



MECHANOBIOLOGY MODULE 1 (MechB1): Scanning Epithelial Cells in Air

Location: L512 Digital Computing Laboratory (DCL)

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Purpose and Expected Outcome:

Examine epithelial cells extracted from cheeks of participants. Students will measure and analyze mechanical properties of the surface of epithelial cells as well as observe optical and topographical appearance.

Overview of AFM:

The structure of eukaryotic cells is controlled by a dynamic balance of mechanical forces exerted by the cytoskeleton. Growth, cell cycle progression, gene expression, and other cell behaviors are sensitive to changes in the cellular mechanical force balance. Measurements of the spatial distribution and changes in viscoelastic properties of living cells will provide valuable insights into these processes. The atomic force microscope (AFM) can be used to image living cells under physiological conditions in a nondestructive manner [1]. The AFM can also be used to study material properties by collecting force curves on the surface of the cell. A force curve is a plot of the force applied to the AFM tip as the sample is approached and pushed against the tip. In principle, this plot gives the force required to achieve a certain depth of indentation (deformation) from which viscoelastic parameters can be determined. By performing multiple force curves along the surface of the cell, we can create high-resolution 2-D maps of mechanical properties such as stiffness and adhesion [2].

Head: this is what contains the optical detection system and controls the Z piezo actuation of the cantilever; it contains thumbwheel adjustments to position the laser and zero the deflection on the position sensitive diode/ detector (PSD); the top view optics position knobs and focus wheel to view the tip from above; has three independent legs that adjust height of head relative to sample. Use two hands to lift the head due to its weight.



Controller: The digital controller contains a DSP and FPGA, and software controlled analogue 'cross point switch' for rerouting internal and external signals for custom experiments. The front panel of the controller is where: the power switch is; the key to turn the 'laser' on/ off; the 'Hamster' wheel is located to fine tune imaging/ control parameters; BNCs for advanced input/output signal access. The controller communicates with the PC via USB interface.



Software

To use the Mode Master, just click on a function button- for example, say you want to imaging in Contact mode: Click 'Topography' button; this will bring up a panel with all the different topography acquisition modes.

Module Outline and Workflow:

For the first experiment, we will simply image epithelial cells in air. The sample preparation is very simple and requires almost no facilities. Take a Q-tip and scrape the inside of your cheek (some physicists have been known to use their finger). Rub the saliva-soaked Q-tip on a glass slide. Use a marker to delimit the area of interest, drawing a circle on the other side of the glass. Let the sample dry for five minutes. If you inspect the slide under an optical microscope and a 10X objective, you should be able to see the cells.

After drying, you can position the cells under an AC mode cantilever and image them using the following standard protocol for air imaging.

Equipment, Materials, and Supplies:

Cotton swabs

Glass slides

AFM Tips – AC mode 150, Resonant Frequency – 150 KHz, Force constant 5 N/m

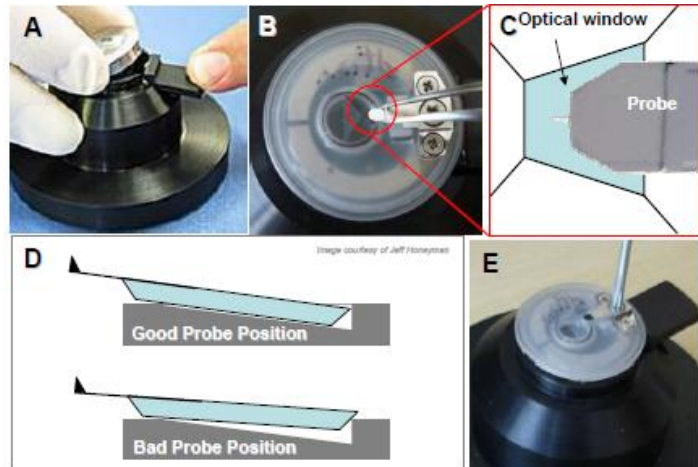
Procedure:

Step 1: Loading the probe

The MFP-3D™ cantilever holder accepts most brands of commercially available probes. The quartz window is resilient from tweezer scratches. It can be cleaned by spraying with 70% Ethanol and spraying dry with canned air.

Proper probe loading:

- A. Load the cantilever holder into the cantilever holder stand.
 1. Loosen tongue clamp screw with provide Phillips head screwdriver
- B. Use tweezers to position cantilever in middle of polished quartz window.
- C. Proper position of probe in quartz window.
- D. Proper position of probe in pocket. For best results, DO NOT push probe chip substrate all the way back in the pocket: it can cause the probe chip to lift off the floor of pocket, compromising the deflection signal



- E. Screw tongue clamp- *no more than finger tight* - with a Phillips (00x40) screwdriver.

Step 2: Install cantilever holder into MFP-3D head:

- Put cantilever holder into the MFP-3D™ head- it is easiest to put the ball bearing on release lever side (red arrow in figure) first, then ease the holder in from the back. Make sure the cantilever holder is parallel to the top of the head; otherwise it is not properly seated. The 'pogo' pins (red circles) used to get signals from the cantilever holder can be easily bent with excessive force.



Step 3: Instrument set-up and imaging

Keep in mind that these values are only guidelines, and need to be adjusted to each sample, each condition (air, buffers...) and each cantilever. Here are some general guidelines to follow:

Setting Up the AFM for AC Mode in Air

For AC imaging in air, we will use a silicon cantilever (AC150)

1. Center the cantilever in the holder as in "Loading the Probe".
2. Return the cantilever holder to the head.
3. Flip the head over and place head on scanner stage.

Placing Head on Scanner Stage:

Once a probe is properly installed in the cantilever holder, the superluminescent diode (SLD) can be aligned using the CCD camera.

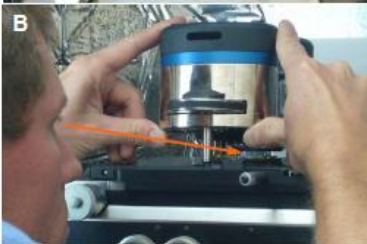
1. Lift the head with two hands and place the back two legs in the kinematically machined divots on the MFP-3D baseplate.



2. Move hands so thumbs are under the front of head, and slowly lower head towards stage using back legs as pivot point; continually monitoring the tip – sample separation. If it looks as though the tip will crash, lift/ pivot head back up and adjust legs down to increase tip-sample separation, and repeat process.

Installing the head onto base plate over sample:

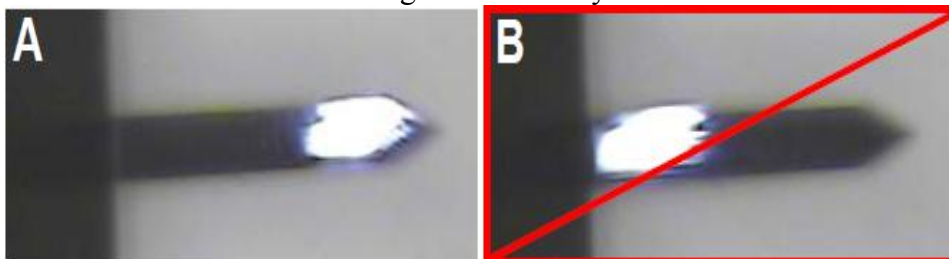
- A) use two hands to lift head, set back two legs onto base plate first
- B) gently lower front leg down, constantly monitoring tip sample distance to avoid tip crash. * Position the head above the sample, lowering the head until it is 1-2 mm above the sample surface. Make sure the head is approximately leveled.



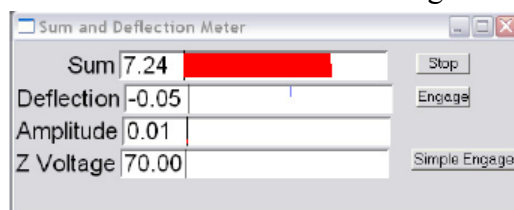
3. Position the spot on the end of the cantilever. We will use top view optical alignment.

4. Locate the SLD spot on the substrate

- Move LDX thumbwheel towards probe chip- the SLD spot is now reflecting off the cantilever; there is a great deal of refracted light, presumably due to the low fiber light illumination level. Slight adjustment in LDY may also be needed to maximize 'Sum' voltage in S&D meter.
- When increasing the fiber light illumination, the spot is more apparent, and the amount of refracted light in the CCD image decreases.
- Regardless of how the SLD spot is aligned on the back of the cantilever, what is desired is to have the spot towards the end of the cantilever to maximize the optical lever sensitivity. The sum should be set to roughly 95% of the maximum value for the highest sensitivity.



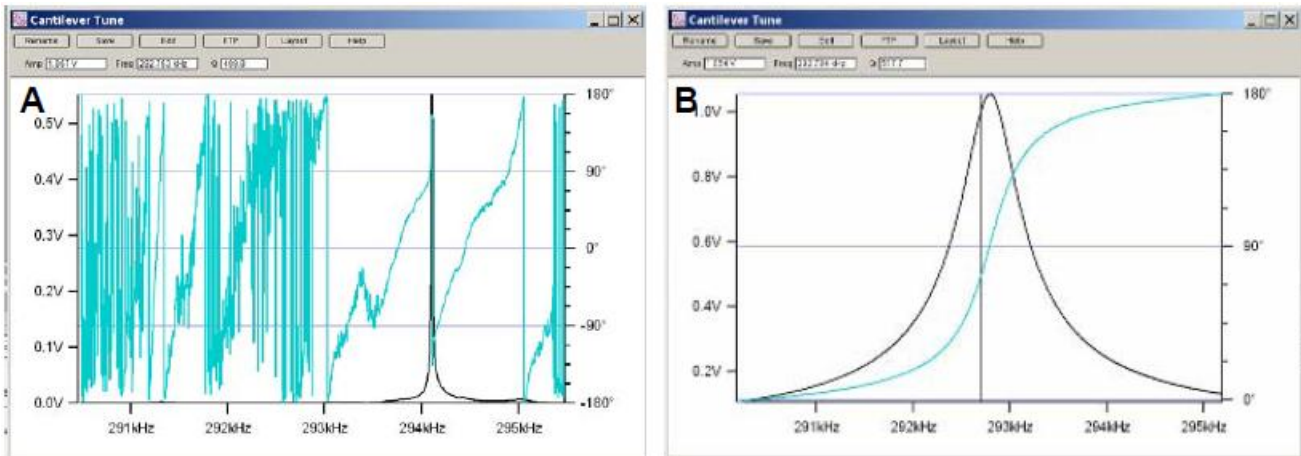
5. Zero the deflection signal using the Photo-Detector (PD) thumbwheel on the side of the head. At this point, the sum and deflection meter should look something like the following.



6. The next step is to tune the cantilever. First, click on the “Tune” tab on the Master Panel. You should see the following panel appear:

For an AC150 lever, set the “Auto Tune Low” value to 250 kHz and the “Auto Tune High” value to 400 kHz. Enter “-5” for the target percent. Once those values have been entered, click on the “Auto Tune” button. After a few seconds, a graph similar to the one below should appear:

Note that the target amplitude can be chosen before tuning by entering a value in the “Tune” panel. *This value should be of about 1V in the case of biological samples.*



This is what a proper tune should look like. **A** shows how it will look during the tune, **B** shows the final product.

7. In the Main panel, select a setpoint of 0.8 Volts (if your target was 1V) and a feedback IGain of 10. Click the “Engage” button on the “Sum and Deflection Meter” panel to turn on the feedback loop. You should see the Zpiezo voltage extend towards the surface (red bar) as the feedback loop attempts to drive the amplitude towards the setpoint of 0.8 Volts

8. Using the large front thumbwheel, lower the head. When the amplitude reaches 0.8 Volts, the computer beeps. You should see the Z Voltage indicator on the “Sum and Deflection Meter” panel decreased from its maximum value of 150 volts. Lower this value until the red line is almost completely gone. This allows the greatest range of motion in the positive and negative directions.

At this point you are ready to start imaging. Click “Do Scan” on the “Main” panel and off you go!

ATTENTION:	If the Sum drops more than 50%, you have crashed the tip. Time to replace it and try again!
NOTE:	The system beeps when the feedback determines the Z voltage at <130V, which communicates the tip is engaged.

Is the acoustic cabinet door still open? Probably.

Follow steps to preserve the tip’s apex while shutting the door:

1. Use the Hamster to decrease the Set Point voltage to pop the tip off the surface.
2. Close the door slowly (no whoosh) and latch.
3. Increase the Set Point voltage with the Hamster again to hard engage.
4. Proceed scanning.

Tuning image parameters:

Once the tip is engaged on the surface, imaging can commence.

Click the 'Do Scan' button on the Main tab of the Master panel. This will move the tip to the corner of the scan area, and begin scanning.

Once scanning commences, the tip's tracking of the surface tip is generally very poor. This can be seen in the individual Trace and Retrace fast scan lines below each of the image channels where one side of the feature has very poor tracking. Typically, three parameters should be adjusted first in this order

1. The **Set Point** voltage generally must be adjusted. Decreasing the Set Point voltage value increases the force applied to the sample. Higher Set Point voltages (lower force), will help preserve the tip apex, but may not allow proper tracking of the surface.



2. The **Drive Amplitude** can also be adjusted to increase the amount of Drive Amplitude applied to the shake piezo (and hence, cantilever); advantages of increasing this can be maintaining the tip during the scan (especially when imaging sticky samples).

The trade off by increasing the Drive Amplitude is beating the tip apex harder against the surface causing oscillations in the image (especially at feature edges); The tradeoff of lowering the Set Point voltage is applying more force to the sample.

3. The **Integral Gain** should be adjusted such that the surface is tracking well; things to avoid are decreasing it to a value that doesn't allow the feedback to track the surface well, OR too high that oscillations are apparent in the image and trace/retrace scan lines below the images. One of the best approaches to adjusting the Integral gain is to increase it until there is a 'ringing' seen in the (re)Traces lines below the height image. Then decrease it until this ringing goes away.

Monitor how the tip's tracking improves by looking at how well the trace and retrace line scans compare to each other. Note that they do not have to overlap exactly (because they are slightly offset in the software display), but they should have similar shapes/slopes per given surface feature.

What were your final settings for

Setpoint: _____

Drive Amplitude: _____

Integral Gain: _____

Other

observations: _____

► Perform a 50 μm scan and a 10 μm scan within that scan area.

► Make a 3D plot from your height data by clicking "3D" on the image menu bar once scan has completed.

Igor Layout

We will create reports using the Igor layout feature of the software. All windows have a send to layout option either through “export to layout” buttons or “Ly” on any scanned image.

Save Graphics:

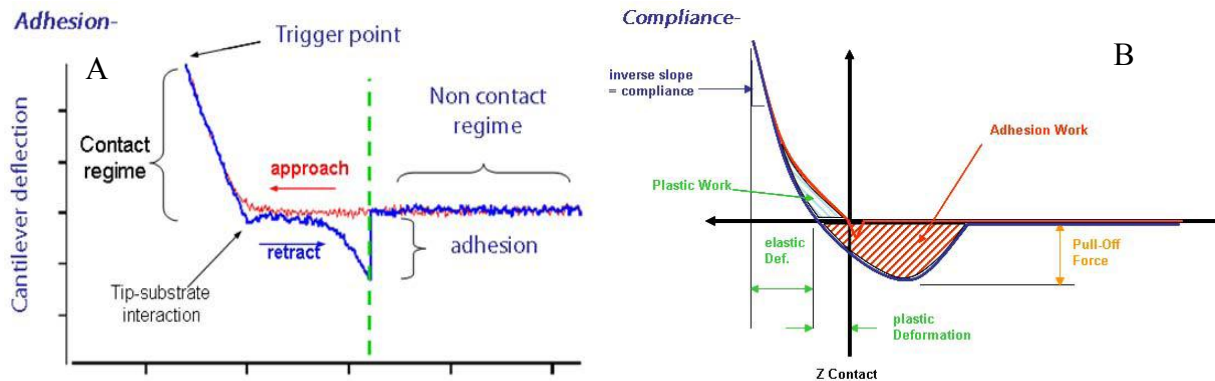
▪ If you happen to just want a screen shot of one of the windows, you can make it the forward most window, and goto File **Save Graphics**. You can alter the size and resolution in the panel.

► Include images of your thermal tune, height trace, and 3D plot in your Igor Layout

Step 4: Force Mapping

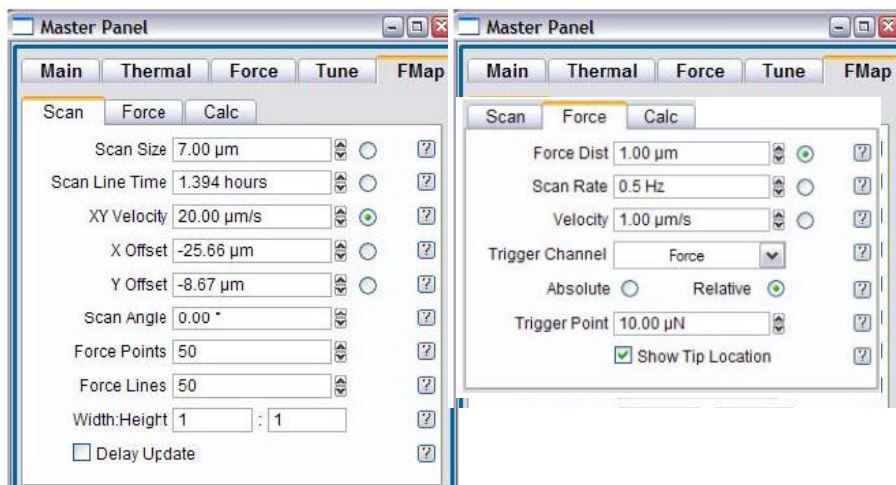
There are two major classifications that most force spectroscopy experiments measure:

1. A pulling event in which the tip interacts with the surface, and some adhesion dissociation event between the tip and surface is measured on the retract cycle (A).
2. The tip pushes into the (material on the) surface to measure compliance (B).



Using the Mater Panel, go to FMap. Select a scan size of 10 μm . Add a name to the file such as “Last_First_FMap”.

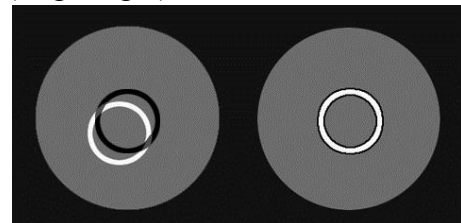
Adjust the settings on the Force subtab to look like the following: We will now create a image using the height data and FMap coloring to see any correlations between topography and stiffness.



Step 5: Combine AFM images with microscopy images.

Phase-contrast imaging is useful to see more details on a translucent sample, such as a cell. Remove the standard light illuminator and replace it with the phase annulus (finger tight).

Switch the filter wheel to CT for phase imaging. You should now see two rings when looking through the binoculars (a light one and a dark one). Adjust the knobs on the illuminator to adjust the floating ring and align them.



Once aligned, you can switch the filter wheel back to observe the cells in phase mode. You should see raised cells with clearly marked edges when compared to the standard light illumination. Using the software for the Olympus X71 camera, obtain an image of the cells in phase.

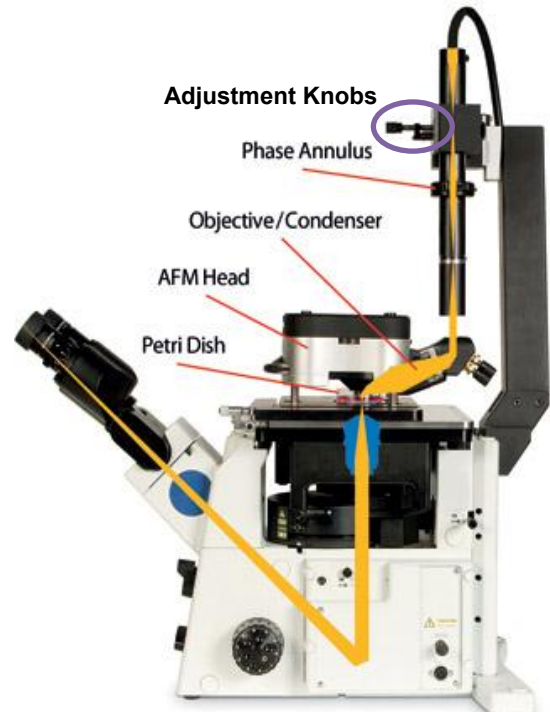
- ▶ Save file as ECPH_Lastname_Date.jpg
- ▶ Include images of your phase image in your Igor Layout

IMAGE OVERLAY

Opening and Scaling Images

- 1) With the AFM software running, open the Video Overlay panel by selecting “Video Overlay” from the Image Processing menu. The Video Overlay panel will appear. Set the Mode to “offline”.
 - 2) Select the appropriate magnification for your image.
 - 3) Acquire or open the optical image.
 - 4) You are now ready to perform the registration. The best method for this is to identify a feature that you can clearly see with both images. Select the feature to be aligned by placing a cursor on the location in both images.
 - 6) Once common features have been selected on each image, you need to perform the alignment. Under the “Align” section of the panel, ensure that the method drop---down box is set to “Feature”. Click the “Do It” button to begin the automated alignment process; this should only take a second or two. At this point there will not be any change in the displayed images.
 - 7) To display the overlaid image, click the “Overlay” button in the Overlay section of the panel. If the alignment was successful, you will see your AFM image overlaid onto the optical image.
- *To correct for misalignment, you can slightly move the cursors on either the AFM or the optical image using the arrow keys.

- ▶ Include images of your overlay (AFM + phase) in your Igor Layout



Related References:

1. Schaus and Henderson, 1997 S.S. Schaus and E.R. Henderson, Cell viability and probe-cell membrane interactions of XR1 glial cells imaged by atomic force microscopy, *Biophys. J.* **73** (1997), pp. 1205–1214.
2. Emad A-Hassan, William F. Heinz, Matthew D. Antonik, Neill P. D'Costa, Soni Nageswaran, Cora-Ann Schoenenberger, Jan H. Hoh, Relative Microelastic Mapping of Living Cells by Atomic Force Microscopy, *Biophysical Journal*, Volume 74, Issue 3, March 1998, Pages 1564-1578.
3. Fuierer, Ryan, Procedural Operation ‘Manualette’, Asylum Research, Santa Barbara, CA, 2008.