The teaching AFM: Part 1
Alignment, Calibration, and Noise

1 Objectives

1. Learn the function of our class AFM system's components and the relationship between them.
2. Practice aligning the AFM optics.
3. Learn how to calibrate the AFM to relate its output signal to physical cantilever deflection.
4. Measure the mechanical vibration noise in the AFM system with the cantilever free and in contact.

2 The Atomic Force Microscopy (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. During the lab, we will talk about the operational principles of a standard AFM. It may also be helpful to review some of the References in Section 4 at the end of this module. A photo of our AFM setup is provided in Figure 4 at the end of this section for you to refer to as you learn about the instrument.

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 4 at the end of this section 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: laser power supply, photodetector, and piezo power supply.*

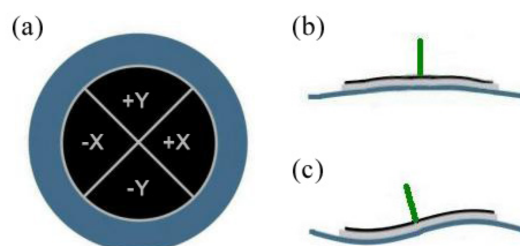


Figure 1: This is a schematic of the piezo disk used to actuate the AFM's sample stage. The circular electrode is divided into quadrants as shown in (a) to enable 3-axis actuation. When the same voltage is applied to all quadrants, the disk flexes as shown in (b), giving z-axis motion. Differential voltages applied to opposite quadrants, produce the deflection 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.

2.2 Scanner 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, so whenever we talk about moving the tip relative to the sample, 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 1, which is divided

into quadrants and flexes to move the post on which the sample stage rests. The piezo disk is controlled from the MATLAB scanning software, and you will learn more about this in the next module.

For motions along the z-axis (vertically), 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 coarse positioning along the x- and y-axis, the micrometers on the positioning stage are used.

WARNING: The AFM probes can be broken by running them into the sample — avoid "crashing" the tip into the surface, or worse bumping the stage into the die or fluid cell. Use caution when moving the sample up and down.

2.3 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.

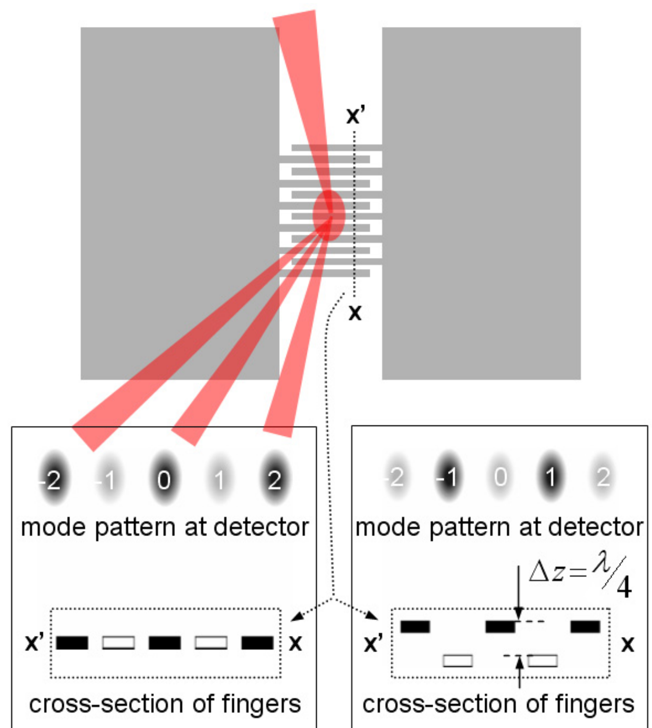


Figure 2: A drawing of the interdigitated (ID) interferometric fingers, with the detection laser shown incident from the top of the figure. When the finger sets are aligned, as in the left box above, the even-numbered modes are brightest, and odd modes are darkest. When they displace relative to each 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 2. 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

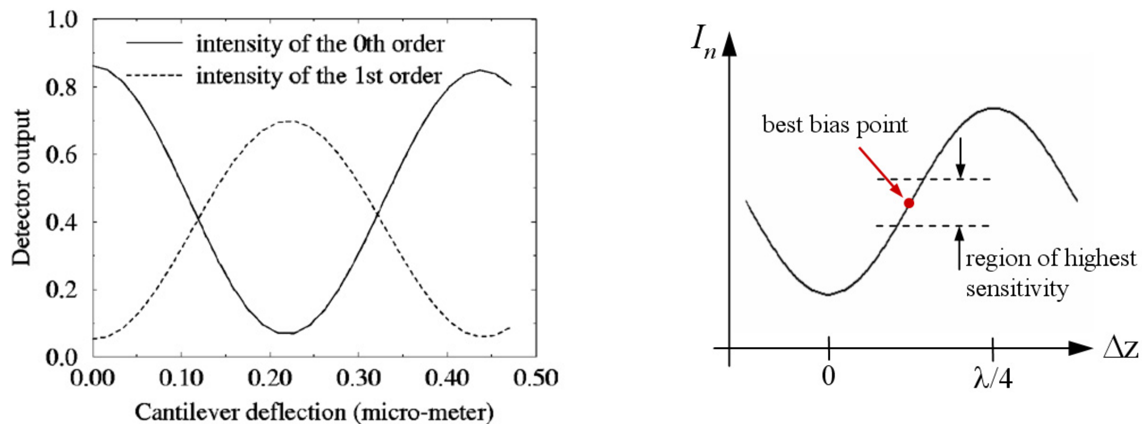
intensity I of odd order modes vs. finger deflection Δz has the form

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

and for odd modes, the sine is replaced by a cosine. The plot in Figure 3(a) shows graphically the intensity of two adjacent modes as a function of displacement.

This nonlinearity causes the sensor's sensitivity to 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 3(b). This can be done with our devices by simply adjusting the position of the laser spot side to side on the finger grating – the grating is not perfectly flat due to residual stress, and thus provides a simple biasing method.

At this point, it's worth remembering the distinction between *calibration*, *sensitivity* and *resolution* – terms which will be used frequently in the context of the AFM, but whose precise meaning isn't always made explicit. Be sure you're clear on the differences between them.



(a) The non-linear intensities of the 0th and 1st order modes plotted as a function of cantilever displacement.

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

Figure 3: The characteristic output of the ID interferometric sensor.

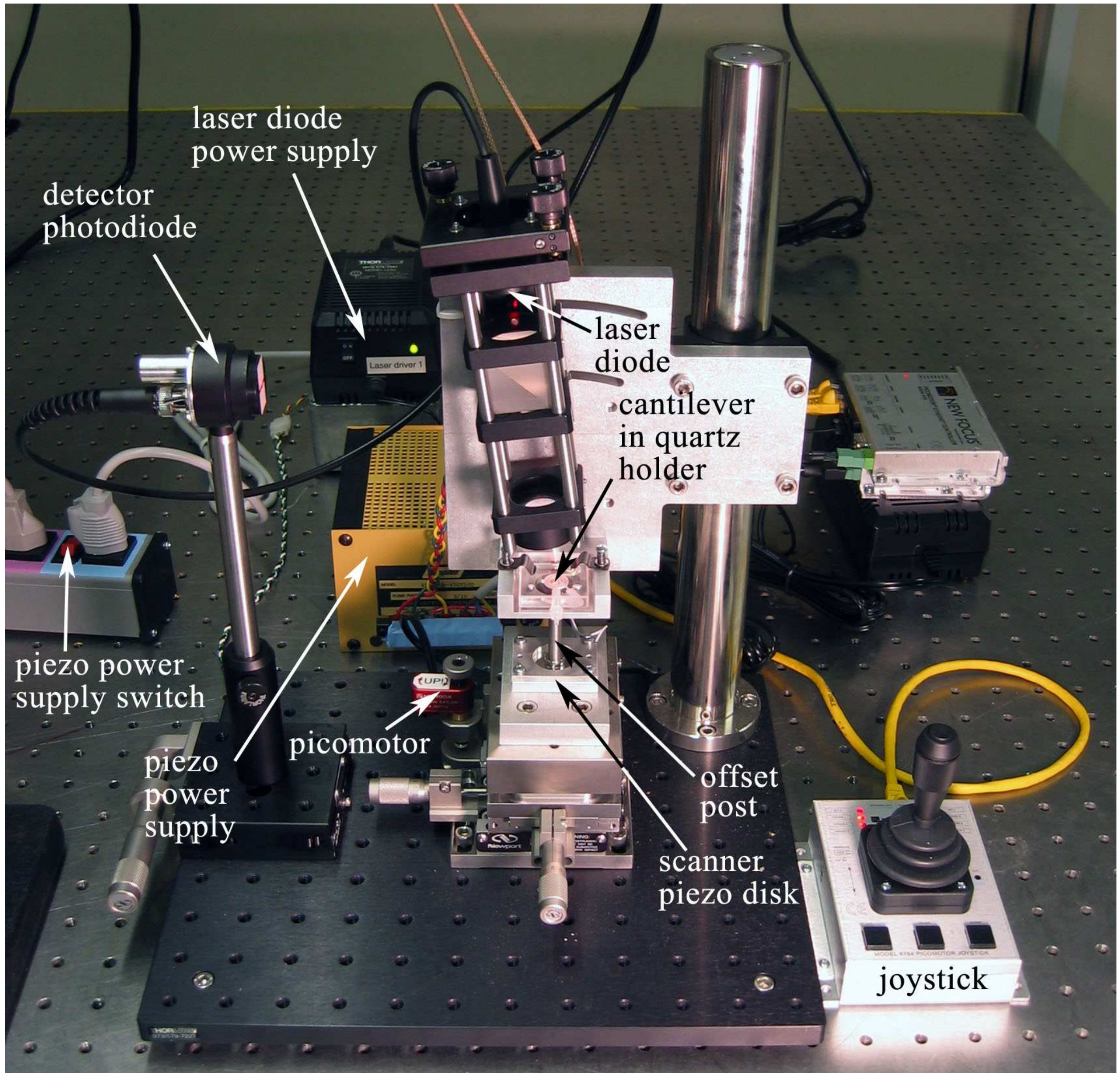


Figure 4: The AFM setup, with major components indicated.

3 Lab Procedures

3.1 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 laser mount, until it hits the interdigitated fingers (use the cantilever schematic in Figure 5 as a reference).

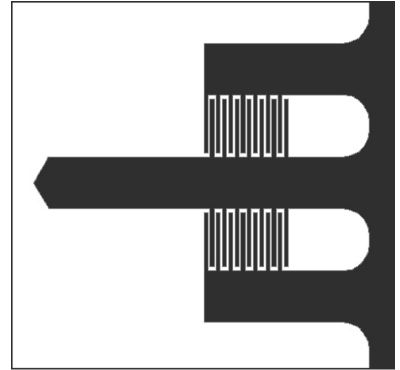


Figure 5: Plan view of the imaging cantilever geometry. The central (imaging) beam dimensions are length $L = 400\mu m$, and width $b = 60\mu m$. The finger gratings begin $117\mu m$ from the base and end $200\mu m$ from it.

When the laser is focused in approximately the right position, the paper "screen" around the slit on the photodetector will allow you to see the diffraction pattern coming out of the beam splitter. Observe the reflections 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.

Before engaging the AFM, start the piezo z-modulation scan in the MATLAB software using the default frequency and amplitude of 2Hz and 8V. (Note: Be sure the mode switch on the rear of the AFM head is flipped down to "force spec. mode," and make sure to turn on the piezo power supply using the color-coded switch on the table.)Carefully bring the tip near the surface, first 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.

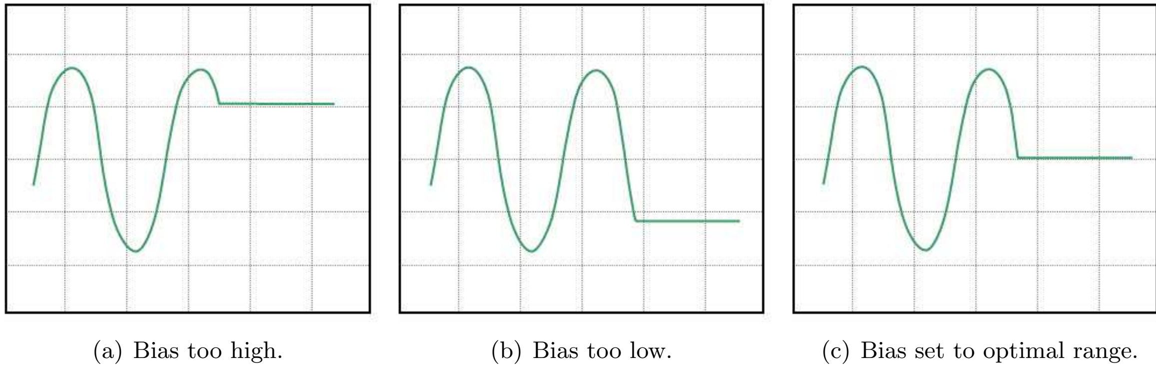
3.2 Calibration and biasing

If you observe the intensity signal on the oscilloscope in x-y mode, you should see something like the plots shown in Figure 6: 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.

For the types of noise measurement that we will do, the signal needs to be at the maximum-slope position along the output curve when it's not in contact with the surface. If necessary, use the offset on your 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 6(c).

To relate the mode intensity output to a physical deflection, we can take advantage of the fact that a mode's brightness goes from fully bright to fully dim as the fingers deflect through a distance of $\lambda/4$. This way, by relating this displacement to the amplitude of the sin^2 curve, you can determine the cantilever sensitivity in nm/V.

You will also need to multiply the calibration by a correction factor to account for the location of the diffraction fingers with respect to the tip of the cantilever (you can assume that the deflected shape of the cantilever fits a second-order polynomial).



(a) Bias too high. (b) Bias too low. (c) Bias set to optimal range.

Figure 6: Proper setting of the bias point for the measurements we'll make in this lab.

4 Helpful References

1. Basic Operating Principles of AFM.

   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/`

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

3. 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.

4. 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.

The teaching AFM: Part 2
Imaging with the AFM

1 Objectives

1. Learn to set up and prepare the AFM for imaging.
2. Image several different samples with the AFM.
3. Use the AFM to measure physical dimensions of imaged features.
4. Use the AFM to measure the elastic modulus and surface adhesion force of a sample.

2 The AFM Scan Control Software

The software that interfaces with the AFM is an application that runs in MATLAB. It 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 1 shows a screen capture of the scanner control window, and an overview of its operation is provided below.

2.1 Overview of Controls

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 2.4 below.

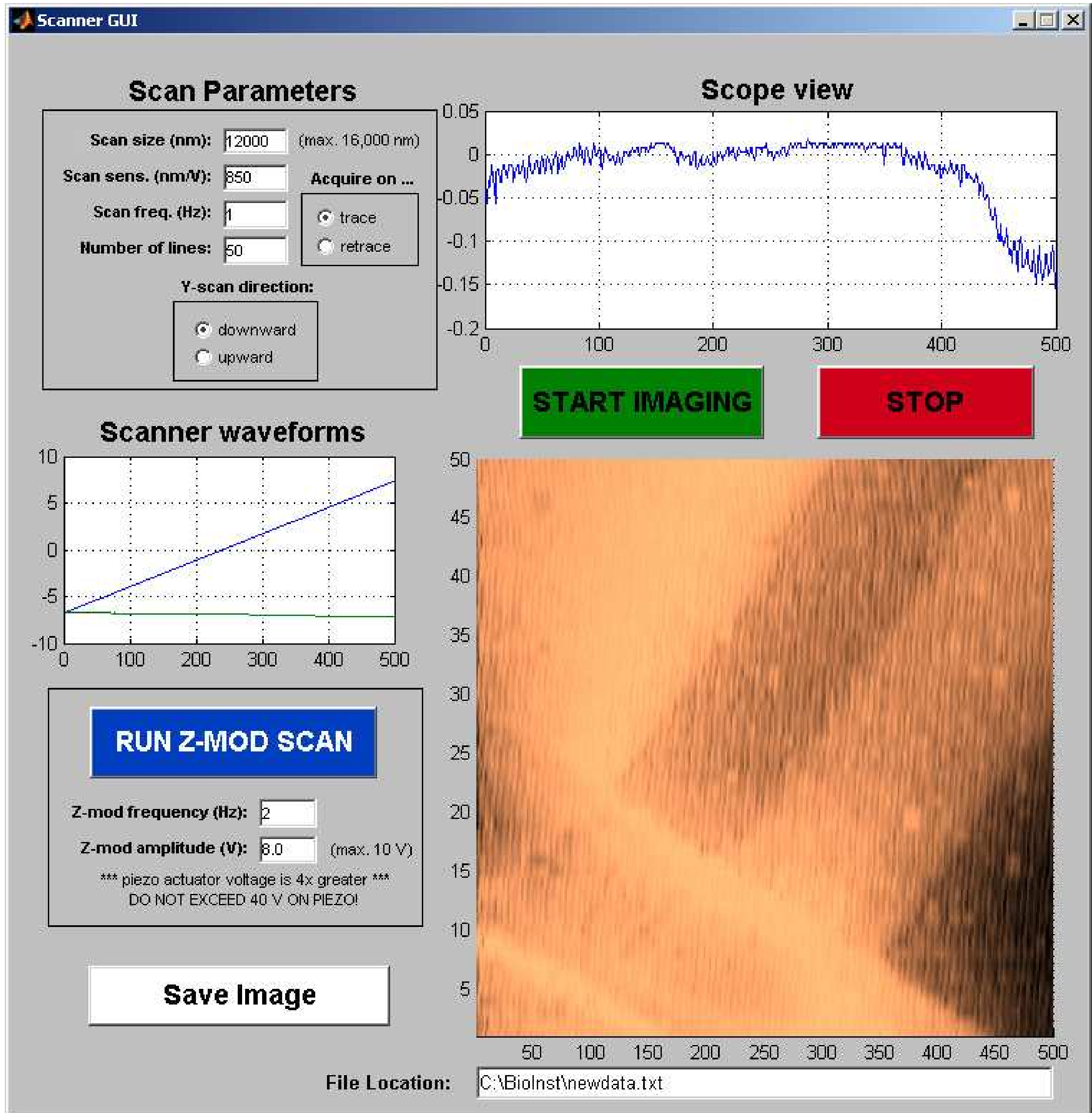


Figure 1: The Scanner GUI window. The AFM is scanning a $12 \times 12 \mu m$ area, at a rate of one line per second, and is currently near the bottom of the image.

2.2 Cantilever probes for imaging

The probes we will use for imaging are shown in Figure 2 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.

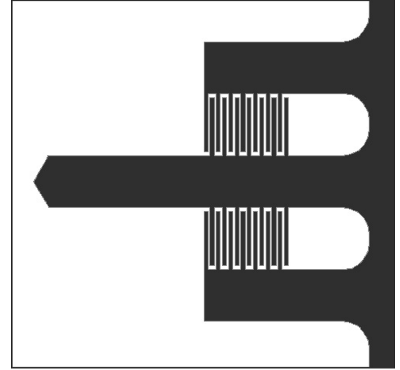


Figure 2: Plan view of the imaging cantilever geometry. The central (imaging) beam dimensions are length $L = 400\mu m$, and width $b = 60\mu m$. The finger gratings begin $117\mu m$ from the base and end $200\mu m$ from it.

2.3 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 gradually rastered up and down the image area. To use this mode, the switch on the back of the AFM must be flipped upward. It is important to remember that the maximum scan area is only abut $15\mu m$ square, and adjusting the position of the sample under the tip requires only the smallest movements of the stage micro-positioners. Also keep in mind that there is a delay after START IMAGING is pressed and before the scan begins, as the actuator drive signals are buffered to the I/O hardware.

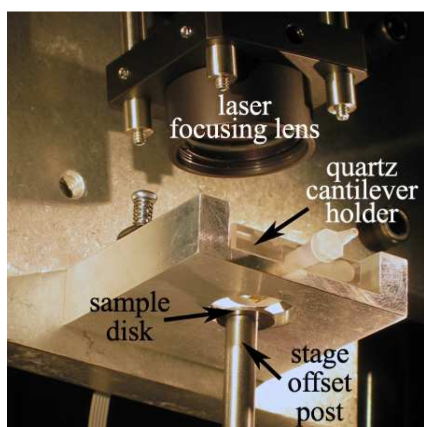
2.4 Z-mod (force spectroscopy) Operation

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. Besides being helpful during calibration and biasing of the readout laser, 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).

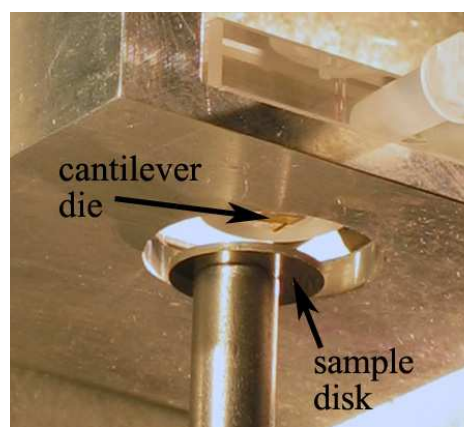
3 Lab Procedures

3.1 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 damage the probe.

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 3(b) above the sample disk is visibly off-center).

Once the sample is in proximity to the cantilever, follow the procedures that you learned in the previous lab module to align the laser, engage the tip with the surface, and view a force curve.

3.2 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 bring the probe into contact 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 the previous module.

For imaging, the bias does not necessarily need to be set at maximum sensitivity when out of contact. Rather, the signal should be at *maximum slope (in the middle of its travel range) when the probe is engaged, and exerting a small amount of force on the surface.* Remember that 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 and *E. coli* bacteria on a silicon dioxide surface. 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 also 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).

Measuring Image Dimensions

When it's necessary to determine the size of imaged features, the *Scan size* control is your most handy reference. This gives the size of the image square, which can be quickly related to features in the image. If needed, the scan size can be changed to make imaged features fit in the image window as desired.

NOTE: the *Scan sensitivity* is pre-set to an approximate value, but if you would like more precise lateral feature measurements, it's worth verifying the calibration of the *Scan sensitivity.* 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, for feature height (z-axis) measurements, they can be made by observing the Scope View of the AFM software. The waveform displayed as the AFM scans over a feature shows the feature height dimensions as voltages — the calibration that you've done to these voltages to nanometers of tip displacement will give you feature sizes.

3.3 Elastic Modulus Measurements

[*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 2, 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.* in *Langmuir.*

As seen in this paper, samples with different elastic moduli change the slope of the in-contact portion of the force curve, when using the optical lever sensor. For our non-linear ID sensor, the equivalent of the changing 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 4(a) below).

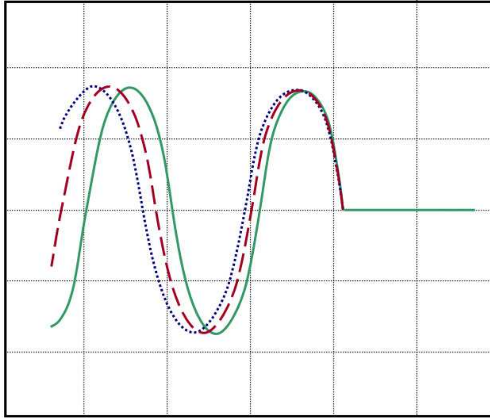
The approach for measuring modulus is to first take a force curve on a hard reference sample, considered to have infinite hardness. We will use a bare silicon nitride surface. This allows us to determine how the x-axis signal corresponds to stage movements.

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. 4(b)).

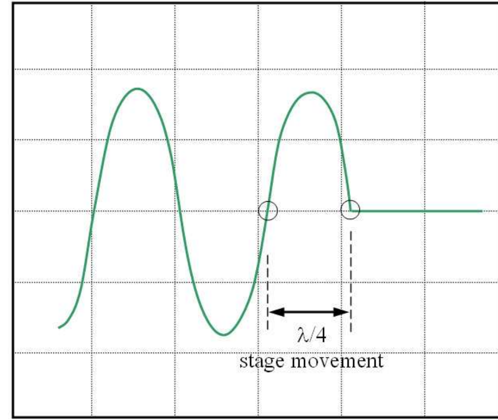
After your measurement of the hard sample, switch to the more compliant PDMS elastomer samples, and run force curves on them.

Make sure to capture the plots of the force curves for later analysis, described in Section **??**.

To get good force curves:



(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

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.

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.

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 $250\mu m$ 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.

The teaching AFM: Part 3
Thermomechanical noise and Boltzmann's constant

1 Objectives

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

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

2 Background

2.1 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:

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

where $\langle \Delta z^2 \rangle$ 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

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

in whichω_0 and Q are the (angular) resonant frequency and quality factor, respectively. At low frequencies, ($\omega \ll \omega_0$) this expression yields what's called the "thermomechanical noise limit" (see Figure 1 for an illustration):

$$\delta = \sqrt{\frac{4k_BT}{Qk\omega_0}}.$$

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 $\langle \Delta z^2 \rangle$, and (3) its spring constant k.

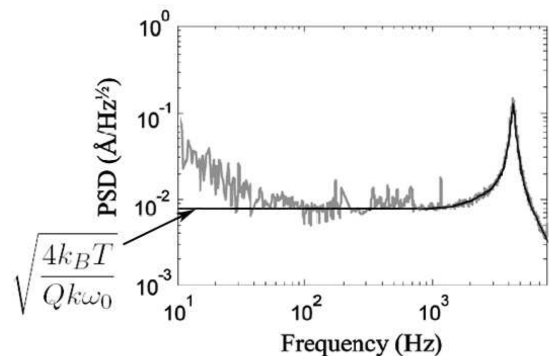


Figure 1: A data plot of a cantilever's noise spectrum, with an ideal transfer function $G(\omega)$ fit on top (dark line). Note that $G(\omega)$ is flat at low frequencies, at the thermomechanical limit, as indicated. In contrast, real data has more $1/f$-type noise present at lower frequencies (see Section 3.2).

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 **??**).

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 measurements.

3. The **spring constant** (stiffness) can be analytically calculated from geometrical parameters in two ways (see Section 2.2 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[1], and it relies on measuring the cantilever's resonant frequency:

$$k = 0.2427 \rho_c h b L \omega_{vac}^2,$$

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 $E = 250\text{GPa}$. As mentioned before, for complete cantilever dimensions, see Figure 2 in Section 2.2.

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

2.2 Cantilevers for Thermal Noise Measurements

The probes you will use for this lab are different than what you've used for imaging and force measurements. Their plan view is shown in Figure 2.

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 in Figure 2 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.

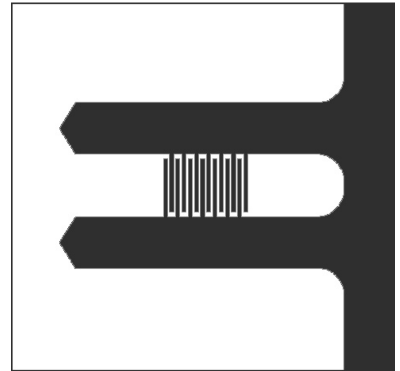


Figure 2: Plan view showing the geometry of a differential cantilever pair. We assume that because the beams are fabricated so close together, their material properties and dimensions are identical.

There are two sizes of cantilever pairs available. Choose either size to make your measurement. 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 fingers are between $93\mu m$ and $175\mu m$ from the base. The width and thickness of all of the cantilevers is $b = 50\mu m$ and $h = 0.8\mu m$, respectively.

3 Lab Procedures

3.1 Alignment, Calibration, Biasing

By now you're familiar with aligning, calibrating, and biasing. The major difference in this case is performing the z-modulation scan for 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. To do this, you'll have 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. A z-mod scan should then deflect only one of the beams, giving us the calibration curve we want.

A few additional remarks to guide you:

– Remember to flip the "imaging/z-mod" mode switch on the back of the AFM to the proper position.

– Reflections of the laser from the edge of the substrate can 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.

– As you've done several times, bias the force curve for maximum sensitivity when out of contact — the flat portion of the curve should be placed midway between the maximum and minimum.

3.2 Recording Thermomechanical Noise Spectra

Once you're happy with your calibration and biasing, withdraw the lever's tip from the surface, making sure that the bias point stays where you set it. 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 50-100Hz. 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 $2\times$ higher than the highest frequency of interest, or about $10\times$ 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.

3.3 Data Analysis with MATLAB

Once you bring your saved PSD data 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, which does a least-squares curve fit, as you may have guessed. We're aiming to do something similar to what you see in Figure 1, 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 on page 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);
```