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.
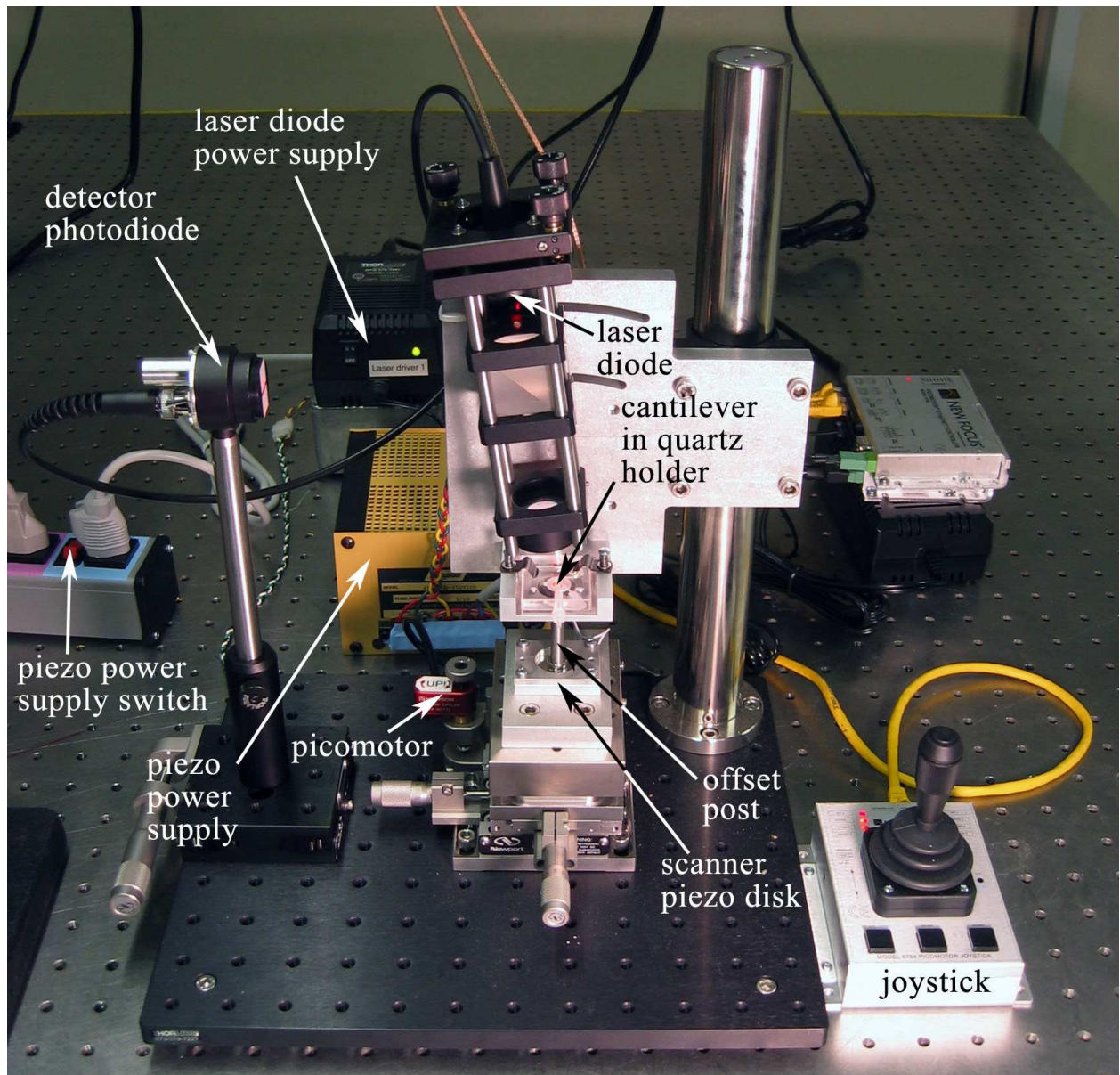
Figure 1: The AFM setup, with major components indicated.

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 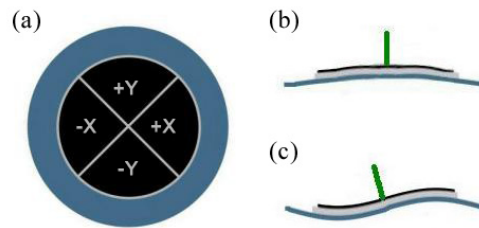in (b), giving z-axis motion. Differential voltages applied to opposite quadrants, produce the flexing shown in (c), which moves the stage along the x- and y-axes, with the help of the offset post, represented here by the vertical green line.

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.
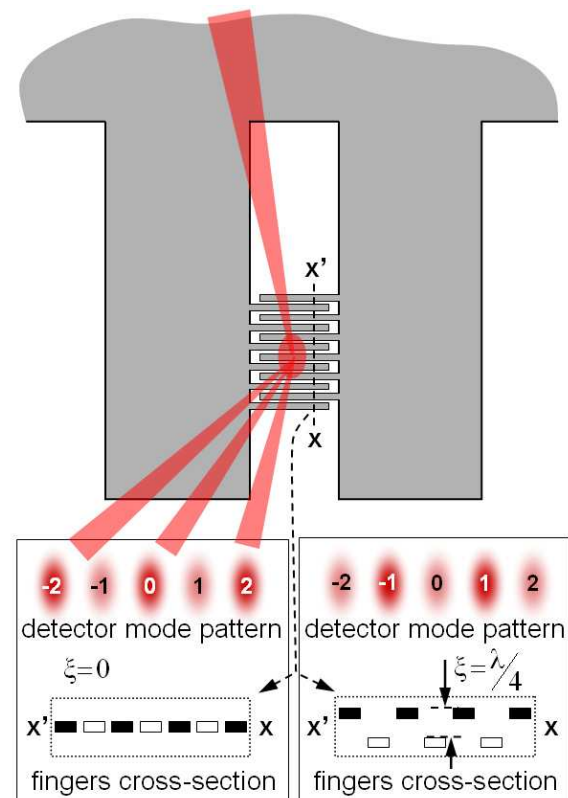
Figure 3: A sketch of the interdigitated (ID) interferometric fingers, with the detection laser shown incident from the top of the figure. When the finger sets are aligned, as in the left box above, the even-numbered modes are brightest, and odd modes are darkest. When they displace relative to each 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.

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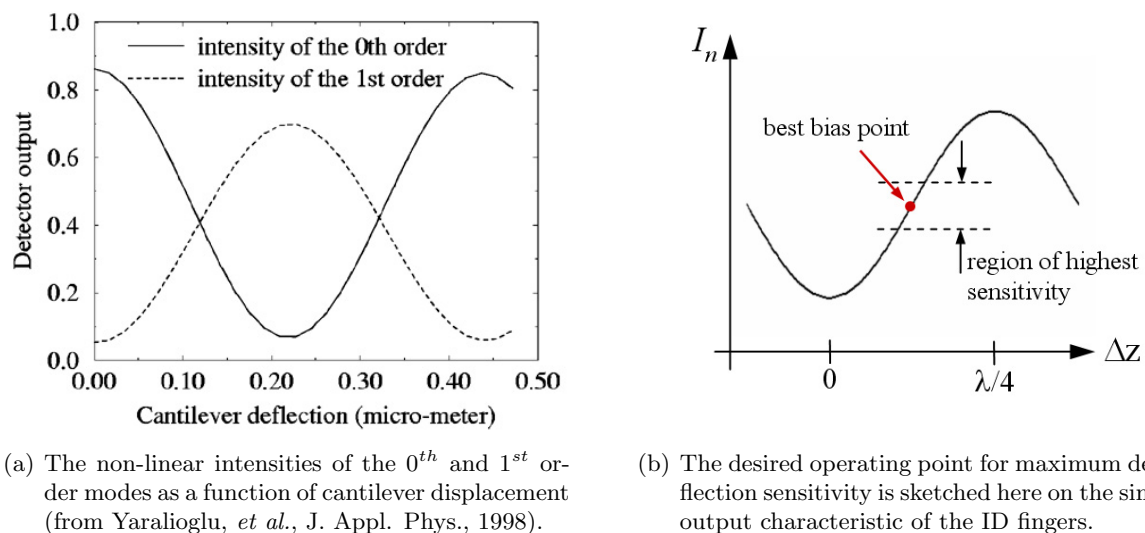
Figure 4: The characteristic output of the ID interferometric sensor.

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.
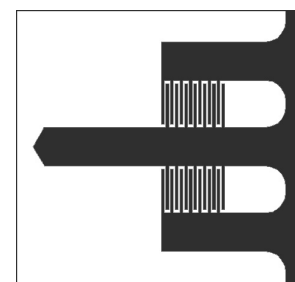
(a)
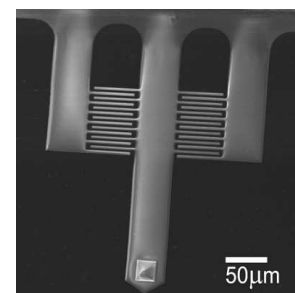
(b)

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.
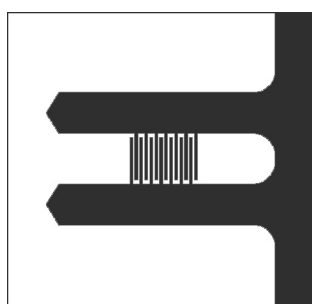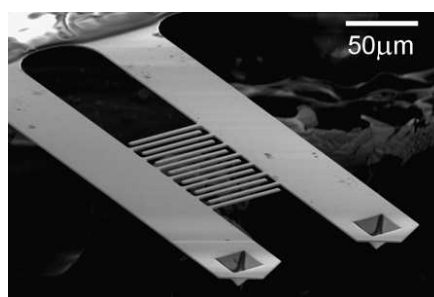
(a)   (b)

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.
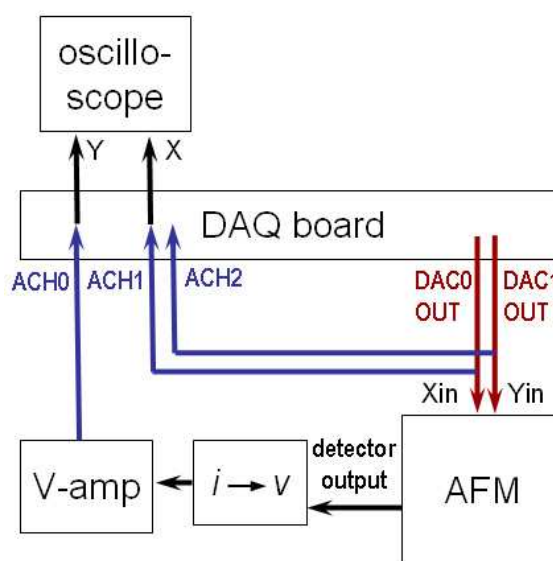
3.2.3 Laser alignment and diffractive modes

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 pie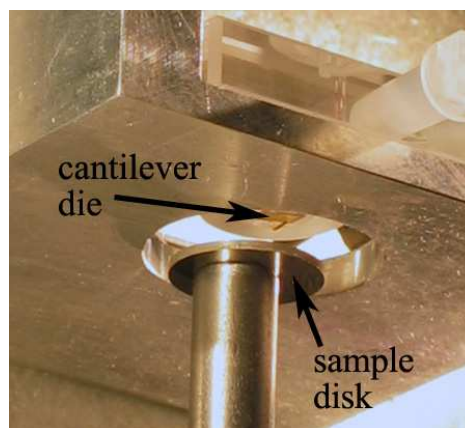zo actuator offset post. The AFM can image only a small area near the center of the opening in the metal cantilever holder, so be sure that the area of interest for imaging ends up there.
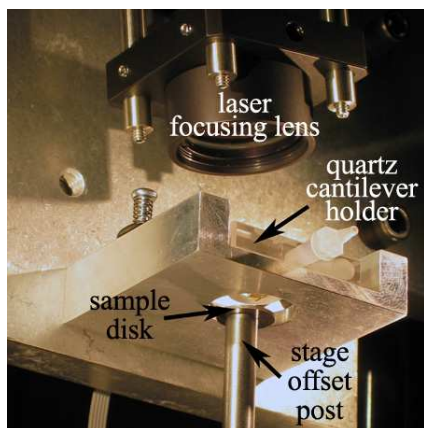
(a) A photo of the underside of the cantilever mount, with the sample disk lowered for changing samples.

(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. |
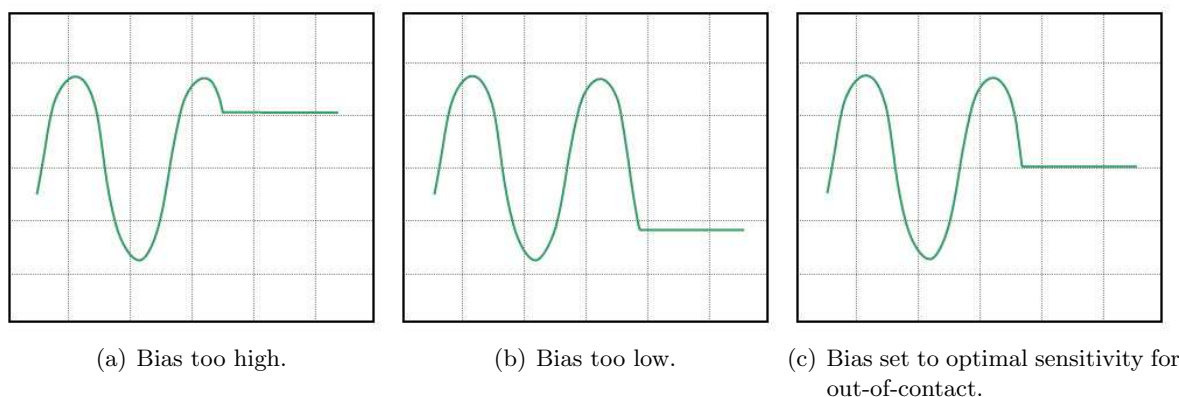
Figure 9: Proper setting of the bias point.

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.
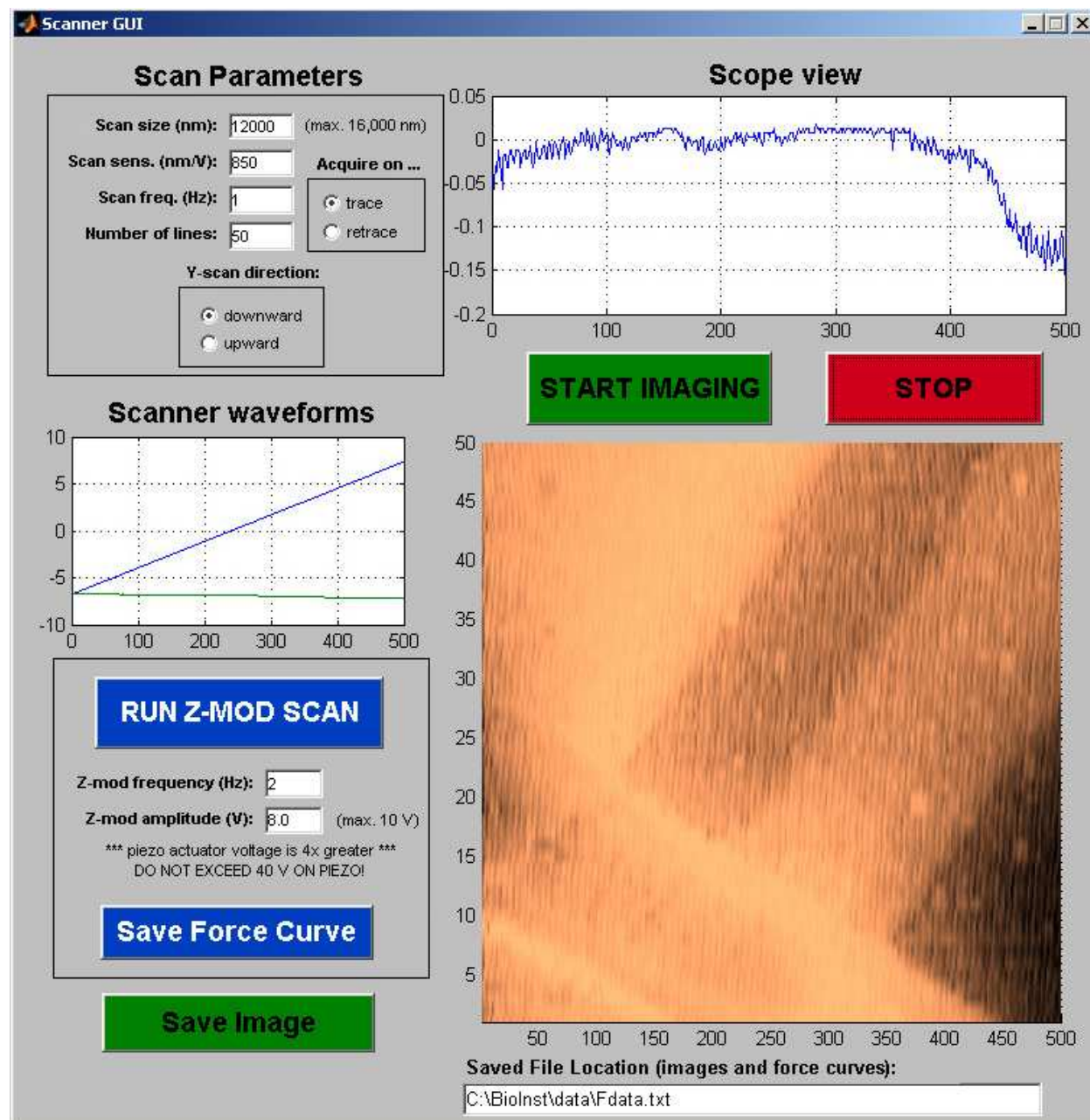

Figure 10: The Scanner GUI window. The AFM is scanning a $12 \times 12 \mu m$ area, at a rate of one line per second, and is currently near the bottom of the image.

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.
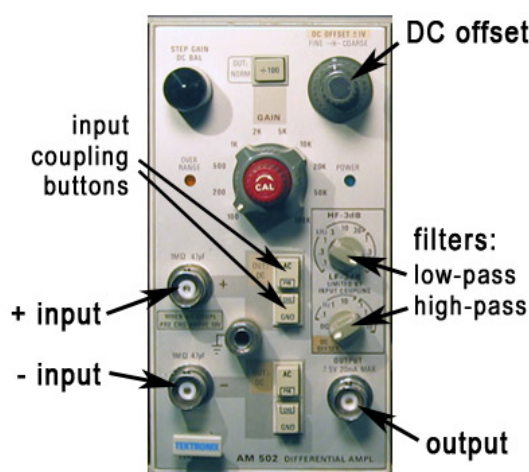

Figure 11: An AM502 differential amplifier. The gain is determined by the central red knob, together with the ÷100 button in the center above it.

3.4.2　LabVIEW VIs for signal capture

The spectrum analyzer
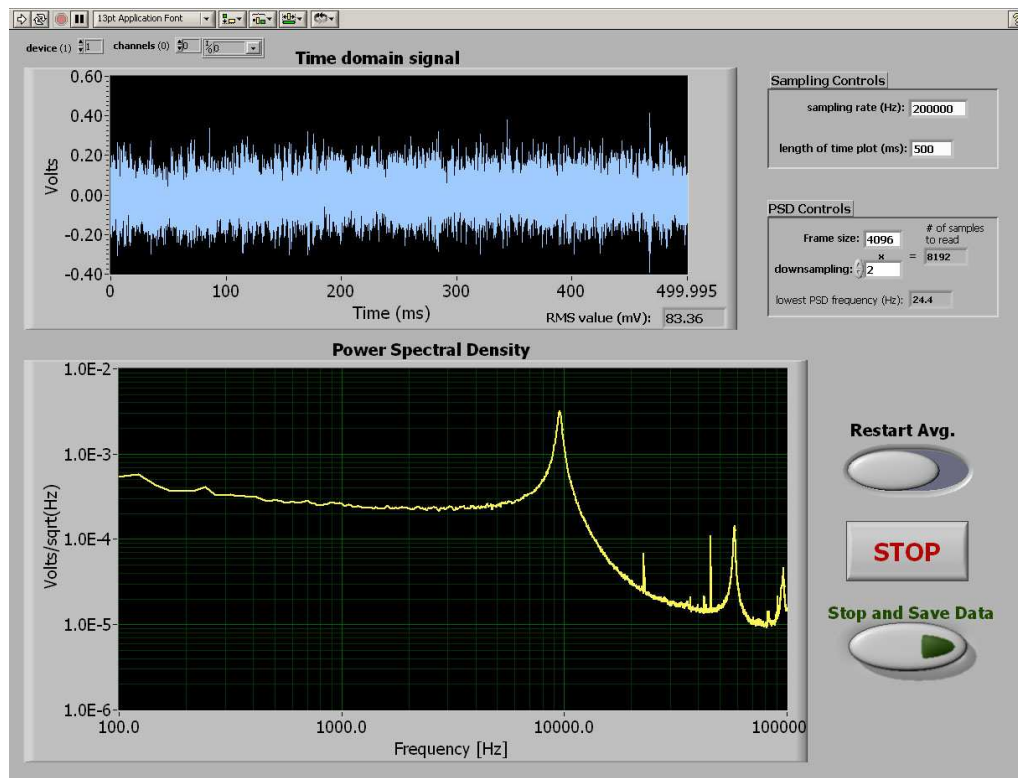
Figure 12: The LabVIEW Spectrum Analyzer VI runs continuously until the STOP button is pressed. Each PSD is averaged with all the data that precedes it, so the spectrum gets cleaner the longer you acquire. Note that the "PSD controls" (Nfft, downsampling) can be changed on the fly, but to change the 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
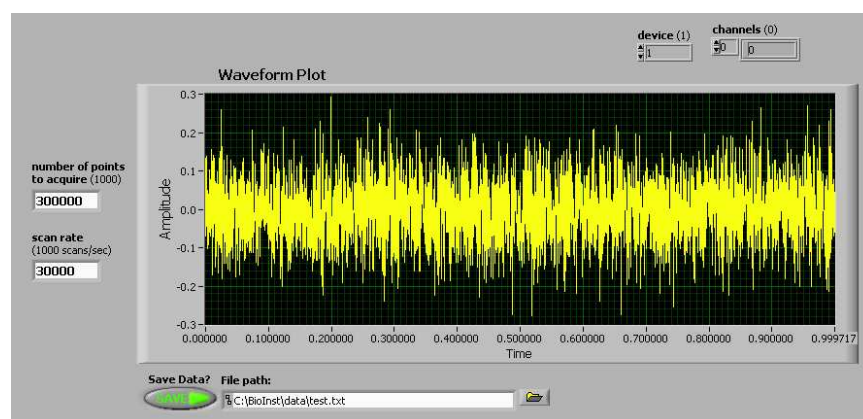
Figure 13: The LabVIEW Waveform Acquisition VI does a "one-shot" waveform capture in the time domain. Pre-set your desired parameters, including sampling rate, length of captured waveform, and filename to save to, then press the Run (arrow) button to do the data capture. What you've captured appears 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).
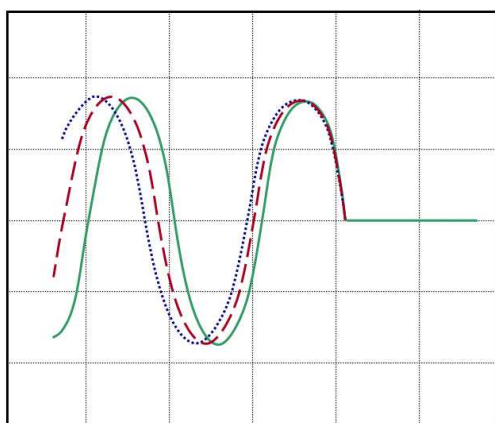
(a) Force curves for samples of varying hardness. The green (solid) curve is for a hard sample, the red (dashes) curve is softer, and blue (dots) curve is softest.
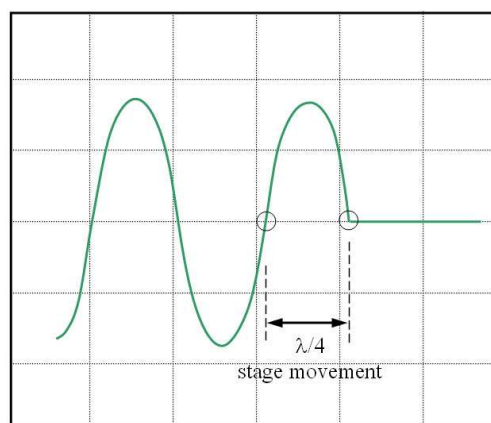
(b) Preferred biasing and calibration on a hard surface for measuring elastic modulus, and corresponding physical stage movement

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

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

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.
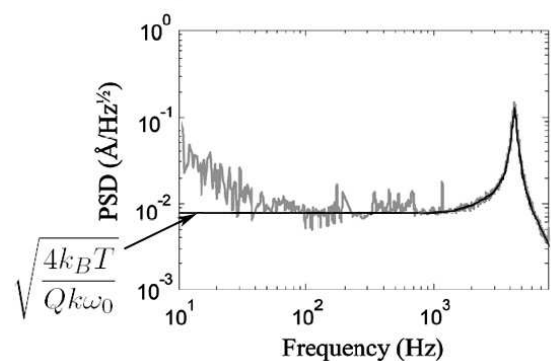

Figure 14: A data plot of a cantilever's noise spectrum, with an ideal transfer function $G(\omega)$ fit on top (dark line). Note that $G(\omega)$ is flat at low frequencies, at the thermomechanical limit, as indicated. In contrast, real data has more $1/f$-type noise present at lower frequencies (see 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 ($Si_x N$), out of which these cantilevers are made are $\rho_c = 3400 kg/m^3$, and $E = 250 GPa$. 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
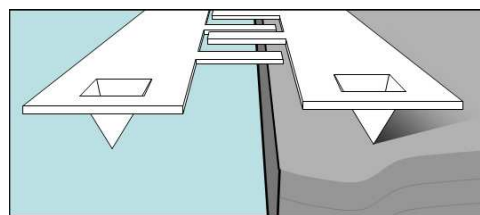
[3]J. E. Sader, *et al*, "Calibration of rectangular atomic force microscope cantilevers," *Review of Scientific Instruments*, **70**(10):3967-3969, 1999.

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);
```

7   Report Requirements

This lab report is due by noon on Friday, Oct. 27.

7.1   Calibration and noise

1. For each measurement you perform, briefly describe the calibration you carried out, and the calculations for the calibration factor in each case. Remember to take into account any amplifier gain, geometric factors, etc. that affect the value of the signal.

2. Using your calibration factor, determine the RMS noise of the system both in and out of contact, in nanometers. Are there particular spectral regions at which noise is greatest?

3. Based on your calculations, can these AFMs resolve atoms? What about DNA? Large globular proteins? Cells?

7.2   Imaging

1. Choose 2-3 samples from those available. For each one, center on an area or feature of interest (e.g. a single *E. coli* bacterium) and take a high-resolution scan (at least 1Hz and 32 lines - but go as fine as you like). This scan should be approximately 3-5μm square. Include a capture of this scan in your report.

2. Determine the lateral (x- and y-axis) dimensions and the vertical (z-axis) height of the object or feature. Indicate these either directly on the image, or in the image caption/description.

3. Comment on what you think the resolution limit of your AFM is (lateral and vertical). Your noise measurements should help your thinking. How small of a "bump" can be resolved?

4. (BONUS) Time permitting, try scanning very fast (10Hz or more — WARNING, this may cause some unpredictable results). How does this affect resolution, image quality and stability, and other performance considerations?

7.3   Elastic modulus

Here, you just need to calculate the elastic modulus of the two elastomer samples (we'll call them PDMS-10 and PDMS-25), and to determine which is softer.

7.4   Measuring Boltzmann's constant

1. Carry out the data analysis of Section 6.4 and summarize your results with a plot showing the noise spectra for both cantilever sizes, and the fit functions for them. Include the relevant MATLAB code.

2. Your report should include the values of spring constants k, resonant frequencies $f_0 = \omega_0/2\pi$, and quality factors Q, that you found for both cantilevers. Explain which method of calculating spring constant you preferred, and why. Finally, what value of Boltzmann's Constant k_B did you find from each device size.

3. How close were you able to come to the actual value of k_B, which is 1.38×10^{-23}J/K?

   (You will be graded much less on the accuracy of your k_B value than on the quality of your analysis.)

4. To understand what factors influence the accuracy of your measurement, consider the sources of uncertainty and "noise" in this experiment:

   – Calibration accuracy

   – Geometrical correction factor

   – Spring constant calculations, including geometrical (L, b, h) and material (ρ_c, E) parameters

   – Parameters derived from the PSD curve fit (Q, ω_0)

   – Any noise in the system other than thermally-driven vibrations of the cantilever beams

   You should quantitatively estimate the uncertainty/noise in these various components and steps of the k_B calculation (if you aren't sure how to approach this, you'll probably find it helpful to discuss with your lab instructor). Paragraphs of explanation for each item are not necessary - briefly mention key considerations, and *focus on the major sources of error/uncertainty.*

   Questions that'll help you: How confident can you be of the various values? Within $\pm 10\%$ or $\pm$ a factor of 2. If a particular variable has a 20% error, how does that propagate to k_B? i.e. what's the dependence of k_B on that variable? Based on these estimates, which one or two items *dominate* the error in the measurement? (i.e. improving anything else won't help until this dominant source of error/uncertainty is dealt with).

Lab Problem

You learned in lecture about the minimal force detectable with a microcantilever. The force was assumed to have a white spectrum, and its PSD (at all frequencies) had a value of:

$$S_{F0} = \frac{4 k_B T \cdot k}{Q \, \omega_0} \ .$$

Using analytical expressions for spring constant and resonant frequency, given by

$$k = \frac{E b h^3}{4 L^3} \quad \text{and} \quad \omega_0 = \frac{h}{L^2} \sqrt{\frac{E}{\rho_c}} \ ,$$

answer the following questions:

(a) Calculate the minimum detectable force in a 100 Hz bandwidth using the 350 μm beams from this lab.

(b) How does this value compare to typical forces in biological systems (e.g. antibody/antigen binding, DNA hybridization, interdomain forces in proteins, etc.)? Use whatever knowledge you may have, or find one or two examples, but don't spend too long doing literature searches.

(c) Which cantilever geometrical parameters affect the minimal detectable force? I.e. for smaller forces, do you need a cantilever that's shorter? thinner? wider? Comment on which parameter variations will have the greatest effect.

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.