

# CNS Institute for Physics Teachers

<b>Title:</b>	<b>Atomic Force Microscopy</b>
<b>Original:</b> <b>Revision:</b>	18 March 2003 23 June 2008
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<b>Appropriate Level:</b>	All Levels
<b>Abstract:</b>	Topographic imaging at the molecular level involves an indirect mapping of the surface of a material with a probe that follows the shape of the surface. In this activity, each team of students will use a simulated topographic scanner with a platform mounted laser probe to attempt to discern the structure of an unknown block of Legos built by another team. At the conclusion of this activity, students will be asked to apply the knowledge gained to develop an understanding of the atomic force microscope.
<b>Time Required:</b>	Two to three 40 minute class periods
<b>NY Standards Met:</b>	M3.1 Apply algebraic and geometric concepts and skills to the solution of problems. S3.1 Use various means of representing and organizing observations and insightfully interpret the organized data. T1.1 Engineering design is an iterative process involving modeling and optimization which is used to develop technological solutions to problems within given constraints.
<b>Special Notes:</b>	<b>Atomic Force Microscopy</b> is a kit available from the CIPT Equipment Lending Library, <a href="http://www.cns.cornell.edu/cipt/">www.cns.cornell.edu/cipt/</a> . It is also available commercially from West Hill Biological Resources, Inc., <a href="http://www.westhillbio.com">www.westhillbio.com</a> .

**Behavioral Objectives:**

Upon completion of this lab a student should be able to:

- Explain the concept of topographic imaging of unknown surfaces.
- Identify factors that can limit the accuracy of topographic imaging.
- Identify the concept of resolution and its role in limiting topographic imaging of unknown surfaces.
- Explain how an atomic force microscope works.

**Class Time Required:**

- 2 periods for introduction, mapping of the topography and analysis
- 1 period (or homework) to complete post-lab worksheet on atomic force microscopy

**Teacher Preparation Time: 5 minutes**

Set out kits. If necessary, restock kits with six new data cards each (available from [www.westhillbio.com](http://www.westhillbio.com)).

**Materials Needed:**

- Topographic imaging kits (available from the CIPT Lending Library at [www.cns.cornell.edu/cipt](http://www.cns.cornell.edu/cipt))
- Scissors
- Straight edge
- Fine-tipped felt pens or markers are best for recording on data cards

**Assumed Prior Knowledge of Students:** None.

**Background Information for Teacher:**

Since the beginning of the 17<sup>th</sup> century, physicists have continued to improve on our ability to see objects both distant (telescopes) and small (microscopes). By the 18<sup>th</sup> century, technology had reached its limits of magnification. Lenses that used light were subject to laws of physics that limited the effectiveness of high power optical lenses and objects smaller than about one millionth of a meter could not be seen.

In the 20<sup>th</sup> century, the limitations of light were overcome with the invention of the transmission electron microscope, which used electrons to probe specimens. Eventually in the best microscopes objects smaller than one-billionth of a meter could be imaged, approximately the distance between individual atoms. However, there were limitations to this technique too. The electrons that were beamed through the sample often damaged it, especially biological materials. The sample had to be cut thinly enough to allow electrons through making preparation challenging. The electron beam needed to be sent through a vacuum. Despite these limitations, it was and still is an outstanding tool for the study of the structure of many types of materials.

The first micro-imaging device used to study the surface, or topography, of a material was the scanning electron microscope. This allowed a beam of electrons to be bounced

off the surface of an object. As electrons penetrated nooks and crannies of the surface, they were reflected. The depth of the penetration and the angle of reflection were used to assemble an image of the surface. Again there were limitations. The material had to be coated with a thin layer of metal so that electrons could be reflected. The coating of course could obscure some features and modify the sample.

During the last decade, a new microscope called the atomic force microscope was developed. It uses a very fine tipped probe to gently touch the surface of individual molecules and in some cases individual atoms. By measuring how much the tip moves up or down while dragging it across the surface, an image can be formed. Its simplicity allows a wide range of materials to be studied, since it works in air and requires no special sample preparation. One powerful capability is that this microscope allows biological molecules to be imaged in aqueous solution, which is their natural environment. Biological applications are currently a very active area of research. The atomic force microscope has become a very versatile tool in several fields of science and engineering.

### **Answers to Questions:**

#### **Lab Analysis:**

1. Was your model identical to the unknown? Only with a lot of luck will the model students construct be identical to the unknown block of Legos. This is because not enough information is available. However, if students are careful they should get something that resembles the original structure.
2. Talk to other teams about their results. For those teams that did not accurately replicate the molecule and did the procedure correctly, what went wrong? There are several things that can hinder accurate reproduction of the "molecule." They include: (1) inability to detect surfaces below the top surface, (2) physical damage to the structure while probing, and (3) the grid spacing is larger than the smallest horizontal features. All of these difficulties arise in real atomic force microscopy.
3. What topographical features were easy to map? Which aspects were difficult or impossible? A feature that is relatively easy to discern is the number of Lego bricks stacked vertically at a certain location. Also, gross horizontal features are relatively easy. Features that are difficult include narrow depressions, overhangs, and the width and angle of Lego blocks (basically fine horizontal features and sub-surface structure).
4. What is "resolution"? Explain this term in your own words. "Resolution" is defined as "the minimum difference between two discrete values that can be determined by a measuring device." For example, it is the distance between two pixels in a TV picture. The resolution is also the error term in measuring height or width.
5. Is the horizontal resolution different than the vertical resolution? If so, which is better? Explain your answer. The horizontal resolution of the Unit is about one inch. The distance between holes in the lid is the main limiting factor and limits horizontal measurements to the nearest inch. The vertical resolution is much better than the horizontal resolution because the height of the probe can be varied continuously. Factors limiting vertical resolution include the roughness of the end of the probe

stick, the precision of the level alignment system for the probe, and the diameter of the felt tip marker. The combination of these effects can be estimated by looking at the variation in several measurements of the same height, such as the baseline, typically 1/4."

6. Was the resolution of this Laser Guided Topographic Visualization Unit a factor in building an accurate model? Yes. The fact that the horizontal resolution is larger than the smallest features of the Lego structure prevents one from accurately reconstructing it.
7. How would you redesign the Unit to get better resolution? There is much room for creativity here. A common response is to decrease the distance between holes in the lid. Other possibilities include a smaller diameter probe, designing a probe to access the sides of the "molecule," and improving the alignment system for the probe.

### **Post-Lab Worksheet:**

1. Laser
  - a. What will happen to the cantilever and tip when a tall part of the sample passes beneath it? The tip will rise and the cantilever will bend upwards.
  - b. How will the reflected laser beam move on the photodiode as the tip rises? The reflected beam will move higher on the photodiode.
2. Cantilever
  - a. What might happen if the force between the tip and the sample gets too large? The tip or the sample or both might get damaged.
  - b. How would you increase the force between the tip and the surface? Decrease it? To increase the force, lower the cantilever and tip; to decrease force, raise it.
  - c. What will happen to the position of the reflected laser beam on the photodiode as you increase the force? Decrease it? Move higher. Move lower.
  - d. Describe how you would keep the force between the tip and the sample constant while the sample was scanning under the tip. Monitor the position of the reflected laser beam on the photodiode to keep it the same (indicates constant force). Adjust the cantilever up or down as needed to keep the force constant while scanning.
3. Tip
  - a. If a DNA molecule was scanned under the tips above, what could be resolved clearly and what couldn't be? The position of the molecule, its shape and length could be resolved to within a few nanometers. The width of the molecule and the positions of the individual atoms could not be resolved. (Note: the height of the molecule would equal the diameter since vertical resolution is much better than horizontal resolution and typically a small fraction of a nanometer.)
  - b. What differences would you expect between images generated with the two tips? The tip with the smaller radius of curvature would give a sharper image.

Analysis:

1. In your own words describe the similarities between the Laser Guided Topographic Visualization Unit and the AFM. The list of similarities includes: (1) both give topographic information about surfaces, (2) both use a laser, (3) both have better vertical resolution than horizontal resolution, (4) both acquire data one point at a time (serially), and (5) both use contact or normal force for imaging.
2. In your own words describe the differences between the Laser Guided Topographic Visualization Unit and the AFM. The list of differences includes: (1) the LGTVU laser is mounted to the probe, but the AFM laser is reflected from the probe, (2) the AFM has nanometer scale resolution while the LGTVU has centimeter scale resolution, (3) the AFM scans the surface continuously while the LGTVU samples discrete points, (4) the AFM adjusts the amount of force at the probe using the deflection of the cantilever while the LGTVU requires the operator to adjust the force by hand.
3. Describe the general principles of how an AFM works. An AFM uses a sharp tip attached to a springy cantilever to contact the surface to be imaged. The tip is scanned over the surface while the deflection of the cantilever is monitored using a reflected laser beam that falls on a position sensitive detector. Typically, the height of the tip is adjusted during scanning to keep the cantilever deflection constant, ensuring a constant contact force. A computer tracks the height adjustments and displays this data as the topography of the surface.
4. What is “resolution” and what determines the resolution of an AFM? Resolution is the width of the smallest feature that can be imaged. The resolution of an AFM is usually determined by the radius of curvature of the tip.
5. Besides the size of the molecules, can you think of another limitation of using the AFM to investigate biological materials? Biological materials are not very rigid and deform or break under too much force, so the act of imaging may modify the sample. Some biological materials are mobile and if they move too fast it is not possible to image them.

### **Tips for the Teacher:**

- During the activity, students will tend to ask specific questions that are not addressed by the written procedure such as what to do when the probe is hitting the edge of a Lego, how to connect their data points, or how to interpret small variations in the baseline data. Since a major goal of this activity is to get students to think about some of the challenges a modern microscopist faces, it is useful to refer such questions back to the students. Challenge students to give their own interpretation and to agree on a procedure with their team that addresses the question.
- Some students may break the Lego “molecule” inside the box and want to open the box to fix it. Remind them not to open the box under any circumstances until they have completed their model. Microscopists do not usually have the option of repairing a damaged sample.
- Remind students to keep Lego structures intact for the analysis section.

## **References:**

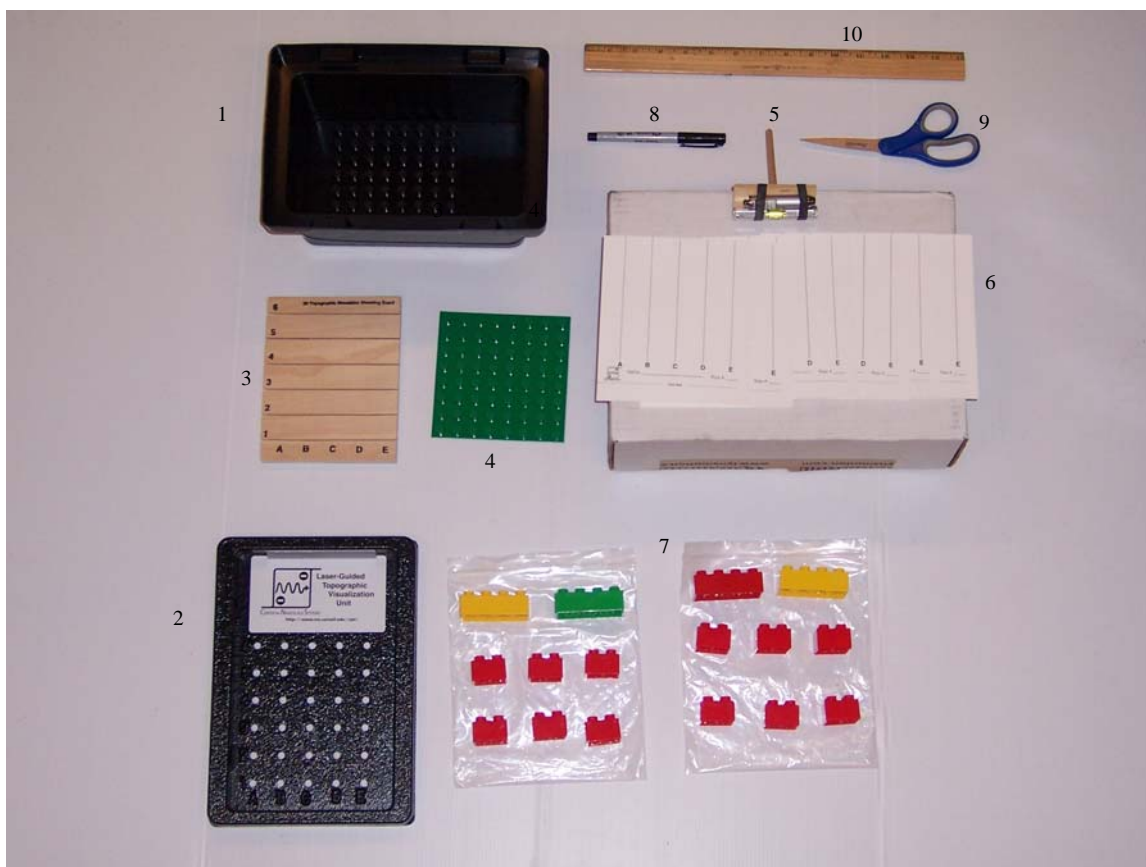
Basic operating principles of the atomic force microscope:

- <http://stm2.nrl.navy.mil/how-afm/how-afm.html>

Biological applications of the atomic force microscope:

- [http://www.physics.ucsb.edu/%7Ehhansma/afm-acs\\_news.htm](http://www.physics.ucsb.edu/%7Ehhansma/afm-acs_news.htm)
- <http://www.mih.unibas.ch/Booklet/Booklet96/Chapter3/Chapter3.html>

## Equipment List



Item Number	Quantity	Item
1	1	Laser Guided Topographic Visualization Unit (plastic box)
2	1	Perforated lid to plastic box
3	1	Projection screens (cards) holder
4	1	Molecular Mounting Plate (Lego) 5"x5"
5	1	Probe - red laser pointer, line level, rubber bands
6	6	Projection screens (cards)
7	2 sets	Molecular Building Blocks (Legos) each bag containing 6 - 1.25"x1.25" and 2 - 1.25"x 2.5"
8	1	Marker
9	1	Scissor
10	1	Straight edge

# ATOMIC FORCE MICROSCOPY

## Introduction

In this activity you will use a special probe to “feel” the shape of a “molecule” that is invisible to you, the microscopist. In more technical language, you will map the topography of the surface of an unknown structure. First, you will use Lego blocks to build a “molecule” or “molecules” within an enclosed box. You will then trade boxes with another team and try to discern the shape of the “molecule” or “molecules” that the other team designed. Feel free to try to make your “molecule” as difficult to map as possible.

**\*\*\*CAUTION: Never look into the laser beam or point it at someone’s eye!\*\*\***

## Prepare your Sample

1. Open and unpack your Laser Guided Topographic Visualizing Unit. Within it you should find the following:
  - a. 2 bags of molecular building blocks (Legos)
  - b. A Molecular Mounting Plate (Lego base plate)
  - c. A probe (dowel rod mounted to a platform with a laser and level)
  - d. A 3-D Topographic Simulation Mounting Board
  - e. Projection Screens (Cards with lines)
2. Using one bag of Legos build a molecule or several separate molecules on the molecular mounting plate (Lego base plate) in the bottom of the unit. Do not build on the areas with black X’s. If you choose to overhang any blocks, make sure that at least half of the block is supported by blocks below it.
3. Once you have designed your molecule, put the cover on. Make sure that row 1 on the cover is above the single row of X’s on the molecular mounting plate.
4. Find another team and trade boxes.

**\*\*\*From this point on, do not open the lid under ANY circumstances until you have completed building a model of what is inside.\*\*\***

## Collect Baseline Data

1. Find a Projection Screen (card with vertical lines), fold it on the dotted line, and insert the folded edge in the slotted pegs on the top of the Laser Guided Topographic Visualization Unit. Make sure the vertical lines are aligned with the rows of holes.
2. You can begin taking your readings by inserting your probe and platform mounted laser in hole A1. Aim the laser toward the screen. You know that there will be no molecules in row 1 as it was a restricted area. Press the platform down until the probe touches the surface.
3. Once the probe is touching the surface, you may click the laser on and project it onto the screen. You will need to align it in two directions:

- a. Orient the laser beam so that it falls on the appropriate vertical line of the Projection Screen (the line marked A).
- b. Gently tilt the platform until the bubble in the level is centered.
4. Once you have aligned the probe, mark the spot on the Projection Screen where the laser hits it.
5. Repeat the above procedure for all of the remaining points in row 1 (B1, C1, D1 and E1). This will be your baseline, or the topography where no blocks are present.
6. Remove this card and write "1" for the row number.

### **Collect Topographic Data**

1. Place a new Projection Screen in the holders and repeat the above procedure in columns A-E, row 2. When you are done, put "2" for the row number.
2. Repeat in each row up to and including row 6. Remember to label each card.

### **Display your Data**

1. Go back to your baseline card (Row 1). The 5 points should be roughly horizontal. Connect the points using a pencil. Discuss with your lab partners how to do this. Using a scissors, cut the card along this line.
2. Connect the points on the other data cards. Once you and your lab partners have agreed upon the data cards, you may cut each one along the line you drew and keep the bottom part.
3. You can now make a paper model of your molecular topography by using the 3-D Topographic Simulation Mounting Board. Start out by inserting your cut out screen for row 1 in the slot labeled "1." Remember that this is your baseline, or the topography where no blocks are present.
4. Insert the remaining cut out screens for each row into the Mounting Board. Be sure to get each screen into the correct slot.

### **Assemble a Molecular Model**

Now that your team has assembled the data on the unknown "molecule," you can use this to build a model with the spare bag of Molecular Building Blocks and Molecular Mounting Plate. Do not look into the box until your entire team agrees on the model. When you agree, open the box and compare the formation inside with your model. Keep all Lego structures intact for the analysis section.

## ANALYSIS

1. Was your model identical to the unknown?
2. Talk to other teams about their results. For those teams that did not accurately replicate the molecule and did the procedure correctly, what went wrong?
3. What topographical features were easy to map? Which aspects were difficult or impossible?
4. What is “resolution”? Explain this term in your own words.
5. Is the horizontal resolution different than the vertical resolution? If so, which is better? Explain your answer.
6. Was the resolution of this Laser Guided Topographic Visualization Unit a factor in building an accurate model?
7. How would you redesign the Unit to get better resolution?

**(POST-LAB WORKSHEET)**

**AFM: A MICROSCOPE THAT CAN IMAGE MOLECULES**

In the previous activity, you used a probe attached to a laser to determine the topography of a Lego molecule. You probably found that although the imaging unit was able to detect large features in the topography, it did not allow you to resolve the fine details of the molecules such as narrow crevices and the exact locations of edges of the blocks.

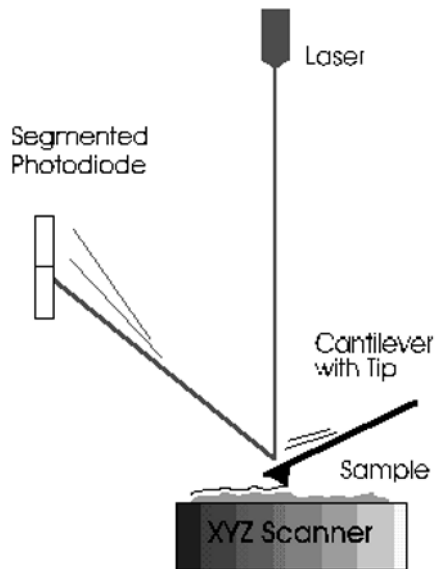
Imagine what it would be like if we could shrink our probe to be less than 100 atoms wide. What advantage would this give?

Imagine that instead of taking readings every inch, we could drag this probe across the surface of the molecule. What advantage would this give?

If the probe was being dragged across the surface of the material (or the material was being dragged under the probe), how would you design the probe in order to read all heights?

If you answered the questions above correctly, you have designed the rudiments of the Atomic Force Microscope (AFM). If you could not answer them, read on, perhaps soon you can go back and answer them.

The basic design of an Atomic Force Microscope (AFM) is illustrated below:



Atomic Force Microscope

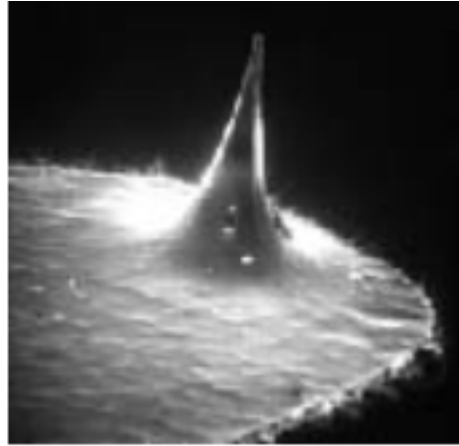
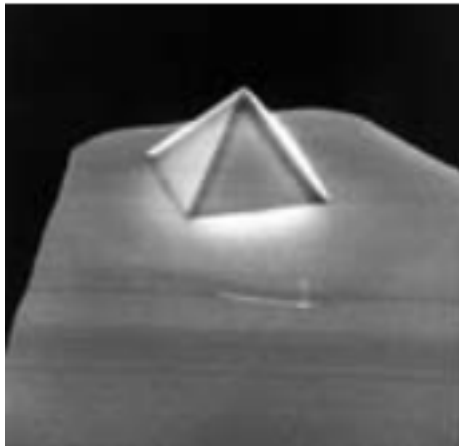
Source: PROBING BIOMOLECULES WITH THE ATOMIC FORCE MICROSCOPE. Helen G. Hansma, Department of Physics, University of California, Santa Barbara, CA 931106 [http://www.physics.ucsb.edu/%7Ehhansma/afm-acis\\_news.htm](http://www.physics.ucsb.edu/%7Ehhansma/afm-acis_news.htm)

### How the AFM works

The sample in the above illustration corresponds to the Lego molecule you built. The cantilever with tip is like your probe. This is where the similarity ends. Let's take a look at each aspect of the AFM.

1. **Laser** - In the AFM the laser is stationary. The sample moves under the tip and the tip rises or falls based on the topography of the sample. The laser reflects from the top of the cantilever and falls on the photodiode, which senses its position.
  - a. What will happen to the cantilever and tip when a tall part of the sample passes beneath it?
  - b. How will the reflected laser beam move on the photodiode as the tip rises?

2. **Cantilever** – The cantilever is shaped like a diving board and it acts like a very soft spring. When the cantilever is lowered and the tip makes contact with surface, it bends so that the force between the tip and the surface is very low (nano-Newtons).
- What might happen if the force between the tip and the sample gets too large?
  - How would you increase the force between the tip and the surface? Decrease it?
  - What will happen to the position of the reflected laser beam on the photodiode as you increase the force? Decrease it?
  - Describe how you would keep the force between the tip and the sample constant while the sample was scanning under the tip.
3. **Tip** - The tip at the end of the cantilever is very important to the resolution of this microscope. The sharper the tip, the finer the features it can detect. The tips pictured below have a radius of curvature of 30 nm and 10 nm respectively. They are made by patterning and etching silicon.

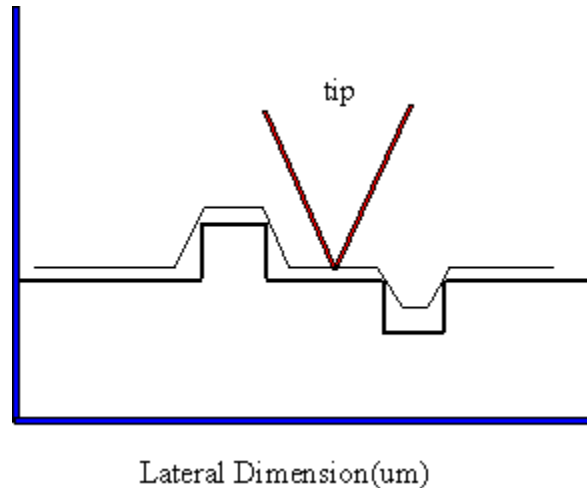


"The tip-sample interaction in atomic force microscopy and its implications for biological applications ", Ph.D. thesis by David Baselt, California Institute of Technology, Copyright © 1993 by David Baselt.  
<http://stm2.nrl.navy.mil/how-afm/how-afm.html>

You may remember from biology that DNA is a double helix, the width of which is 2 nm. You may also remember that DNA is itself thousands of nanometers long.

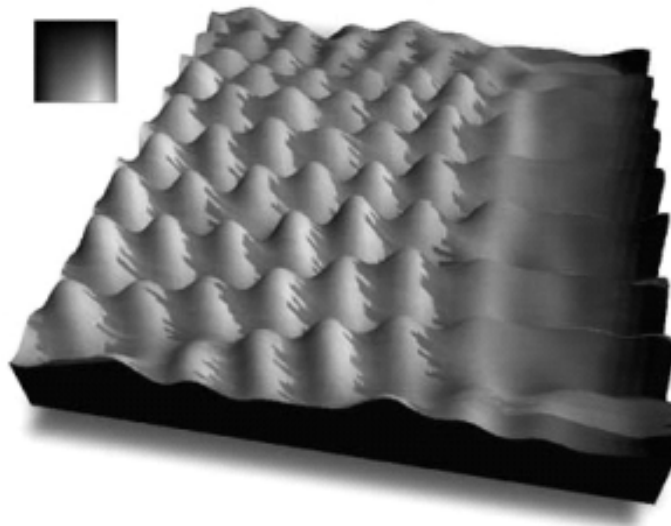
- If a DNA molecule was scanned under the tips above, what could be resolved clearly and what couldn't be?
- What differences would you expect between images generated with the two tips?

Like your Lego experiment, even the AFM has limits of resolution. If the tip is wider than the features of surface being studied, then the tip cannot resolve the fine details of surface (see diagram below). In general, the narrower and sharper the tip, the better the resolution.



Source: PROBING BIOMOLECULES WITH THE ATOMIC FORCE MICROSCOPE. Helen G. Hansma, Department of Physics, University of California, Santa Barbara, CA 931106 [http://www.physics.ucsb.edu/%7Ehhansma/afm-acis\\_news.htm](http://www.physics.ucsb.edu/%7Ehhansma/afm-acis_news.htm)

Despite many challenges, atomic resolution is sometimes possible. For example, the figure below shows the extremely regular pattern of graphite. The bumps in the topographic image correspond to individual carbon atoms. The AFM has made possible the imaging of surface topography at the nanometer scale and has provided a new tool for scientists and engineers.



"The tip-sample interaction in atomic force microscopy and its implications for biological applications ", Ph.D. thesis by David Baselt, California Institute of Technology, Copyright © 1993 by David Baselt. <http://stm2.nrl.navy.mil/how-afm/how-afm.html>

## ANALYSIS

1. In your own words describe the similarities between the Laser Guided Topographic Visualization Unit and the AFM.
2. In your own words describe the differences between the Laser Guided Topographic Visualization Unit and the AFM.
3. Describe the general principles of how an AFM works.
4. What is “resolution” and what determines the resolution of an AFM?
5. Besides the size of the molecules, can you think of another limitation of using the AFM to investigate biological materials?