breadboard (http://www.thorlabs.com/thorProduct.cfm?partNumber=MB1224) . The breadboard has a grid of tapped holes for mounting all kinds of optical and mechanical hardware. ThorLabs (http://www.thorlabs.com) manufactures most of the hardware stocked in the lab. A few of the components you will certainly use include: 1/2" diameter posts (http://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=1266) , CP02 cage plates (http://www.thorlabs.com/thorProduct.cfm?partNumber=CP02) , and 1" diameter lens tubes (http://www.thorlabs.com/NewGroupPage9.cfm?ObjectGroup_ID=1521) .

Use optical rails (http://www.thorlabs.com/thorProduct.cfm?partNumber=RLA0300) and rail carriers (http://www.thorlabs.com/thorProduct.cfm?partNumber=RC1) or optical bases (http://www.thorlabs.com/NewGroupPage9.cfm?ObjectGroup_ID=47) to mount 1/2" posts on the breadboard. RA90 (http://www.thorlabs.com/thorProduct.cfm?partNumber=RA90) right angle post clamps and post holders (http://www.thorlabs.com/thorProduct.cfm?partNumber=PH2-ST) can also be useful.

There are a variety of ways to construct the apparatus. A good design will be compact, stable, and simple. It will be necessary to shield the optical system from ambient light, so a small footprint will be advantageous.

Illumination

Begin by mounting the LED on your breadboard. Note that there are two styles of LEDs. The Lamina LED Array (http://www.laminaceramics.com/docs/BL_2_Blue.pdf)
is mounted on an aluminum heat sink and bolted to a CP02 cage plate. The CP02 attaches to the top of a post. It has an SM1 threaded hole through the middle that connects to 1" diameter lens tubes. The Cree LEDs

(http://www.allelectronics.com/spec/LED-112.pdf) are already mounted in a 1" lens tube.

Both styles of LED emit a range of wavelengths with a peak at 475 nm. A Chroma Technology D470 (http://web.mit.edu/~20.309/Students/Datasheets/Chroma%20D470-40.pdf) filter eliminates unwanted parts of the spectrum that might interfere with detection of the fluorescence signal. The filters have exposed, delicate coatings and must be handled carefully (http://www.chroma.com/index.php?option=com_content&task=view&id=56&Itemid=65) In addition, the filter works better in one direction than the other (http://www.chroma.com/index.php?option=com_content&task=view&id=57&Itemid=66) .

Light from both kinds of LEDs diverges in a cone with an angle of about 100 degrees, so place the device close to the sample. You can also use a lens to concentrate the LED's output. Several lenses are available in the lab:

- f=25.4mm (http://www.thorlabs.com/thorProduct.cfm?partNumber=LA1951)
- f=50mm (http://www.thorlabs.com/thorProduct.cfm?partNumber=LA1131)
- f=100mm (http://www.thorlabs.com/thorProduct.cfm?partNumber=LA1509)
- f=200mm (http://www.thorlabs.com/thorProduct.cfm?partNumber=LA1708)

Fluorescence detection

The SM05PD1A (http://www.thorlabs.com/Thorcat/8700/8770-D02.pdf) photodiode is mounted in a short tube with SM05 threads. Use a SM1A6 (http://www.thorlabs.com/thorProduct.cfm?partNumber=SM1A6) adapter to mount the photodiode in a CP02 cage plate. Mount the photodiode assembly to the breadboard at 90 degrees to the LED. Build a system to hold the Chroma E515LPV2 (http://web.mit.edu/~20.309/Students/Datasheets/Chroma%20E515lpv2.pdf) emission filter in front of the photodiode. You can use a lens to focus light from the sample on to the detector to improve performance, if you like.

Put an optical quick connect at the end of the photodiode assembly to facilitate easy attachment of the heating block during experimental runs. The other half of the quick connect goes into the CP02 cage plate mounted on the heating block.

Electrical System

LED driver

Or: how to make a current source

Drive the LED array with an LM317T (http://www.national.com/ds/LM/LM117.pdf) variable voltage regulator as

Current feedback to the *adjust* pin of the LM317T variable voltage regulator provides a steady source of illumination. (Note that 4.2Ω should read 4.3Ω)

shown. The LM317T has a feedback circuit that strives to maintain 1.25 volts between its output and adjustment pins. Thus, in the circuit shown, the LM317T sources a constant current of approximately .29A through the load (and the feedback resistor).

It is possible to drive an LED with a voltage source; however, the steepness of a diode's I-V curve results in large current swings for small changes in supply voltage. LED brightness is proportional to current. A current source will provide a more stable light output.

The LM317T and 4.3Ω resistor both dissipate quite a bit of power in this connection. They will become toasty. Use a heat sink on the LM317T. Double check your wiring before connecting the LED array. The array can be damaged by excessive current. Remember the rule of finger: if you can't keep your finger on a component indefinitely, it is too hot. Use a larger feedback resistor to keep the electronics cooler (at the expense of light output), but never a smaller one.

Temperature

The electrical resistance of most materials varies with temperature. An RTD is a special resistor (usually made out of platinum) that exhibits a nearly linear change in its value with temperature. An RTD may be used to accurately measure temperature by including it as an element in a voltage divider. As the resistance of the RTD changes, so will the voltage across it.

A PPG102A1 RTD (http://www.ussensor.com/prod_rtds_thin_film.htm) has been pre-mounted to the DNA heating block. This RTD has a nominal resistance of 1 KΩ and its value increses with temperature. Note that the maximum current carrying capacity of this device is 1 ma. Hook up the RTD in a voltage divider. Make sure the divider has no more than 1 mA flowing through it. Use freeze spray or heat the block on the warmer to test the circuit.

Fluorescence intensity

Amplification circuit

The photodiode produces only a tiny current – on the order of nano

Schematic diagram of a high gain transimpedence amplifier.
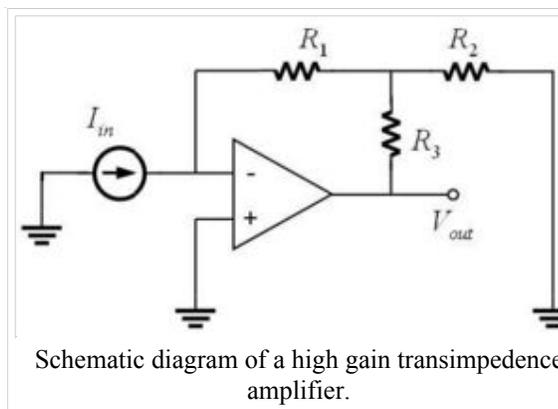
a voltage measurable by the PC data acquisition system. A transimpedance amplifier (sometimes called a current-to-voltage converter) with a gain of approximately 10^8 V/A will be required. The circuit considered in Homework 1 is capable of providing this gain. (Optional question: why not simply use a resistor, and omit the op-amp?)

Photodiode amplifiers can be fiddly under the best of circumstances. At such high gain, many of the non-ideal behaviors of op amps become apparent. It will be important to keep your wiring short and neat. The amplifier and witing will also be susceptible to physical movement, so prevent things from getting bumped during experimental runs. In addition select an op amp that has a very low input bias current as possible. (Why?) Op amps with JFET inputs like the LF411 (http://www.ortodoxism.ro/datasheets/nationalsemiconductor/DS005655.PDF) and LF351 (http://www.ortodoxism.ro/datasheets/nationalsemiconductor/DS005648.PDF) generally have the lowest input current

Offset circuit

The positive and negative input channels of an op amp cannot be perfectly matched during manufacturing. Because the open loop gain of an op amp is huge — usually 10^5 or more — even a slight mismatch will cause a non-ieal behavior called input offset voltage. (In other words, if you apply a zero voltage to the across the plus and minus pins by shorting them together, the output will probably saturate at the full positive or negative output limit.) V_{os} is the voltage that must be applied across the inputs to achieve a zero output. Most op amp datasheets specify a maximum value for V_{os}. In terms of the ideal circuit elements, input offset acts like a small voltage source connected i series with one of the input pins. As a real world example, the maximum specified offset voltage of the LF411 is 2.0 mV.

In the lab, you will find it useful to be able to adjust the quiescent output level of the photodiode amplifier. Many op amps provide a means for externally balancing the mismatch between plus and minus inputs. Pins 1 and 5 of both the LF351 and the LF411 are connected to the current sources that drive the differential input stage. As suggested by the name, these balance pins allow slight changes in the balance of current flowing through each side of the input stage. A potentiometer with both ends hooked across these pins and the wiper hooked to the negative supply voltage allows V_o to be virtually eliminated with a single adjustment. See the *Typical Connection* schematic diagram on page 1 of the LF411 (http://www.ortodoxism.ro/datasheets/nationalsemiconductor/DS005655.PDF) datasheet.

Although the primary intent of the balance pins is to null out V_{os}, they will also work quite nicely as an output level adjustment. Use a 10 turn pot so that you can get the output to settle where you want it. Adjust the dark output of the amplifier to be approximately zero.

Unfortunately, input offset voltage varies with temperature. (The LF411, for example, specifies a maximum temperature coefficient of 20μV/°C.) This sensitivity is one of the chief causes of output drift in the high gain amplifier, which you will undoubtedly notice in the lab. Try spraying a little freeze spray on the op amp to observe the effect. (Don't freeze your op amp right before you do an experimental run — it takes quite a while to stabilize.)

Practical matters

> As with most amplifiers, care should be taken with lead dress, component placement and supply decoupling in order to ensure stability.
>
> —LF411 Datasheet (http://www.ortodoxism.ro/datasheets/nationalsemiconductor/DS005655.PDF)
>
> In theory, there is no difference between theory and practice. But, in practice, there is.
>
> —Jan L. A. van de Snepscheut (http://en.wikiquote.org/wiki/Jan_L._A._van_de_Snepscheut) /Yogi Berra (http://en.wikiquote.org/wiki/Yogi_Berra)

The universe is rife with electrical noise. Keeping the noise out of sensitive electronic instruments requires a great dea of care. Unfortunately, electronic breadboards are a poor environment in which to construct high gain amplifiers. A fe simple tricks can improve things.

- Strap the ground of your breadboard to the optical table by connecting it with a short wire to a screw in the table
- Use power supply bypass capacitors. Connect a large capacitor between all supply voltges and ground. Large, electrolytic capacitors of at least 0.1 μFd work well for this purpose. Electrolytic capacitors are polarized. Make sure to put them in the right direction.
- What happens when the shield of a BNC cable touches the optical table? If you notice an effect, take precaution to prevent this from happening during an experimental run.
- Move your hands around dfferent parts of the circuit. What effects do you see?

PC Data Acquisition System

Each lab PC is equipped with a PCI-MIO-16E-1 (http://www.ni.com/pdf/manuals/370503k.pdf) data acquisition (DAQ) card. (National Instruments (http://www.ni.com) renamed the PCI-MIO-16E-1 to PCI6070E.)

The PCI-MIO-16E-1 is a PCI card that has a single, 12-bit analog to digital converter with a maximum sample rate of 1.25 MHz. A multiplexer selects from among 16 single-ended or 8 differentail input signals. In addition, the card includes an instrumentation amplifier with a programmable gain of 0.5 to 100. The card also supports two 12 bit analog output channels, 8 digital input and output lines, and two 24-bit counter/timers with a maximum clock rate of 20 MHz.

A 10 meter cable runs from the DAQ card to a BNC-2090 (http://www.ni.com/pdf/manuals/321183a.pdf) signal breakout box. The BNC-2090 provides BNC type connectors for each of the DAQ board's analog inputs and outputs.

LabVIEW VI

The DNA Melting LabVIEW VI is located in the `Students/Labs/DNA Melting` folder of the course locker. Double click to launch the VI. (The current version is R1.0)

Click the run arrow or select Operate->Run from the menu to start the VI. The top two charts show the digitized voltage at the RTD and diode inputs over time. Use the range settings to get a good view of the signal.

Press `Start Recording`
to begin taking data. The sample rate for recorded data can be set in increments of 0.1 seconds. Press `Stop Recording` at the end of an experimental run and use the `Write Data`
button to save the most recent result in a comma delimited file that can be read into Matlab or Excel.

Debugging the apparatus

1. Use freeze spray and the heat gun to make sure the temperature circuit is working properly.
2. Cover and uncover the photodiode to verify operation of the fluorescence measurement system.
3. Use a box and a piece of black cloth to shield your apparatus from ambient light. Can you measure the difference between a cuvette filed with water and one with DNA and SYBR Green?
4. Observe every electrical signal node with the oscilloscope. Are any signals noisy? Is there a way to improve the quality of poor signals?
5. Watch the fluorescence readout over time. Is it stable or does it drift?

Experimental procedure

Once your instrument is running to your satisfaction, measure melting curves each of the 5 conditions:

- 40bp perfect match
- 3 unknown 20 bp sequences (perfect match, single mismatch, and complete mismatch)
- 20 bp perfect match at different ionic strength

If you have time, you can run additional experiments. For example, you could gather additional ionic strength data points.

The DNA melting apparatus will generate the best data when both the amplifier circuit and LED have been on for a while and all the components have reached their steady state temperature. Make sure the outups of the system are stable before you begin taking data. Turn your apparatus on and measure the difference between a cool DNA sample and water. Run the DNA melting LabVIEW VI in the DNAMelting directory of the course locker. Adjust the range

controls for each channel to provide the greatest measurement resolution.

The steps for each experimental run are:

1. Heat up the sample on the hot plate
2. Quickly transfer the sample to your setup
3. Cover the apparatus to block out ambient light
4. Start recording RTD and photodiode output with the LabVIEW VI.
5. Wait for the block to cool to below 40°C

Prepare your apparatus

Use the potentiometer to adjust the amplifier voltage offset until it reads close to 0 Volts in the dark. Make sure your apparatus has reached the steady state and the fluorescence readout is stable.

Make a sample

SYBR Green I in DMSO is readily absorbed through skin. Synthetic oligonucleotides may be harmful by inhalation, ingestion, or skin absorption. Wear gloves when handling samples. Wear safety goggles if there is a danger of liquid splashing into your eyes. Do not create aerosols. The health effects of SYBR Green I have not been thoroughly investigated. See the SYBR Green I and synthetic oligonucleotide MSDS in the couse locker for more information.

Pipet 500µl of DNA plus dye solution into a disposable plastic cuvette. Pipet 20µl of mineral oil on top of the sample help prevent evaporation. Put a top on the cuvette and mark it with a permanent marker. Keep the sample vertical to make sure the oil stays on top. You should be able to use the same sample for many heating/cooling cycles. Only discard it if you lose significant volume due to evaporation. If you need to leave the sample overnight, store it in the la refrigerator. If you finish with a sample and it is still in good shape, pass it on to another group.

Heat up the sample

Place your heating block and sample in the hot water bath. You can use a DVM to monitor the temperature of the holder. It takes longer than you think to reach equilibrium. The block will cool down a bit while you transfer it to you setup, so heat it to a temperature well above where the DNA melts (at least 85°C, preferably 90°C). The double boiler arrangement will not allow the sample to boil.

Transfer the sample to your apparatus and take data

Use tongs to remove the heating block from the bath. Remember to keep everything upright. Set the block down on a paper towel. Use leather gloves to pick up the sample and connect it optically and electrically to your apparatus. Once everything is hooked up, press the *Start Recording* button on the LabView DNA Melting VI.

Discard pipette tips with DNA sample residue in the *Biohazard Sharps* container. Do not pour synthetic oligonucleotides or SYBR Green down the drain. Empty the liquid into the waste container provided. Dispose of plastic cuvettes in the *Biohazard* container.

Module 2: Atomic Force Microscope

Contents

1 Objectives and Learning Goals

- Learn the function of the 20.309 AFM and the relationships between its components.

- Understand how to extract quantitative information from this tool.

- Use the AFM to take images, probe sample stiffness, and estimate the value of a fundamental physical constant.

- Analyze the sources of uncertainty and noise in the system that limit the accuracy of measurements.

2 Roadmap and Milestones

The AFM will be the basis of three weeks' worth of experiments, and the roadmap below is subdivided into weeks to help you gauge your progress.

Week 1:

1. Learn the signal paths and connections of the system.

2. Practice aligning the AFM optics.

3. Learn how to calibrate the AFM to extract useful physical data.

4. Measure the vibrational noise floor in the AFM system.

5. Set up and prepare the AFM for imaging.

Week 2:

1. Image several different samples with the AFM and measure the physical dimensions of imaged features (Experiment 1).

2. Use the AFM to measure the elastic modulus and surface adhesion force of several different samples (Experiment 2).

Week 3:

1. Use your knowledge of the AFM system and associated instrumentation to record the vibrational noise spectrum of a cantilever probe (Experiment 3).

2. Calculate the value of Boltzmann's Constant k_B from the vibrational spectrum.

3 The 20.309 AFM System

This section describes the various components of the AFM you will use in the lab, and particularly how they differ in operation from a commercial AFM. In lecture we will discuss the operational principles of a commercial AFM. You may also find it helpful to review some of the References in Section 8 at the end of this module.
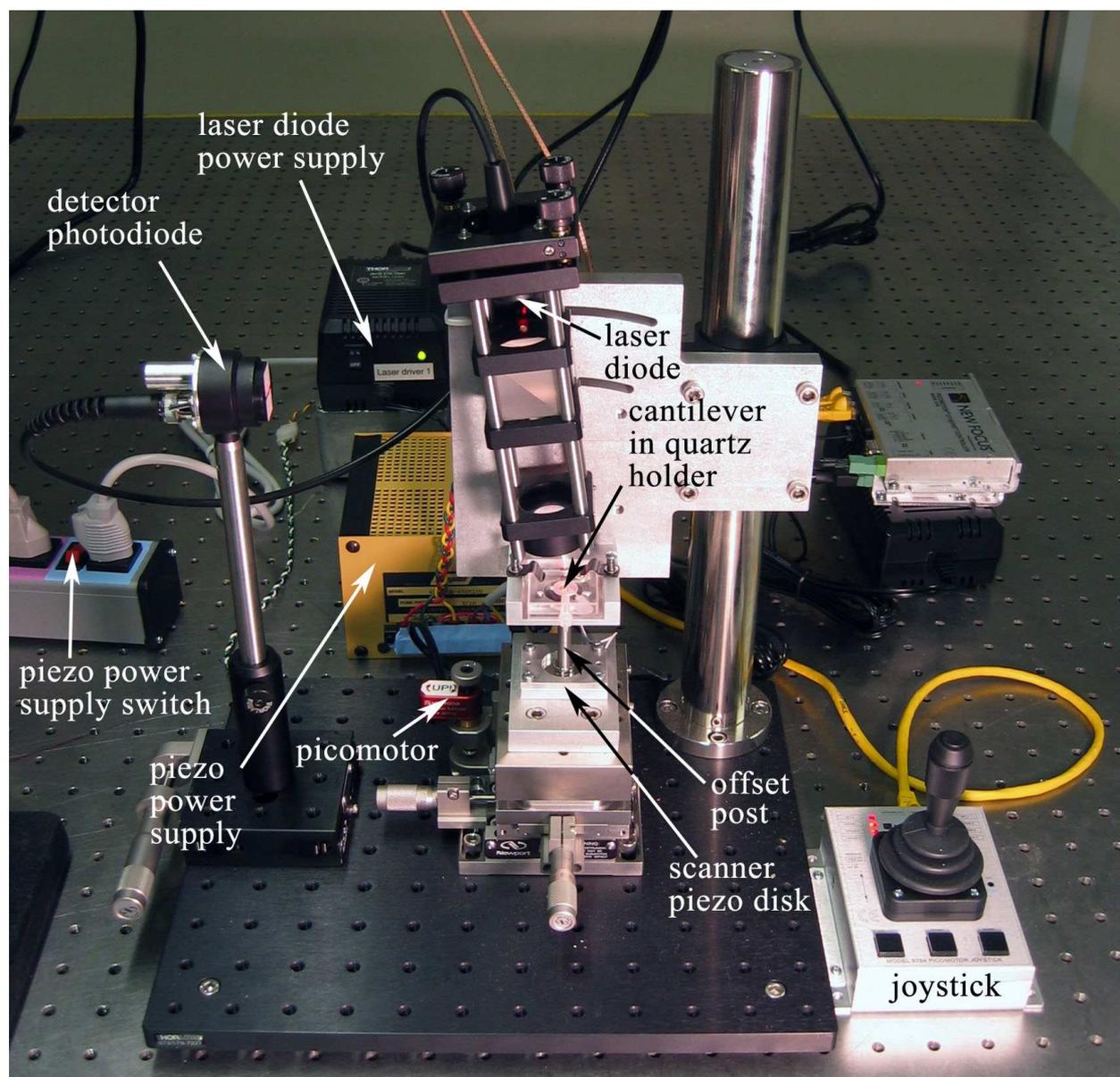
Figure 1: The AFM setup, with major components indicated.

3.1 Hardware

A photo of our AFM setup is provided in Figure 1 for you to refer to as you learn about the instrument. Start by physically examining the setup and identifying all the parts described below.

3.1.1 Motion control system

To be useful for imaging, an AFM needs to scan its probe over the sample surface. Our microscopes are designed with a fixed probe and a movable sample (also true of some, but not all, commercial AFM systems). Whenever we talk about moving the tip relative to the sample, in 20.309 we will always only move the sample. The sample is actuated for scanning and force spectroscopy measurements by a simple piezo disk, shown in Figure 2. The piezo disk is controlled from the
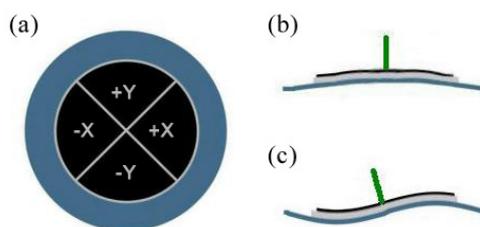
Figure 2: Schematic of the piezo disk used to actuate the AFM's sample stage. The circular electrode is divided into quadrants as shown in (a) to enable 3-axis actuation. When the same voltage is applied to all quadrants, the disk flexes as shown in (b), giving z-axis motion. Differential voltages applied to opposite quadrants, produce the flexing shown in (c), which moves the stage along the x- and y-axes, with the help of the offset post, represented here by the vertical green line.

MATLAB scanning software, which is described in Section 3.3.

For vertical motion along the z-axis, there are three regimes of motion:

Manual (coarse): turning the knob on the red picomotor with your hand (clockwise moves the stage up).

Picomotor (medium): using the joystick to drive the picomotor (pushing the joystick forward moves the stage upward).

Piezo-disk (fine): actuating the piezo disk over a few hundred nanometers using the MATLAB software.

For x- and y-axis positioning (in the sample plane), coarse movements are accomplished with the stage micrometers, and fine (several μm) movements are also attained using the piezo disk.

3.1.2   Optical system

Our microscopes use a somewhat different optical readout from a standard AFM to sense cantilever deflection. Rather than detecting the *position* of a laser beam that reflects off the back surface of the cantilever, we measure the *intensity* of a diffracted beam. To do this, a diode laser with wavelength $\lambda = 635nm$ is focused onto the interdigitated (ID) "finger" structure, and we observe the brightness of one of the reflected spots (referred to as "modes") using a photodiode. This gives us information about the *relative displacement* of one set of fingers relative to the other — this is useful if one set is attached to the cantilever, and the other to some reference surface.
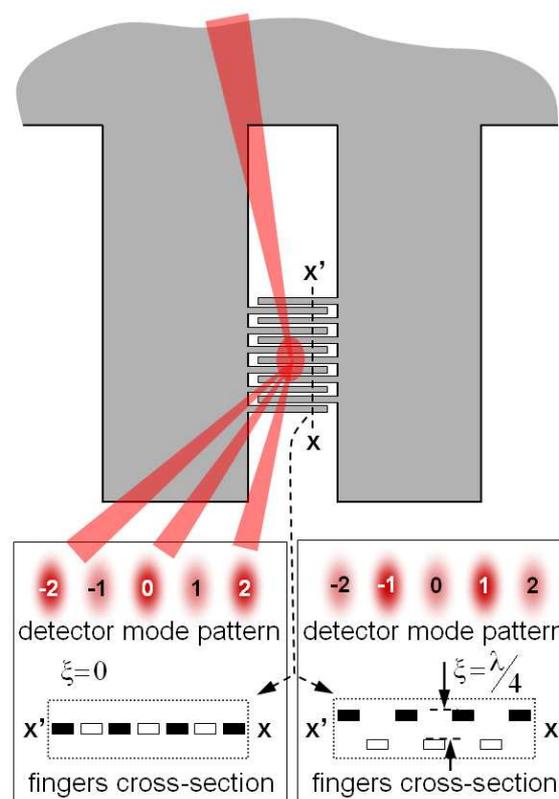
Figure 3: A sketch of the interdigitated (ID) interferometric fingers, with the detection laser shown incident from the top of the figure. When the finger sets are aligned, as in the left box above, the even-numbered modes are brightest, and odd modes are darkest. When they displace relative to each other by a distance of a quarter of the laser wavelength λ, the situation reverses, shown on the right. This repeats every $\lambda/4$ in either direction.

As the cantilever deflects, and the out-of-plane spacing between the ID fingers changes, the reflected diffractive modes change their brightness, as shown in Figure 3. However, a complication of using this system is the non-linear output characteristic of the mode intensities. As the out-of-plane deflection of the fingers increases, each mode grows alternately brighter and dimmer. The intensity I of odd order modes vs. finger deflection Δz has the form

$$I \propto sin^2 \left(\frac{2\pi}{\lambda} \Delta z \right),$$

and for odd modes, the sine is replaced by a cosine. The plot in Figure 4(a) shows graphically the intensity of two adjacent modes as a function of displacement.

This nonlinearity makes the sensor's sensitivity depend critically on the operating point along this curve at which a measurement is done. To make useful measurements, the ID interferometer therefore needs to be *biased* to a spot on the sin^2 curve where the function's slope is greatest - midway between the maximum and minimum, as sketched in Figure 4(b).

Due to residual strain in the silicon nitride from which the cantilevers are fabricated, the relative planar alignment of the two finger sets varies slightly over the area of the grating. This variation is typically a few hundred nanometers in the lateral direction. Therefore, the bias point of the detector's output can be adjusted along the sin^2 curve by moving the incident laser spot side to side on the diffraction grating.
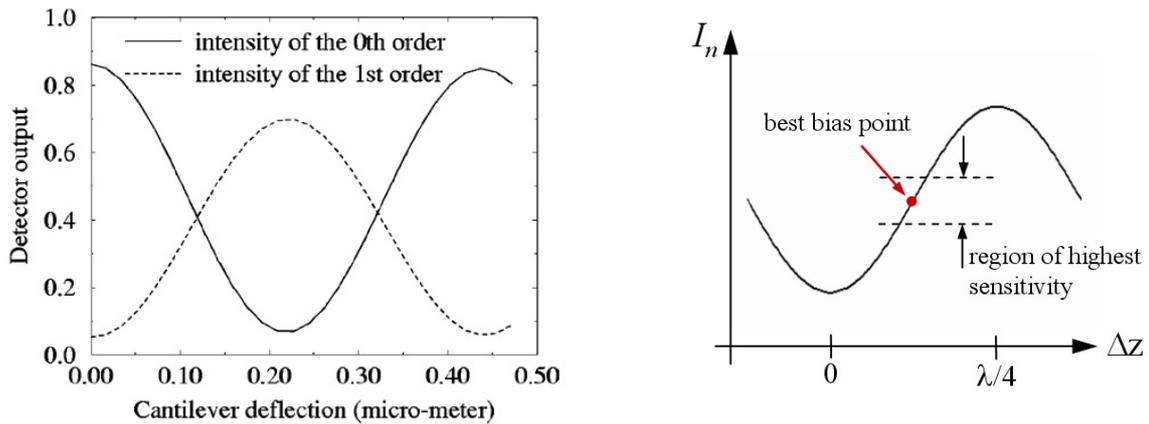
(a) The non-linear intensities of the 0th and 1st order modes as a function of cantilever displacement (from Yaralioglu, *et al.*, J. Appl. Phys., 1998).

(b) The desired operating point for maximum deflection sensitivity is sketched here on the sin^2 output characteristic of the ID fingers.

Figure 4: The characteristic output of the ID interferometric sensor.

3.1.3   Cantilever probes for imaging

A few words about **probe breakage:** you **will** break at least a few probes – this is a normal part of learning to use the tool. We have a large, but not infinite supply of replacement probes. The cost of an individual AFM probe is not large, and the greater problem with breaking them is the time lost to replacing the probe and realigning the laser.

Exercise caution when moving the sample up and down, but don't let this prevent you from getting comfortable moving the sample around. Under most conditions, the cantilevers are surprisingly flexible and robust. They are most often broken by running them into the sample (especially sideways) – avoid "crashing" the tip into the surface, or worse bumping the stage into the die or fluid cell. Make sure you're familiar with the motion control system (Sec. 3.1.1).

The probes we use for imaging are shown in Figure 5 with relevant dimensions. The central beam has a tip at its end, which scans the surface. The shorter side beams to either side have no tips and remain out of contact. The side beams provide a reference against which the deflection of the central beam is measured; the ID grating on either side may be used. When calibrating the detector output to relate voltage to tip deflection, remember to include a correction factor to account for the ID finger position far away from the tip.

3.1.4 Cantilevers for thermal noise measurements

For noise measurement purposes, we'd like a clean vibrational noise spectrum, which is best achieved using a matched pair of identical cantilevers. The configuration with a central long beam and reference side-beams has extra resonance peaks in the spectrum that make it harder to interpret. With the geometry shown in Figure 6 the beams have identical spectra which overlap and reinforce each other. Using a pair of identical beams also helps to minimize any common drift effects from air movements or thermal gradients.

There are two sizes of cantilever pairs available. For the long devices, $L = 350\mu m$ and the finger grating starts $140\mu m$ and ends $250\mu m$ from the cantilever base. For the short devices, $L = 275\mu m$, and the finger gratings begin $93\mu m$ and end $175\mu m$ from the base. The width and thickness of all of the cantilevers is $b = 50\mu m$ and $h = 0.8\mu m$, respectively.
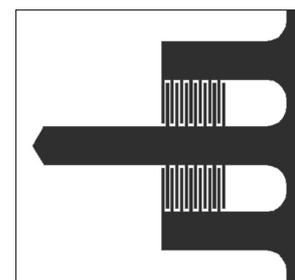
(a)
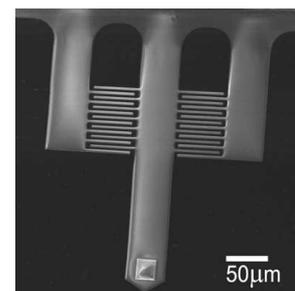
(b)

Figure 5: (a) Plan view and (b) SEM image of the imaging cantilever geometry. The central (imaging) beam dimensions are length $L = 400\mu m$, and width $b = 60\mu m$. The finger gratings begin $117\mu m$ and end $200\mu m$ from the base.
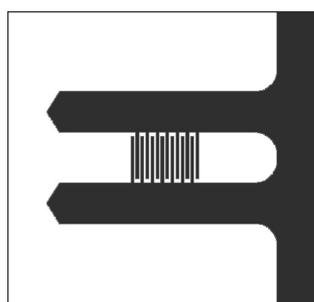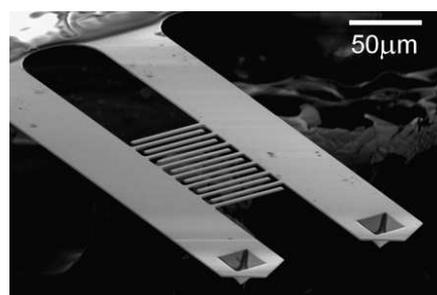
(a)   (b)

Figure 6: (a) Plan view and (b) SEM image of the geometry of a differential cantilever pair. Because the beams are fabricated so close together, we assume that their material properties and dimensions are identical.

3.2   Major operational steps

3.2.1   Power-on

For our AFMs to run, you must turn on three things: (1) the detection laser, (2) the photodetector, and (3) piezo-driver power supply. The photodetector has a battery that provides reverse bias, and the others have dedicated power supplies (refer to Figure 1 for where these switches are located). *When you finish using the AFM, don't forget to turn off the three switches you turned on at the beginning.*

3.2.2   Signal connections and data flow

The first key step to using the instrument is properly connecting all of the components together. Figure 7 will help to guide you.

The AFM itself requires two signal inputs (X_{in} and Y_{in}) to drive the piezo actuator, which connect to the electronics board on the back of the headplate. They are provided by the computer's digital signal outputs (DAC0OUT and DAC1OUT). The computer also needs to read these signals in, together with the AFM signal output, so these become the three DAQ inputs.

The output from the AFM's photodetector is a current signal proportional to the brightness of the laser spot, which needs to be converted to a voltage (a 100 kΩ resistor to ground is sufficient). It's good to be able to amplify and offset this voltage at our convenience, so run this signal through an AM502 amplifier before it enters the DAQ.

Figure 7: Schematic of signal connections.

Finally, during calibration, it's very useful to watch the detector signal as a function of stage movement in realtime, on the oscilloscope screen, so run those signals to the scope as well.

3.2.3   Laser alignment and diffractive modes

To get a cantilever position readout, the laser needs to be well focused on the interdigitated fingers of the cantilever. Use the white light source and stereo-microscope to look at the cantilever in its holder. The laser spot should be visible as a red dot (there may be other reflections or scattered laser light, but the spot itself is a small bright dot). Adjust its position using the knobs on the kinematic laser mount, until it hits the interdigitated fingers (use the cantilever schematics in Figures 5 and 6 as a reference).

When the laser is focused in approximately the right position, the white "screen" around the slit on the photodetector will allow you to see the diffraction pattern coming out of the beam splitter. Observe the spot pattern on this screen while adjusting the laser position until you see several evenly spaced "modes." Make sure you aren't misled by reflections from other parts of the apparatus — some may look similar to the diffraction pattern, but aren't what you're looking for.

When you see the proper diffraction pattern is on the detector, adjust the detector's position such that only one mode passes through the slit. Typically the 0th mode gives the largest difference between bright and dark.

3.2.4   Sample loading and positioning

Correctly mounting a sample in the AFM is a key part of obtaining quality images. Our samples are always mounted on disks, which are magnetically held to the piezo actuator offset post. The AFM can image only a small area near the center of the opening in the metal cantilever holder, so be sure that the area of interest for imaging ends up there.
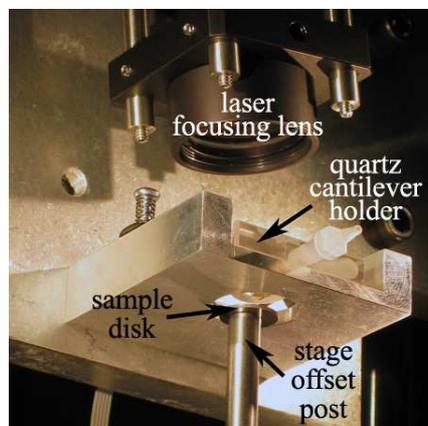
(a) A photo of the underside of the cantilever mount, with the sample disk lowered for changing samples.
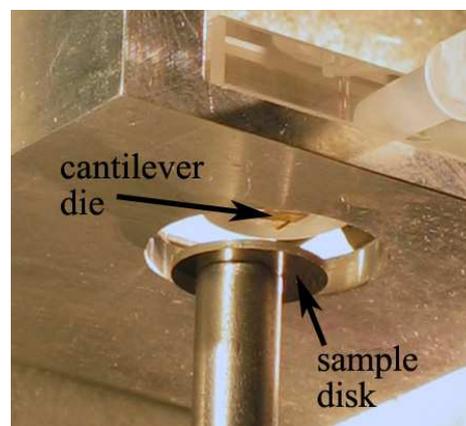
(b) A close-up view of the opening into which the sample rises, showing the cantilever die and sample disk.

When changing or inserting a sample disk, the 3-axis stage must be lowered far enough for the disk to clear the bottom opening of the cantilever mount, as shown in the figures above. This requires a large travel distance, so exercise caution when bringing the sample back up to the cantilever, and take care not to crash the tip.

In addition, as you change samples, it is critical to reposition the offset post as nearly centered as possible on the actuator disk, to ensure true horizontal motion in the x-y plane (Centering the sample disk at the top of the offset post is not critical; rather, it's the position of the **bottom end of the post on the piezo scanner disk**. For instance, in figure 8(b) above the sample disk is visibly off-center, which is not a problem).

3.2.5   Engaging the tip

The process of bringing the probe tip to the sample surface so we can scan images and measure forces is called "engaging." The aim is to get the tip in close proximity so it is just barely coming into contact, and bending only slightly. If the probe does not touch the surface, it is obviously useless, but if it's bent too much against the surface, it is equally useless.

Before engaging, start the piezo z-modulation scan in the MATLAB software (see Sec. 3.3.3). Be sure the mode switch on the AFM electronics board is flipped down to "force spec. mode," and make sure to turn on the piezo power supply. Carefully bring the tip near the surface, first turning the red motor knob by hand, then very slowly with the joystick. When you make contact, you will see the modes on the photodetector fluctuate in brightness. Because of the device geometry, only the central long cantilever with the tip will make contact with the sample surface.

3.2.6   Calibration and biasing

At this point, it's worth pausing to review the definition of *calibration*, as well as the distinction between *sensitivity* and *resolution* – terms which will often used frequently in this context, but

whose precise meaning isn't always made explicit. Be sure you're clear on the differences between them.

Calibration - finding the relationship between the output of your instrument to the physical quantity you are measuring like distance or force; in our case, relating the mode brightness measured by the photodiode to cantilever tip deflections

Sensitivity - a numerical expression for the calibration, the "slope" of a transducer output e.g. mV/N, W/Å, or in our case nm/V

Resolution - the minimal measurement or change in signal that an instrument can detect; depends completely on the noise and the frequency and bandwidth of the measurement

It's a good idea to run a calibration before performing any measurement, because they varies from AFM to AFM, and may be thrown off by drifts or disturbances. We calibrate our AFM in force spectroscopy (or z-modulation) mode, in which the sample is only moved straight up and down (see Section 3.3.3 for details).

Watching the AFM signal on the oscilloscope in x-y mode, (with detector output on the y-axis, and the stage actuation signal on the x-axis) you should see something like the plots shown in Figure 9: a flat line that breaks into a $\sin^2$ function at a certain x-value (whether it starts upward or downward depends on the mode you choose). The flat line is the cantilever out of contact, and the oscillating section is the cantilever bending, after making contact with the sample.

If necessary, use the offset on the voltage amplifier to position the $\sin^2$ so that it is centered around zero. Then, set the out-of-contact bias point by moving the position of the laser focus on the fingers until the flat section of your force spec. curve is approximately at zero volts, halfway between the maximum and minimum, as in Figure 9(c).

| (a) Bias too high. | (b) Bias too low. | (c) Bias set to optimal sensitivity for out-of-contact. |
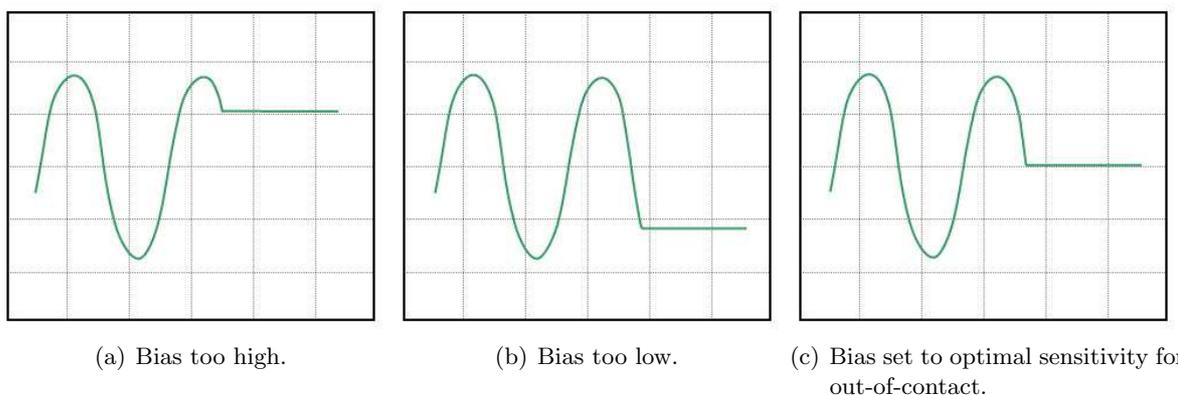
Figure 9: Proper setting of the bias point.

The goal of this calibration process is to relate the detector's voltage signal to physical tip deflection – i.e. how many nm is the cantilever tip bending for every volt of signal. We can take advantage of the fact that a mode's brightness goes from fully bright to fully dim (peak to trough on the $\sin^2$) as the fingers deflect through a distance of $\lambda/4$ (≈ 160 nm). This should allow you to calculate the relationship between deflection and y-axis voltage in nm/V.

Finally, you will also need to multiply the calibration by a correction factor A_{corr} to account for the location of the diffraction fingers with respect to the tip of the cantilever. A_{corr} can be estimated most simply by assuming a quadratic shape for the bent cantilever $A_{corr} = 1/m_{ID}^2$, where $m_{ID} = L_{ID}/L_T$ is the ratio of the distance of the ID fingers from the cantilever base to the total cantilever length. A more precise expression is $A_{corr} = 2/(3m_{ID}^2 - m_{ID}^3)$ (derived from the equation for the shape of a simple rectangular beam, with an applied end-load).

3.3   Software

The software that interfaces with the AFM is an application that runs in MATLAB. Its graphcial user interface (GUI) is launched by typing 'scannergui' in the MATLAB command window. Its main function is to systematically scan the probe tip back-and-forth across the sample, recording the cantilever deflection information at each point, line by line, and assembling that data into an image. Figure 10 shows a screen capture of the scanner control window, and an overview of its operation is provided below.
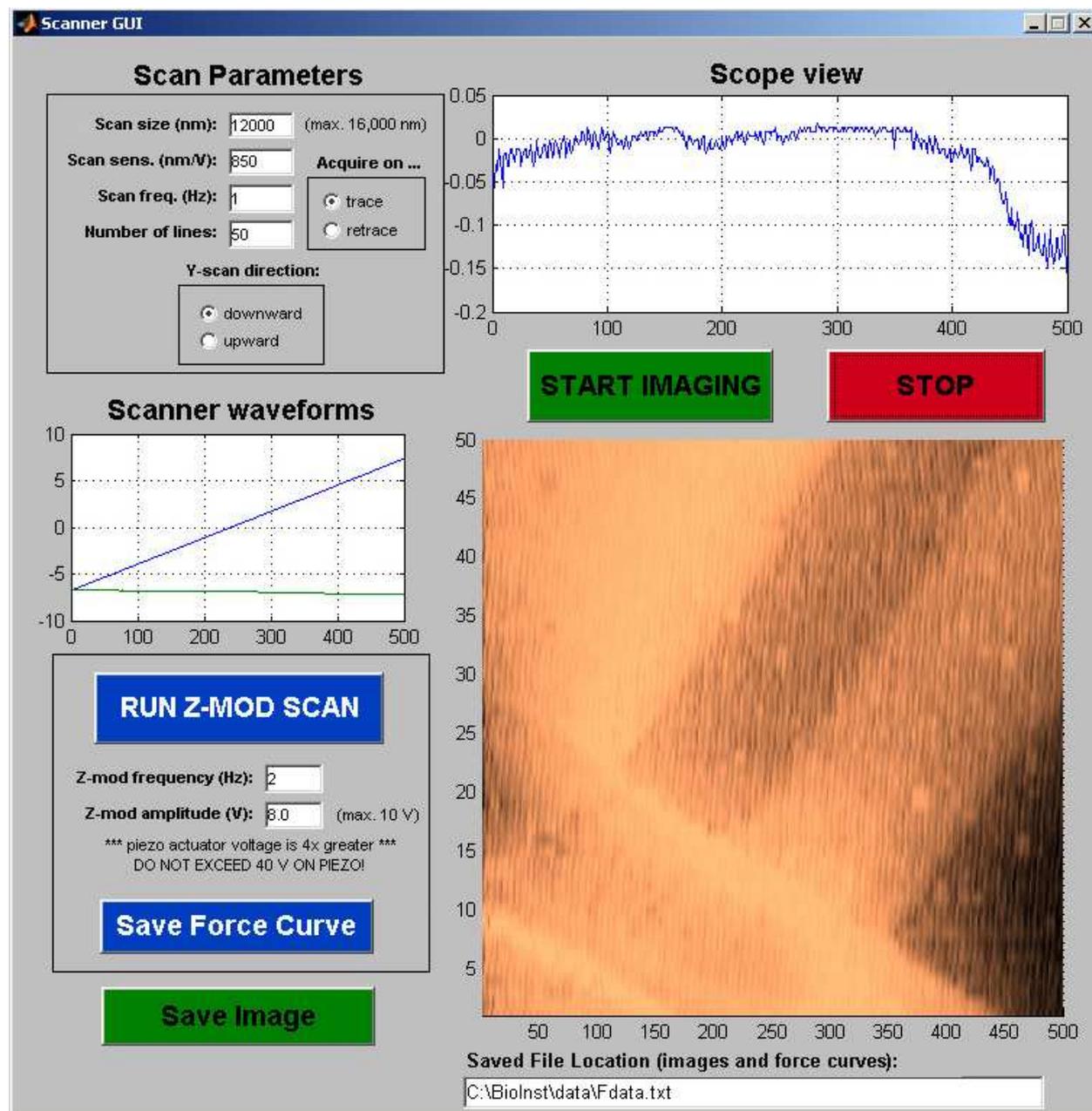
Figure 10: The Scanner GUI window. The AFM is scanning a $12 \times 12 \mu m$ area, at a rate of one line per second, and is currently near the bottom of the image.

3.3.1   Controls overview

Many of these are self-explanatory, such as the START IMAGING and STOP buttons, as well as the image area in the lower right, which displays the image currently being scanned. Some notes are given below on features that are not immediately obvious.

To begin with, it's easiest to simply use the default settings on all these controls, and to experiment with changing them as you become more familiar with the tool.

Scan Parameters - The *Scan size* sets the length and width of the image in nanometers (always a square shape), but its accuracy depends on having the correct value for *Scan sensitivity* (which should already be set for you, but may require calibration). The *Scan frequency* (lines per second) sets the speed of the tip across the surface, and together with the *Number of lines* affects the amount of detail you will see in the image. Setting the *Y-scan direction* tells the scanner whether to start at the top or the bottom of an image, and the *trace/retrace* selector determines whether each line is recorded as the tip scans to the left or to the right.

Scope View - As the tip scans back and forth, this plots the tip deflection data for each line. Useful for quantitative feature height measurements.

Scanner Waveforms - Shows the voltage waveforms driving the piezo scanner, for each scan line that is taken. Helpful for knowing where in the image the current scan line is located, and the output level of the waveforms driving the scanner.

Z-mod Controls - These are only active during a z-mod scan, and have no effect when taking an image. For more on this mode, see Section 3.3.3.

3.3.2   Image mode operation

This is the primary operating regime of the AFM, and provides a continuous display of the surface being scanned, as the probe is rastered up and down the image area. To use this mode, the switch on the back of the AFM must be flipped upward. Remember that the maximum scan area is only abut 15 μm square, and adjusting the position of the sample under the tip requires only the smallest movements of the stage micrometers. Finally, keep in mind that there is always a delay after pressing START IMAGING before the scan begins, as the actuator drive signals are buffered to the I/O hardware.

3.3.3   Z-mod (force spectroscopy) operation

In this mode, the piezo moves the sample only along the z-axis – i.e. straight up and down (hence z-mod, short for z-modulation). To use this mode, the switch on the back of the AFM must be flipped downward. The default frequency and amplitude of 2 Hz and 8 V provide a nice force curve. Besides being critical for calibrating and biasing the readout, this mode is used to perform force spectroscopy experiments, in which tip-sample forces can be measured as the tip comes into and out of contact with the sample.
(Note that the red STOP button is also used to stop a z-mod scan).

3.3.4   Saving AFM data

The software allows you to save the raw data of both images as well as force curves. Unsurprisingly the "Save Image" and "Save Force Curve" buttons do this. In both cases an instantaneous "snapshot" of the current image or force curve is written to the file location specified in the entry box at the bottom of the window.

An image is written to a files as a square matrix (interpolated to have the same number of rows and columns), with the value at each point representing height data. Force curve data is saved as two columns: x-axis (stage deflection) data in column one, y-axis (mode intensity) in column two.

If you intend to save an image, it is best to set the filename before starting the scan – the filename box behaves ... elusively while the scanner is running due to some peculiarities of the software.

As a final note, a "quick and dirty" way to save an image is by simply doing a screen capture while the AFM is scanning (press "Print Screen" on the keyboard). The captured image can then be pasted into MS Paint (Programs → Accessories → Paint) and cropped to leave only the scanned AFM image. These images can be imported into MATLAB for analysis/processing (we'll do this in later parts of the course).

3.4   Additional instrumentation

3.4.1   Differential amplifier

The Tektronix AM502 is a *differential* amp, so it amplifies the voltage difference between the two input signals. Both inputs have DC, AC, and GND input coupling, like the scopes. The amp can be used single-ended if the (–) input is left unconnected and ground-coupled. The **gain** (amplification factor) ranges from 1 to 10,000, and is set by the red knob together with the "÷100" button above it.

The instrument also has high- and low-pass filters. These are somewhat confusingly labeled "HF-3dB" and "LF-3dB" (see Figure 11). This doesn't mean "high-pass" and "low-pass," but refers to the "high [cutoff] frequency" of a LPF and the "low [cutoff] frequency" of a HPF. *Therefore, HF-3dB is the low-pass filter, and LF-3dB is the high-pass filter.*

Both filters are considered "off" when the low-pass is turned all the way up, and the high-pass all the way down.

Finally, the lower (high-pass) filter knob also has two settings for controlling output signal DC level: DC and DC OFFSET. When set to DC, the amp outputs the *actual* DC level of the input signal, multiplied by the gain. Set to DC OFFSET, you can manually adjust the DC level using the knob at the upper right of the AM502. In both cases, the AC component is simply added on top.
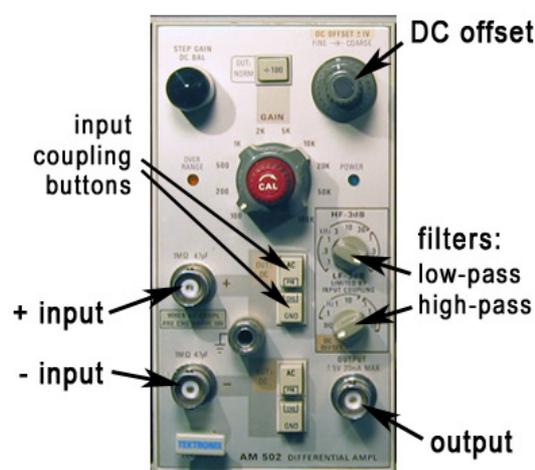
Figure 11: An AM502 differential amplifier. The gain is determined by the central red knob, together with the ÷100 button in the center above it.

3.4.2   LabVIEW VIs for signal capture

The spectrum analyzer
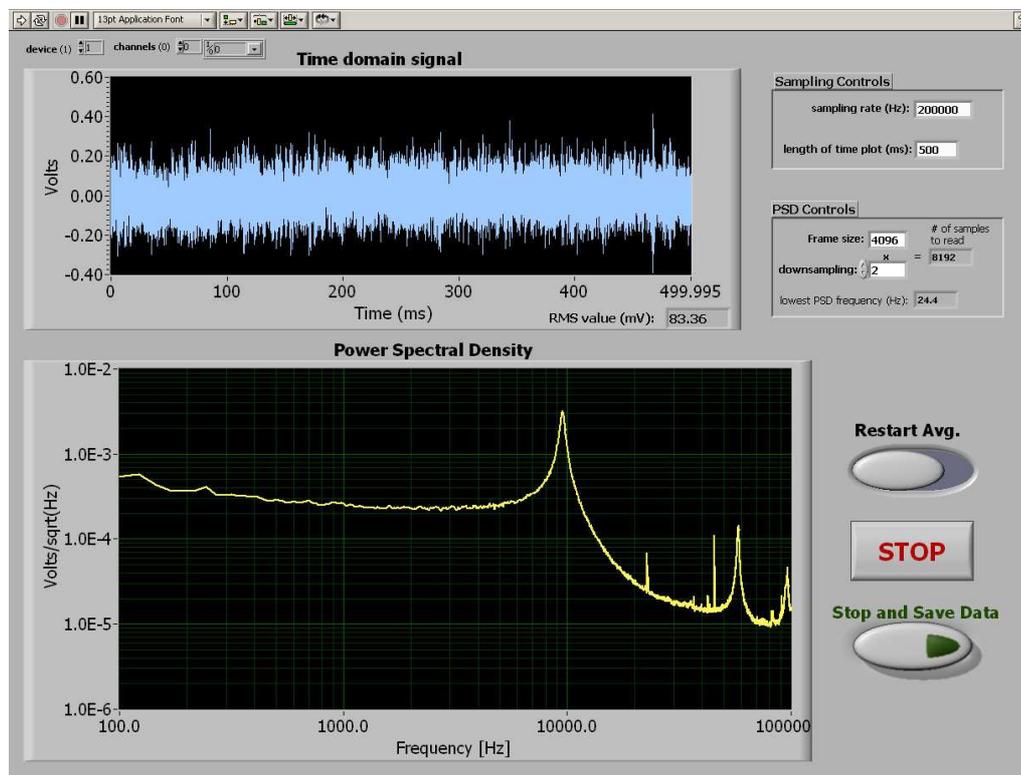
Figure 12: The LabVIEW Spectrum Analyzer VI runs continuously until the STOP button is pressed. Each PSD is averaged with all the data that precedes it, so the spectrum gets cleaner the longer you acquire. Note that the "PSD controls" (Nfft, downsampling) can be changed on the fly, but to change the sampling rate you must stop and re-start the VI. Pressing "Stop and Save Data" pops up a window in which you can choose a file name to store the spectrum currently displayed.

Time-domain signal capture
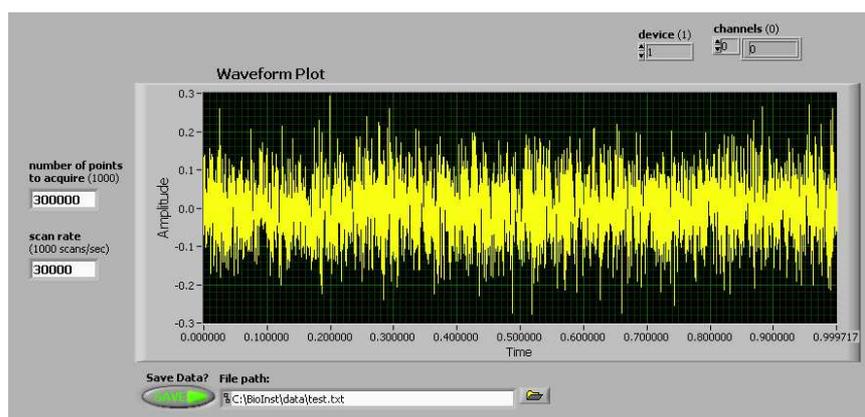
Figure 13: The LabVIEW Waveform Acquisition VI does a "one-shot" waveform capture in the time domain. Pre-set your desired parameters, including sampling rate, length of captured waveform, and filename to save to, then press the Run (arrow) button to do the data capture. What you've captured appears in the waveform plot.

4   Experiment 1: Imaging

The general approach to imaging is to (1) set the overall output signal range and offset while in the z-mod regime, (2) stop the z-mod scan and engage the probe with the surface, and (3) carefully adjust the cantilever deflection to give the desired bias point, and (4) start the image scan.

Correct biasing is key to obtaining good images. If you are not yet comfortable with choosing and setting an appropriate bias point for the cantilever's optical readout, it is worth reviewing that material from Section 3.2.6.

For imaging, maximum out of contact sensitivity is not the goal. Instead, the sensitivity should be greatest *when the probe is engaged, and exerting a small force on the surface.* The more force the probe applies to the surface, the more wear and damage to the probe and surface can result.

The samples available for imaging are calibration gratings, various integrated circuit chips, and *E. coli* bacteria on a silicon dioxide surface. With the latter two, you may need to navigate around the surface in order to find interesting features to image. Unfortunately, unlike commercial instruments, our AFMs do not have an integrated optical viewing system, so when positioning the sample it is difficult to determine exactly what spot on the sample the probe tip will scan. Use the stereo-microscope (at moderate magnification) and a fiber-light to observe the tip and position it as well as you can. Temporarily turning off the sensing laser will make it easier to see. You can move the sample "on the fly", as you're imaging, but it takes some practice to make small enough stage movements. You will also likely have to readjust the bias point (also fine to do on the fly).

4.1   AFM resolution limit

Researchers using the AFM always need to know their noise-limited resolution. Therefore, we will characterize the noise floor of the system, so we know what performance to expect.

The simplest way to do this is to look at the root-mean-square (RMS) noise, which represents the average fluctuations that will obscure features in AFM images. RMS noise data is easily obtained by recording a length of the output signal in the time domain (using the VI from Sec. 3.4.2), and analyzing the data in MATLAB.

You should capture and calculate the RMS noise of the AFM **both out of contact and in contact** with the sample surface. Make sure you don't lose your calibration, especially when you bring the AFM into contact – it's easy to move to a different point on the output curve.

You may also find it instructive to look at the noise output signal using a Spectrum Analyzer (LabVIEW VI in Sec. 3.4.2) to see whether the noise is concentrated in particular spectral regions.

4.2   Measuring Image Dimensions

When it's necessary to determine the size of imaged features, the *Scan size* control is most helpful. This gives the size of the image square, which can be related to imaged feature dimensions.

NOTE: the *Scan sensitivity* is pre-calibrated to 900nm/V, but if you would like more precise lateral feature measurements, it's worth verifying this. This can be done by scanning a reference with precisely known feature sizes (such as a calibration grating), and adjusting the *Scan sensitivity* until the actual image size matches the *Scan size* setting.

Finally, feature height (*z*-axis) measurements can be made by observing the SCOPE VIEW of the AFM software. The waveform display shows the feature height dimensions as voltages – the calculated calibration factor then gives corresponding feature sizes.

5   Experiment 2: Elastic Modulus

[*For these measurements, we'll use the shortest and stiffest cantilevers available to us, which will give the best signal. These have very similar geometry to the long cantilevers in Figure 5, but have a length of 250μm, and a width of 50μm. The fingers begin 43μm from the base and end 125μm from it. Ask your lab instructor to provide you with a short cantilever when you are ready.*]

As you know, some of the most useful applications of AFMs in biology take advantage of their ability to measure very small forces. We'll use this capability to measure the elastic moduli of some soft samples, to simulate mapping cell wall elastic properties, similarly to the 2003 paper by Touhami *et al.*[1]

As seen in this paper, when using the optical lever sensor, samples with different elastic moduli change the slope of the in-contact portion of the force curve. For our non-linear ID sensor, the equivalent of changing the slope is a changing *period* for the $\sin^2$ function. Just as softer samples cause lower slope with the optical lever, softer samples give the output function of the ID sensor a longer period, with greater spacing between the peaks (see figure 14(a) below).
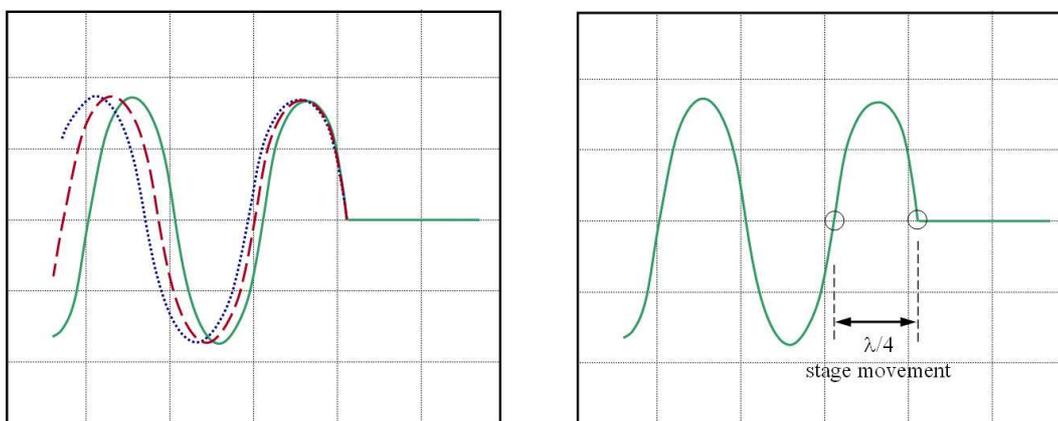
(a) Force curves for samples of varying hardness. The green (solid) curve is for a hard sample, the red (dashes) curve is softer, and blue (dots) curve is softest.

(b) Preferred biasing and calibration on a hard surface for measuring elastic modulus, and corresponding physical stage movement

The approach for measuring modulus is to first take a force curve on a reference sample, considered to have infinite hardness. We will use a bare silicon nitride surface. This allows us to determine how the x-axis signal corresponds to stage movements.

You'll want to bias this measurement similarly to measuring noise – the output should be at the middle of the output range when out of contact (See Fig. 14(b)).

After your measurement of the hard sample, switch to the more compliant PDMS elastomer samples, and run force curves on them.

Make sure to save these force curves for later analysis, described in Section 5.1.

To get good force curves:

1. Don't change the biasing or laser position between samples – if you do, the force curves you get can't be compared one to another.

2. Careful initial biasing at the middle of output range is worth it — this will make a big difference in ease of data analysis.

---

[1]A. Touhami, B. Nysten, and Y. F. Dufrêne, "Nanoscale Mapping of the Elasticity of Microbial Cells by Atomic Force Microscopy," Langmuir **19**(11), 4539-43 (2003).

3. After you've brought each sample into contact and are satisfied with the Z-modulation range, run the scan at slow speed (e.g. 0.5Hz) for the cleanest force curves.

5.1 Data Analysis

According to Touhami *et al.*, the depth δ of an indentation made by a conical tip (approximately true for ours) is related to the applied force F by

$$F = \frac{2}{\pi} \tan \alpha \left(\frac{E}{1 - \nu^2} \right) \delta^2 \ ,$$

where α is the half-angle of the conical tip, and E and ν are the elastic (Young's) modulus and the Poisson's ratio of the substrate material, respectively.

Substituting in appropriate values for α (35.3°) and ν (0.25), we are left with

$$F = 0.60E\delta^2 \ ,$$

in which we need only the force and indentation δ values to calculate modulus.

The force F is calculated by treating the cantilever as a Hookian spring, which obeys the law $F = -kz$, where k is the spring constant and z the tip deflection. For the 250 μm long cantilever, assume a spring constant of 0.118 N/m.

Finally, all that remains is to calculate indentation depths for the soft materials from the difference in the period of the $\sin^2$ output between their force curves and the one for the hard sample. Corresponding forces are derived from the cantilever deflection. Don't forget at all points to include the factor that relates cantilever tip deflection to finger deflection.

6   Experiment 3: Measuring Boltzmann's Constant k_B

6.1   Theory: thermomechanical noise in microcantilevers

For simplicity of analysis, we model the cantilever as a harmonic oscillator with one degree of freedom, similar to a mass on a spring, as discussed in lecture. According to the Equipartition Theorem, the thermal energy present in the system is simply related to the cantilever fluctuations as follows:

$$\frac{1}{2}k_B T = \frac{1}{2}k\left\langle \Delta z^2 \right\rangle,$$

where $\left\langle \Delta z^2 \right\rangle$ is the mean-square deflection of the cantilever, T is the absolute temperature, k is the cantilever spring constant, and k_B is Boltzmann's Constant (yes, this notation can be confusing – take care to keep these ks straight).

The characteristic transfer function of the second-order resonant system has the form

$$|G(\omega)| = \sqrt{\frac{4k_B T}{Qk\omega_0}} \sqrt{\frac{1}{\left(1 - \frac{\omega^2}{\omega_0^2}\right)^2 + \frac{1}{Q^2}\frac{\omega^2}{\omega_0^2}}} \,,$$

in which ω_0 and Q are the (angular) resonant frequency and quality factor, respectively.[2] At low frequencies, $(\omega \ll \omega_0)$ this expression yields what's called the "thermomechanical noise limit" (see Figure 14 for an illustration):

$$\delta = \sqrt{\frac{4k_B T}{Qk\omega_0}} \,.$$

These relations suggest several possible approaches that can be taken for determining k_B, for which you will need the values of several parameters. These include (1) the quality factor Q and resonant frequency ω_0 of the resonator (2) the cantilever's mean-square deflection $\left\langle \Delta z^2 \right\rangle$, and (3) its spring constant k.

Figure 14: A data plot of a cantilever's noise spectrum, with an ideal transfer function $G(\omega)$ fit on top (dark line). Note that $G(\omega)$ is flat at low frequencies, at the thermomechanical limit, as indicated. In contrast, real data has more $1/f$-type noise present at lower frequencies (see Section 6.3).

1. The **quality factor** and **resonant frequency** can be obtained from taking the noise PSD of the vibrating cantilever. If your intuitive sense for them is good, you can estimate these quantities directly from the plot, or determine them more precisely by fitting an ideal transfer function to the noise data, and extracting the fitting parameters (more on this in Section 6.4).

2. The **mean-square deflection** is readily available from either time-domain or PSD data of the cantilever thermal noise. Recall that these are related through Parseval's theorem as follows:

$$\left\langle \Delta z^2 \right\rangle = \int_0^\infty S(\omega)d\omega \,,$$

where $S(\omega)$ is the PSD function of Δz. By now, you know enough MATLAB spectral analysis techniques to make these caculations.

---

[2]If you'd like to understand how this function is derived in greater detail, see M. Shusteff, T. P. Burg, and S. R. Manalis, "Measuring Boltzmann's constant with a low-cost atomic force microscope: An undergraduate experiment," Am. J. Phys. **74**(10), pp. 873-9, (2006).

You will likely find that simply using the mean-square noise value and Parseval's theorem will not give you a good result (why?). Instead, the approach in Section 6.4 will work much better.

3. The **spring constant** (stiffness) can be analytically calculated from geometrical parameters in two ways (see Section 3.1.4 and Figs. 5 and 6 for cantilever dimensions). From basic mechanical beam-bending analysis of a rectangular cantilever the stiffness k can be expressed as

$$k = \frac{Ebh^3}{4L^3} \ ,$$

in which E is the elastic (Young's) modulus of the beam material, and L, b and h are the length, width, and thickness of the beam, respectively (b is used for the width to avoid confusion with angular frequency ω).

This method, however, does not always yield accurate results — can you suggest why? Another analytical model for the spring constant was devised by Sader and coworkers[3], and it relies on measuring the cantilever's resonant frequency:

$$k = 0.2427\rho_c hbL\omega_{vac}^2,$$

where ρ_c is the mass density of the material, L, b, and h are the same geometrical parameters as above, and ω_{vac} is the cantilever's resonant frequency in vacuum. For the purposes of these calculations, you can assume that the resonant frequency that you will measure in air ω_{air} is 2% lower than ω_{vac}. (*remember the factor of 2π when interconverting between ω and f in your equations*).

Suitable material parameters to use for the low-stress silicon nitride (Si_xN), out of which these cantilevers are made are $\rho_c = 3400\text{kg/m}^3$, and $E = 250\text{GPa}$. Dimensions are given in Section 3.1.4

6.2   Calibration and biasing

For the accuracy of this measurement, it's especially important that the signal be biased at the maximum-slope position along the output curve when it's not in contact with the surface.

By now you're familiar with aligning, calibrating, and biasing your AFM. The major difference in this case is how you'll actually perform the z-modulation scan for the calibration.

Because this device is a pair of identical cantilevers, simply bringing it down to a surface will deflect both beams equally. A z-mod scan will show approximately zero deflection of one beam relative to the other. Instead, we want to bend only one of the beams, while keeping the other unbent.

Figure 15: Using the edge of a sample to bend only one beam of a differential cantilever pair.

To do this, you'll use a sample with a sharp step edge. The goal is to position the cantilever pair above this edge such that one of the beams will be on the surface, and the other will hang in free space (sketched in Fig. 15). A z-mod scan should then deflect only one of the beams, giving us the calibration curve we want. (Note: reflections of the laser from the edge of the substrate can

---

[3]J. E. Sader, *et al*, "Calibration of rectangular atomic force microscope cantilevers," *Review of Scientific Instruments*, **70**(10):3967-3969, 1999.

interfere with the diffractive modes. If this is the case, try repositioning the sample edge, perhaps using only the corner to bend one of the cantilevers, until the $\sin^2$ shape improves.)

6.3 Recording thermomechanical noise spectra

[*Of the two cantilever sizes available, choose either size to make your measurement, and work on the second size time permitting. If you do not have enough time to do both, exchange data with a group that measured the other size, and note in your notebook and report with whom you shared data.*]

Once you're satisfied with your calibration and bias point, withdraw the lever's tip from the surface, making sure that the bias point stays where you set it. Gain up the signal using the AM502 voltage amp, and use the LabVIEW spectrum analyzer to record the thermal noise signal coming from the freely vibrating cantilever. Once you are happy with how the spectrum looks, save it to a .txt file of your choise.

You only need to measure the noise spectrum down to about 50-100 Hz. Below this frequency, $1/f$-type or "pink" noise dominates. You are welcome to measure this if you are interested, but it is of limited use for determining k_B. For very low-frequency measurements, anti-aliasing and proper input coupling becomes very important. If you are interested in this, your lab instructor can provide guidance.

Some guidelines for getting a good noise spectrum:

- Choose a sampling frequency at least 2× higher than the highest frequency of interest, or about 10× higher than the first resonance peak of the cantilever.

- Use AC coupling on your voltage amplifier, and use a gain of 100-1000.

- If necessary, add a low-pass anti-aliasing filter (recall Module 1) at an appropriate frequency to eliminate high-frequency components being "folded" over into the frequency region of interest.

- Recall from the previous lab that, if you prefer, you can also measure the time-domain signal directly, and later calculate its PSD in MATLAB. You can decide which technique you prefer.

6.4 Data analysis in MATLAB

Once you bring your saved PSD data from LabVIEW into MATLAB (`[Fvec,PSDvec]=load('filename')` is the syntax you want), you can manipulate it as you wish. To fit the second-order transfer function $G(\omega)$ to the noise data, we'll use the `lsqcurvefit` routine from MATLAB's optimization toolbox. We're aiming to do something similar to what you see in Figure 14, where an ideal function is overlaid on real noise data.

To make the fit converge easily, we'll separate the nonlinear f_0 and Q parameters from the linear scaling factor. When doing the fitting, it is helpful not to use the whole frequency range of your data. Instead, crop your PSD data to a suitable range around the resonant peak – the vectors `xdata` and `ydata` used below are *cropped* PSD frequency and magnitude data, respectively, extracted from `Fvec` and `PSDvec`.

First, you'll need a MATLAB function `transfunc` to generate the *unscaled* transfer function (i.e. the thermomechanical noise scaling factor is 1 here – refer to the equations in Sec. 6.1):

$$|G(\omega)| = \frac{1}{\sqrt{\left(1 - \frac{\omega^2}{\omega_0^2}\right)^2 + \frac{1}{Q^2}\frac{\omega^2}{\omega_0^2}}} \ .$$

The function takes the f_0 (note that this is *real* frequency in Hz, and not angular frequency in rad/sec) and Q parameters as input, with a vector of frequencies, and outputs corresponding PSD magnitude data:

```
function [output]= transfunc(params,xdata)
% params [f_0  Q]
x=xdata/params(1);  % x-matrix to contain freq. points normalized to f/f_0
output=sqrt(1./((1-x.^2).^2 + (x/params(2)).^2));
```

Then, create a function `scaling` to do the linear scaling, and calculate the thermomechanical noise level (the left-divide operation actually does a least-squares fit):

```
function [y]=scaling(params,xdata,ydata)
unscaled=transfunc(params,xdata);
A=unscaled\ydata;  % note the left-divide here!
y=unscaled*A;
```

Finally, use the `lsqcurvefit` routine, supplying an appropriate initial guess for f_0 and Q:

```
options=optimset('TolFun',1e-50,'tolX',1e-30);
p=lsqcurvefit('scaling',[f_guess Q_guess], xdata, ydata, [ ], [ ], options, ydata);
```

This will return the best f_0 (again in Hz, not in rad/sec) and Q parameters after the fit as a two-element vector `p`. Now you just need the scaling pre-factor, which you can get by left-dividing the full-range fit function by the PSD magnitude data (the left-divide again gives you a least-squares fit "for free"):

```
A=(transfunc(p,full_xdata))\full_ydata;
```

Here `full_xdata` and `full_ydata` are the full-range frequency and magnitude PSD vectors, rather than just the cropped sections used for the fit algorithm. You can now see how well the fit worked, by plotting it on top of the original PSD data:

```
Gfit = A*transfunc(p,full_xdata);  loglog (full_xdata, Gfit);
```

20

8 Useful References

AFM basic operating principles

a. A website with a basic description:
http://www.weizmann.ac.il/Chemical_Research_Support/surflab/peter/afmworks

b. One with some more detail:
http://saf.chem.ox.ac.uk/Instruments/AFM/SPMoptprin.html

c. If these really stimulate your interest, this is a more comprehensive site on Scanning Probe Microscopy (SPM), of which AFM is a subset:
http://www.mobot.org/jwcross/spm/

The paper that started it all. G. Binnig, C. F. Quate, Ch. Gerber, "Atomic Force Microscope" *Physical Review Letters* **56**(3):930-933, 1986.

Using interferometric ID fingers for position detection

a. The original paper:
S. R. Manalis, S. C. Minne, A. Atalar, C. F. Quate, "Interdigitatal cantilevers for atomic force microscopy," *Applied Physics Letters* **69**(25):3944-3946, 1996.

b. A more thorough treatment:
G. G. Yaralioglu, S. R. Manalis, A. Atalar, C. F. Quate, "Analysis and design of an interdigital cantilever as a displacement sensor," *Journal of Applied Physics*, **83**(12):7405-7415, 1998.

A nice review of using the AFM in boiology

D. Fotiadis, S. Scheuring, S. A. Müller, A. Engel, D. J. Müller, "Imaging and manipulation of biological structures with the AFM," *Micron*, **33**(4):385-397, 2002.