Atomic Force Microscopy

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The Nuts and Bolts of the Fernandez™ brand Atomic Force Microscope

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Introduction

This manual is for users of the Fernandez™ brand atomic force microscope. Specifically, this AFM is used to publish the works performed by members of the Fernandez Lab, currently at Columbia University in New York City. Several other groups have built and developed their own versions of the AFM. This manual focuses on the current setup that unfolds proteins using several different methods (constant piezo velocity, constant force, and linear ramp of force) and the newest method, protein folding. The plan for this manual is it to be divided into several sections encompassing general usage, development, and troubleshooting. This manual will <u>not</u> be going in to major details in how to perform experiments nor analysis. Please visit the Fernandez Lab website at http://www.columbia.edu/cu/biology/faculty/fernandez/FernandezLabWebsite/.

You may contact the author of this manual at raganr@gmail.com if you have any questions.

Chapter 1

GENERAL USAGE

The atomic force microscope is used to mechanically study single molecules. The lab has developed specific biological tools that allow us to be certain that we are studying single molecules. We use several different methods to study the mechanics of molecules. We stretch molecules using a constant velocity regime in which we attach a molecule to the cantilever and pull away from the surface using the piezo at a constant velocity. We pull on polysaccharides using a linear ramp of force. We unfold proteins by shifting the unfolding probability using a constant force regime. Finally, we investigate the folding pathway of proteins using a double pulse of constant force.

Each method has its own nuances and annoyances. These will only be found by using the machine on a daily basis and developing your experiment. There are really no guidelines in how to proceed with an AFM experiment. The protein, the surface, the concentration, the humidity, the wind velocity, the amount of sleep you had the night before, the speed of a monarch butterfly's wings during migration (ahh, chaos theory) all seem to affect your pulls.

Good Luck.

To use the AFM perform the following:

1. Apply sample to the piezo. It is usually place upon a gold disk which is attached to the top of the piezo.
2. Focus laser. Try to place the laser near the center of the tip of the cantilever. At this point, do not worry about the A+B signal for it will change once the cantilever is in water.
3. Launch Igor. Launch the correct procedure to run the AFM.
4. Lower the sample to ensure that the cantilever will not hit the sample.
5. CAREFULLY place the optical head above the piezo. Ensure that the cantilever does not hit the sample because it may break.
6. Slowly add PBS through the fluid cell holes. DO NOT ADD TOO MUCH. Adding too much will cause the fluid to spill over the disk and onto the piezo. This is not a good thing.
7. Turn on the Black Box (if not already). Lift sample stage (via micrometer) until the cantilever is completely submerged.
8. Change the mirror to get the best A+B signal.
9. Change the photodiodes to get an A-B/A+B signal of around 0 (zero).
10. Get a Power Spectrum. The cursors should flank the first harmonic at the energy minimums on the left and right of the hump.
11. Turn on the oscilloscope and piezo controller. If these were on while you were getting the power spectrum, turn them off and redo the power spectrum.

12. Lift the sample stage until the cantilever is approximately 400nm away from the surface. Use the micrometer, DC offset, and slider in Igor.
13. Get a slope. The best measurement of a good slope is what the FX trace looks like. It should appear behind the CalibrateSlope graph. During contact, the FX trace should be completely vertical. As a general rule of thumb, try to contact with ~5000pN of force. This seems to generate the best values.
14. Make sure the spring constant makes sense. Is it within the range you expect?
15. The system is ready for you to perform experiments.

Here are some simple guidelines to assist you in your experiments:

1. An excursion of 400nm at a rate of 300-400nm/s is a pretty good starting point. These values are dictated by the completely unfolded length of your protein and the condition of the sample.
2. A contact force of 800pN is a good starting point.
3. The "GO" button on the panel is for automatic. To stop it, hold down "Ctrl".
4. If you want to save all FX traces, have the "auto detect" checkbox unchecked.
5. The saving criteria can be changed to reflect your protein, cantilever, and sample.
6. To perform a Force Ramp study, uncheck the "Step?" checkbox.

Chapter 2

STEP-BY-STEP HOW TO BUILD AN AFM

Part I. – Parts to buy
Part II. – Parts to mill
Part III. – Put it together

Part I. – Parts to buy

AFM
1. PicoCube XYZ Positioning System Closed Loop (P-363.3CD).
2. Controller Basic Chassis 19" (E-500.00)
3. Custom Amplifier for +/- 250 VDC (E-509.C3A)
4. Display/Interface Module 20 Bit
5. DI Optical Head Model TVOH-MMAFM
6. DI Contact fluid Cell
7. Vertical linear stage (MVN50/M-MVN-50)
8. Differential Micrometer (DM11-16)
9. Schafter/Kirchoff laser system (items 1-19).
10. Craighead Black Box
11. 15pin min-e-con connector if four quadrant photodiode in AFM optical head (available from DI).

Support
1. Optical Air Table
2. Minus K
3. Instrument rack
4. Two analog filters. We use filters from Frequency Devices™. These are for filtering the signals to the oscilloscope.
5. An oscilloscope. We use a Tektronix 603A for its storage ability.
6. A powered protoboard to help troubleshoot issues.
Note: There are multiple means to isolate the AFM from vibrations.

Connections
1. At least 7 BNC cables
2. Two NI DAQ cables, at least 4'
3. Signal connection to Craighead box (provided by Craighead)

Computer
1. Computer. The more RAM, the better.

2. Big Monitor. Suggestion: 1 20" or bigger or 2 18" or 19"
3. Igor Pro. Current (11/04) version is 5.0 from Wavemetrics
4. NIDAQ XOP from Wavemetrics
5. NIDAQ 6052E card from National Instruments
6. NIDAQ 6703 card from National Instruments
7. NI Measurement and Automation (NIMAX) from National Instruments. This comes with the 6052E.
8. Force_Spectroscopy_3D_XXX.ipf from the Fernandez Lab. "XXX" is a date when the ipf (Igor Procedure File) was made.
9. Force_Spectroscopy_Analysis_XXX.ipf from the Fernandez Lab. This will allow you to analyze your data obtained by the AFM.

Laser Focusing

The laser must be focused onto the tip of the cantilever when it is placed within the AFM head. There are multiple means to do this. Below are two way.
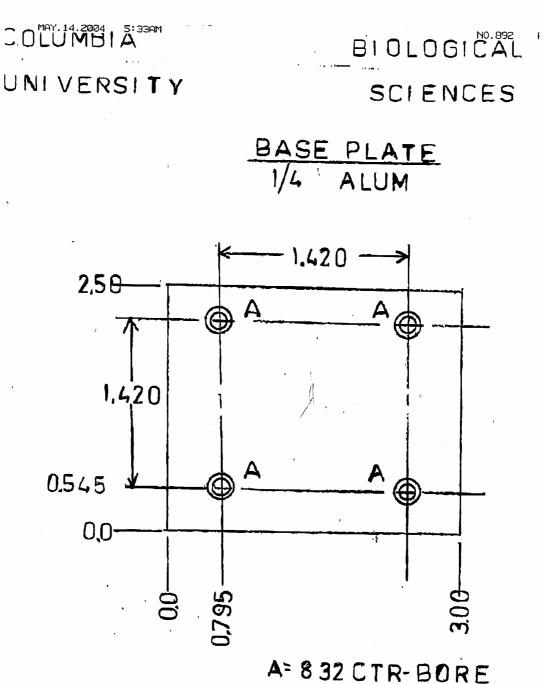
Method 1:
1. 1' optical tower
2. 3 translators
3. 10x objective
4. Milled arm to support optical head above the objective
5. BNC CCD
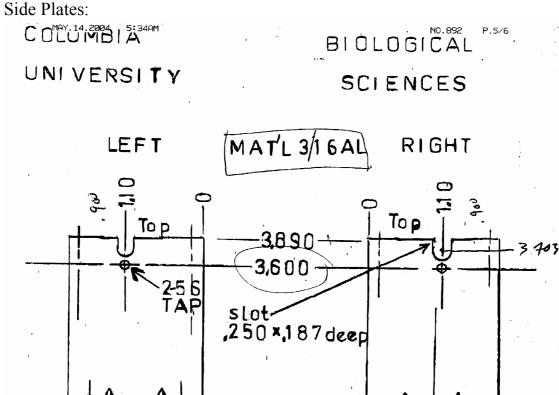6. Newport adjustable magnet model 200
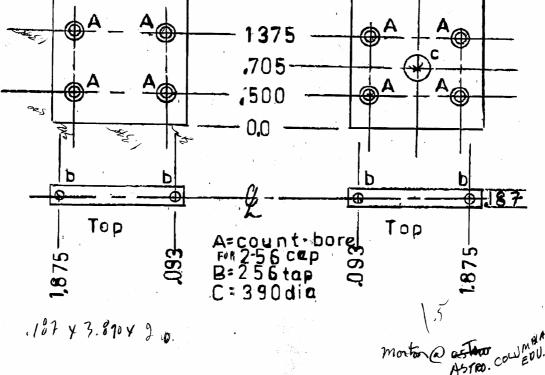7. TV that can accept BNC connection

Method 2:
1. Spare inverted microscope
2. 10x objective
3. CCD (webcam)
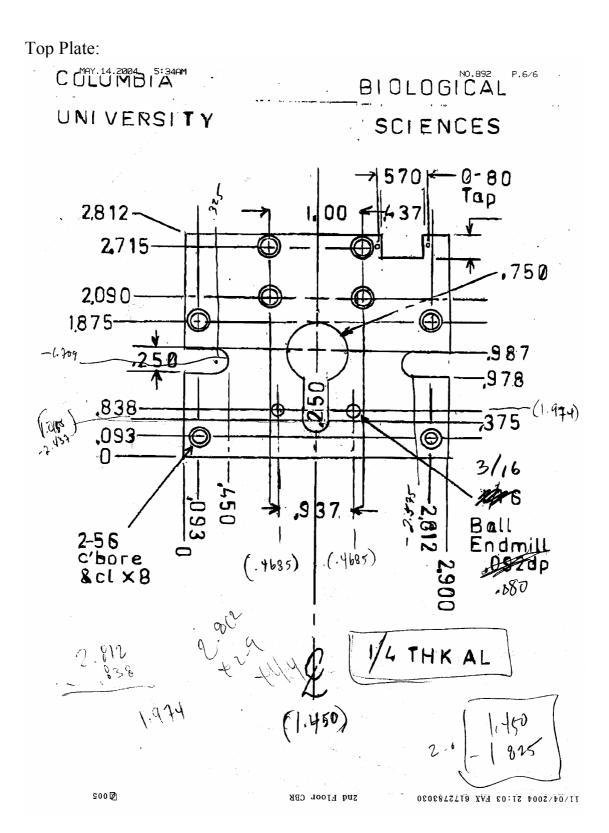4. TV (computer monitor)

Part II. – Parts to mill

Base Plate:

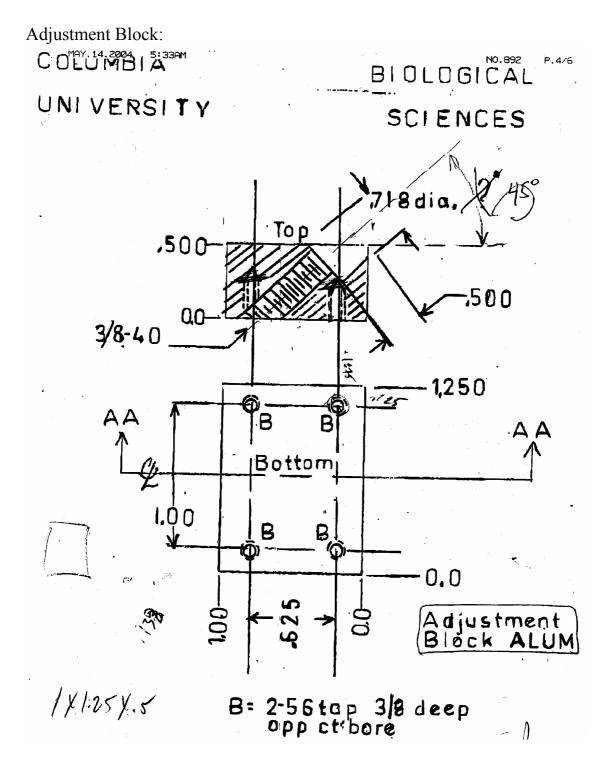COLUMBIA   BIOLOGICAL
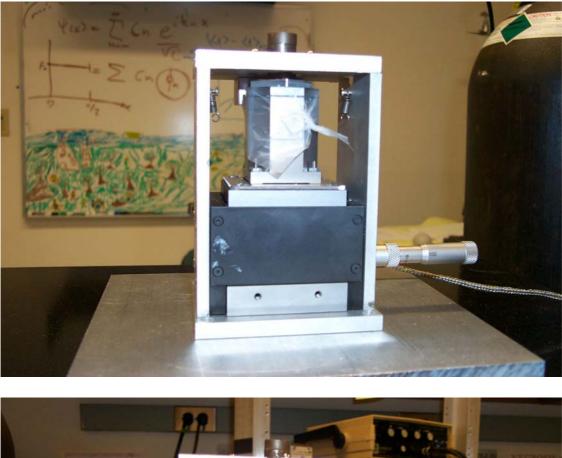
UNIVERSITY   SCIENCES

BASE PLATE
1/4 " ALUM

|← — 1.420 — →|

2.50

A   A

1.420

0.545   A   A

0.0

0.0   0.795   3.00

A = 8 32 CTR-BORE

Side Plates:

COLUMBIA

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BIOLOGICAL

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LEFT   MAT'L 3/16AL   RIGHT

Top

2-56 TAP

3,890

3,600

3,703

slot .250 x .187 deep

A A   1375   A A

.705   c

A A   .500   A A

0,0

b   b   b   b

Top   Top   .187

A = count bore
FOR 2-56 cap
B = 2-56 tap
C = .390 dia

.187 x 3.870 x 2 Ø.

morton @ astro.columbia.edu
astro.columbia.edu

1.875   .093   .093   1.875

1.10   0   0   1.10

Top Plate:

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BIOLOGICAL
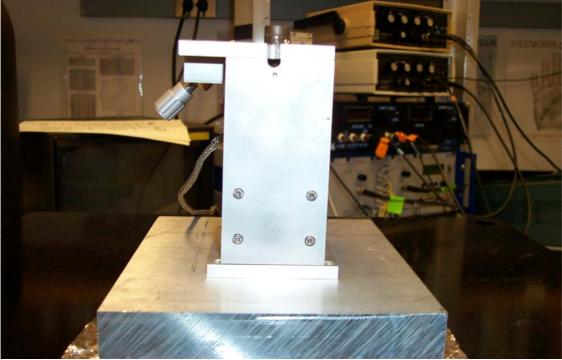
SCIENCES

Adjustment Block:

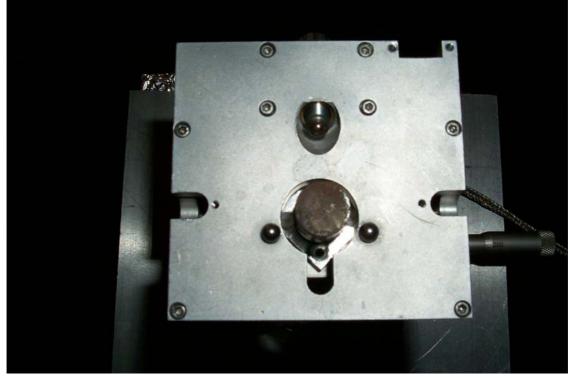Note: These pieces may have to be adjusted to your specific parts.
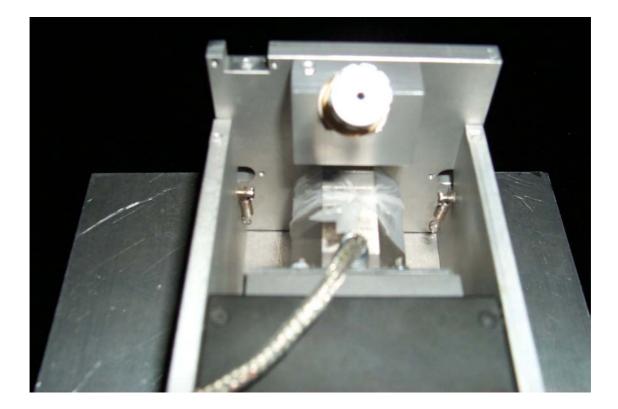
Part III. – Put it together

Chapter 3

TROUBLESHOOTING

First, here is a small essay on the AFM that may help in some troubleshooting. Then there is an FAQ followed by some last thoughts.

What I Need to Know I Already Learned From Building and Running an AFM
Or Top 30 Things (or so) I Need To Know About an AFM

1. The AFM is wise, the AFM is good, but in need of tender loving care.

2. The AFM must be watch for it is a fickle machine.

3. A large A+B is good, but it can be as low as 2 without issue.

4. If the piezo is emitting a high pitched screech, turn down the gain.

5. The head has two knobs to zero the A-B signal.

6. You do not want too big of a DC offset on the piezo controller

7. The DC offset moves the piezo in a smoother fashion than Igor.

8. The head is precariously perched on the picocube – BE CAREFUL

9. The oscilloscope is not the true signal. It is processed to make it look like the true signal.

10. The machine should be manually zeroed now and then. This helps improve the resolution of the AFM.

11. If your trace looks like it saturated (i.e.-flatten out), lower the gain on the panel.

12. There are three ways to get close to the surface: micrometer, Igor slider, and DC offset.

13. If you want a panel to stay in the same place that you placed the panel, change the code.

14. Contact fraction during FX is typically 15%

15. 800pN is a good starting point for Contact Force

16. -100pN is a good starting point of Pulling Force

17. 400nm is a good starting point for excursion

18. 300pN/nm to 400pN/nm is a good starting point for FX rate

19. Folding is called "double pulse" due to historical reasons in the program

20. To see what function a button runs, click on the panel, type Ctrl-T, select the second square down that shows up, select the arrow head, and double click on the button. The name of the function is in the upper right hand corner of the window that opens.

21. The NI Measurement and Automation software that comes with the NiDAQ cards should be installed prior to installing the cards. The software must be run to configure the ID numbers of the cards to 6052E as ID 1 and 6703 as ID 2.

22. Igor must configure the cards by running the NiDAQ Configuration software within Igor to the specification dictated by the program and is beyond the scope of this list. Each AFM should have an image of the configurations.

23. Waves can be deleted using the analysis package, but it is irreversible.

24. The user must sanity-check the results of the analysis since nothing is perfect.

25. To remove/add a button/variable/checkbox/box/slider to a tabbed panel, you must change the disabling procedure attached to the panel to disable the item when the tab is deselected.

26. Before you change ANYTHING in the code, save the procedure file under a new name.

27. The data collection and analysis procedures were improved by Ragan Robertson at Columbia University (raganr@gmail.com). The origination of the code is from Julio Fernandez and his postdocs.

28. If a new magnet must be used, it must be cooked in order to reduce the magnetization.

29. Nulling the head and sense may improve your traces.

30. If your force is not clamping to within 2% of your desired force, turn TP9 to improve the clamp.

31. If your sense signal seems to saturate or seems to constantly change in wide movements, the PID switch may be dead.

32. Instrumentation and Development never ends.

33. The AFM will have problems eventually.

34. Know the circuit diagram.

35. The 6052E is the workhorse, the 6703 is there to move in X and Y.

36. The two switches on the front of the Craighead box should be up (remote).

37. The switches must be down to troubleshoot the box disconnected from the computer.

38. When the computer is not connected to the box during troubleshooting, the PID switches are OPEN, i.e. - the PID is ON.

39. The oscilloscope is not the real signal the computer reads. It is a visual representation only.

40. The DC offset on the piezo controller does affect the extension signal.

41. Keep the piezo controller off while you get a power spectrum.

42. Touch with approximately 5nN when calibrating the slope. The best test of a good slope is that the FX trace is straight up during contact (the FX graph is usually behind the CalibrateSlope Graph).

FAQ

Below are a few questions that have come up in the years that I have been working with the AFMs in regards to the equipment itself.

1. The computer seems to beep when a trace is saved. Why?
        A: It is to alert you that a trace was saved. Under the save functions in the spectroscopy ipf, the word "beep" tells the computer to beep. If you do not want it to beep, comment it out.

2. My trace seems noisy. What can I do to improve it?
        A: This is a tough question. Bad cantilevers, poor samples, interference, poor power source (the power from the wall might have some noise), and vibrations all affect the traces. To fix the problem, it is mostly trial and error.

3. When the machine is just sitting there, the A-B/A+B signal is changing. Why?

A: This is drift. This is an inherent problem in AFMs. The source has as many possibilities as noise. The best fix is a new cantilever.

4. When I get a power spectrum, there seems to be some BIG spikes. Does this affect my calibration?

A: Depends. If the spikes are to the right of the rightmost cursor, then they don't affect the calibration. The largest source of noise that can be removed is to remember to keep the piezo controller OFF while you get the power spectrum.

5.