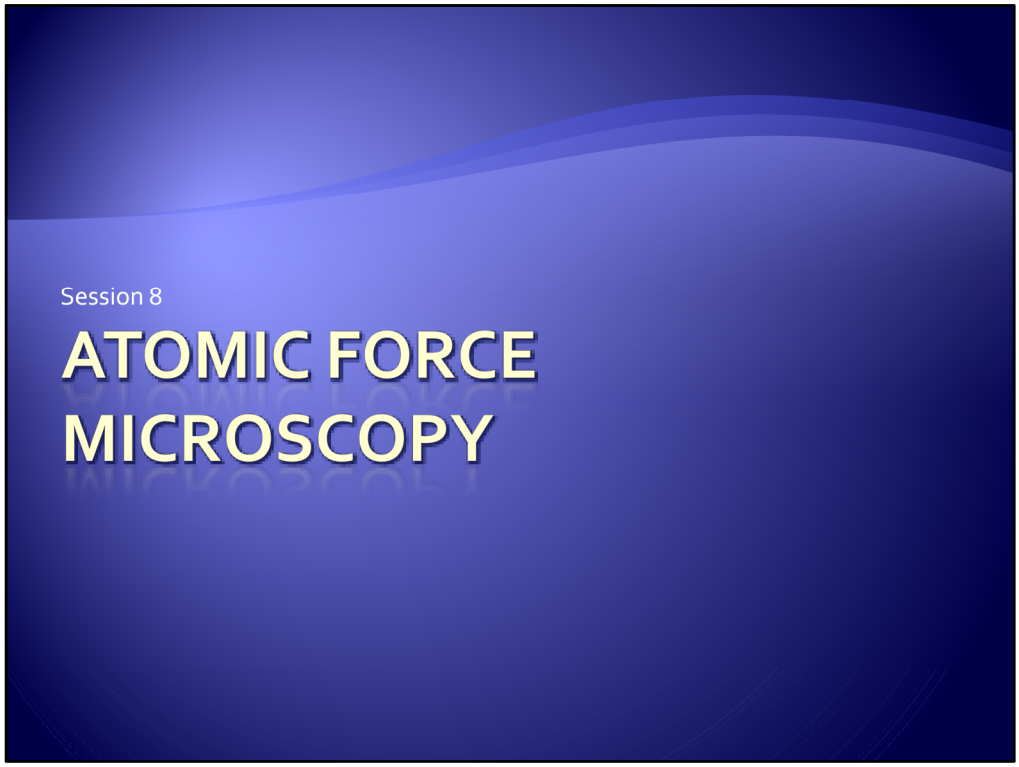
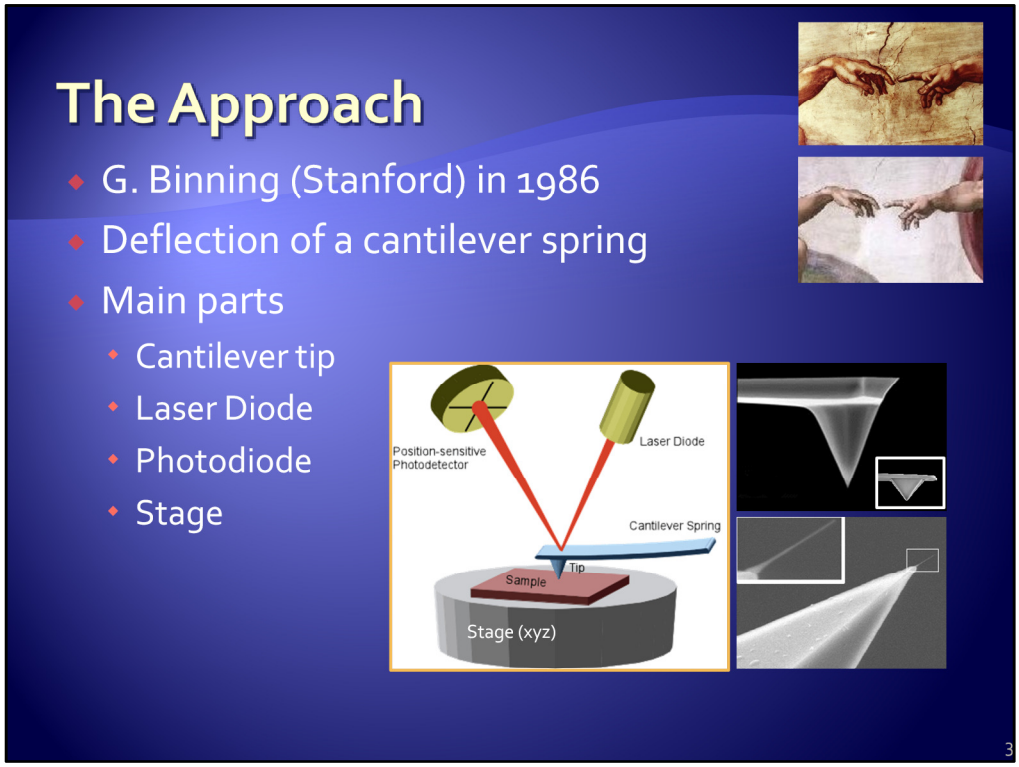


Semiconductor technology meets biomechanics…

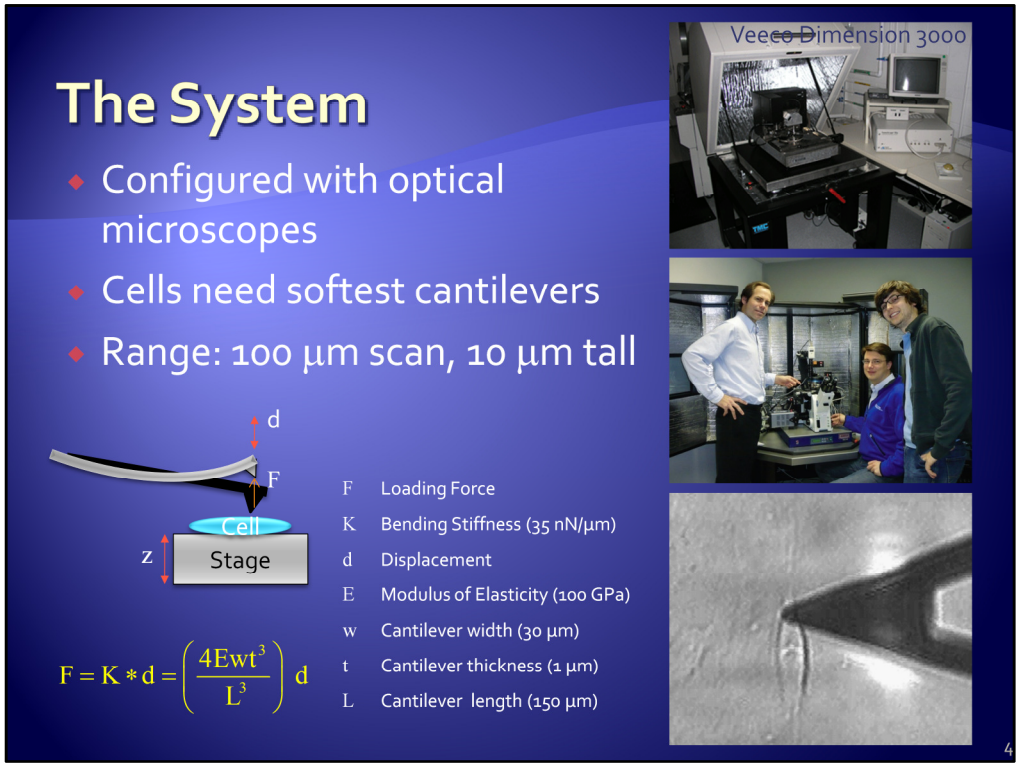


The AFM came from the same minds that developed the scanning tunneling microscope. With AFM, the surface of a nonconducting material can be measured for its height, stiffness, and adhesion. Soon after its conception, it was used to look at biological materials like cells, proteins, and DNA.

There are many different designs of AFMs but the main parts of almost any system are similar: 1) a sharp tip mounted on a cantilever that acts as a spring, 2) a laser diode that is focused on the end of the cantilever, 3) a position-sensitive photodiode that detects the laser beam that is reflected off the back of the cantilever tip, and 4) a piezo stage for positioning the sample relative to the tip in x, y, and z directions.

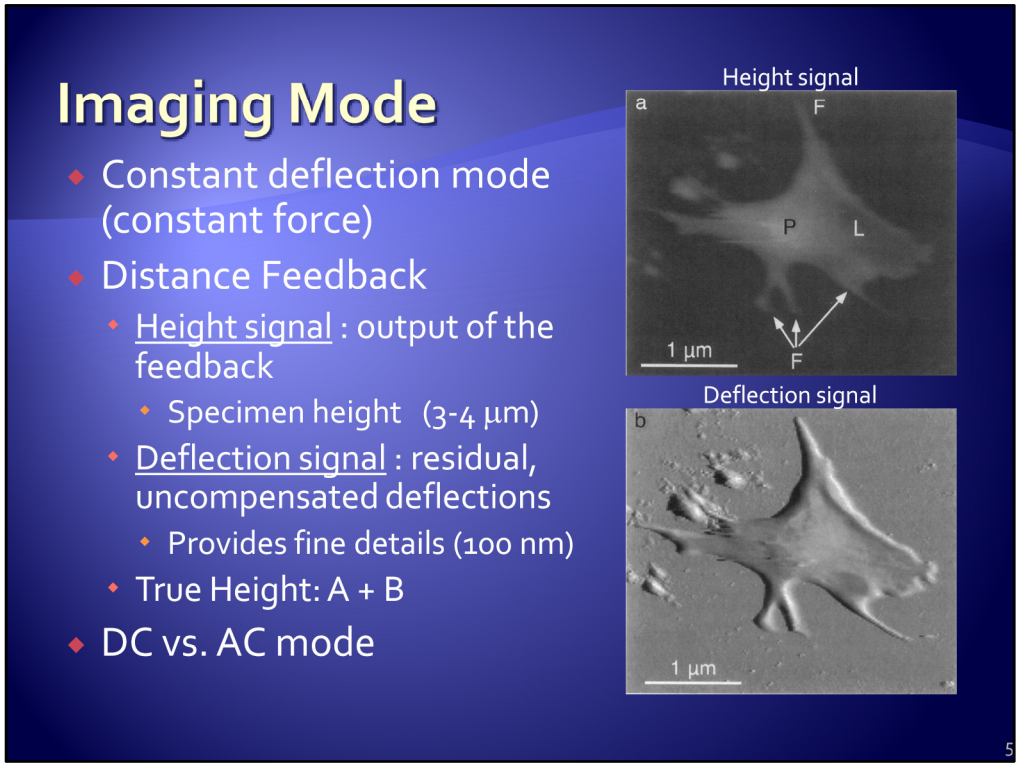
Examples of different tips are shown. They can be made from silicon nitride or pure silicon. There are new efforts to make the tips even smaller but mounting nanowires or carbon nanotubes on the ends of the tip.

The tip To measure the force,



AFM for cell mechanics requires the softest cantilevers available. These systems are more commonly used for measuring nanoscale structures that are not observable with optical microscopy and difficult to characterize with SEM. For cell mechanics, the stiffness or elasticity of a cell can be measured by increasing the height of the stage (z) until the tip makes contact with the surface of the cell. As the stage is brought up further, the cantilever deflects from the loading force on the cell.

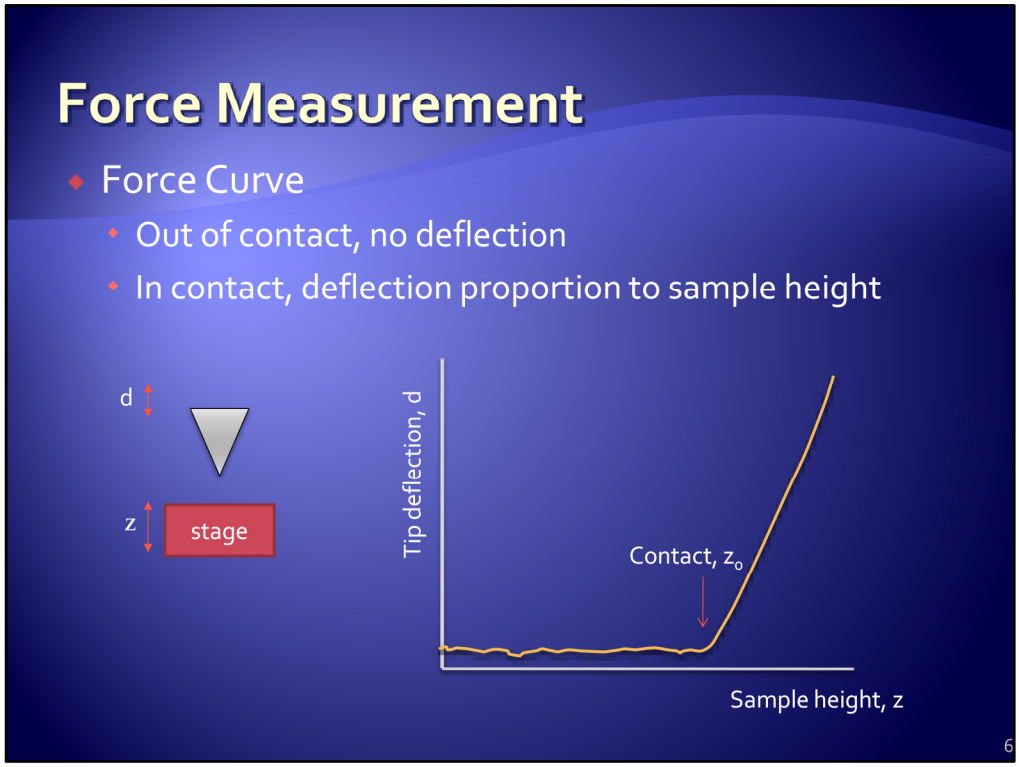
In the video, the peizo stage is very fast and can be used to laterally scan the height or stiffness of a cell.



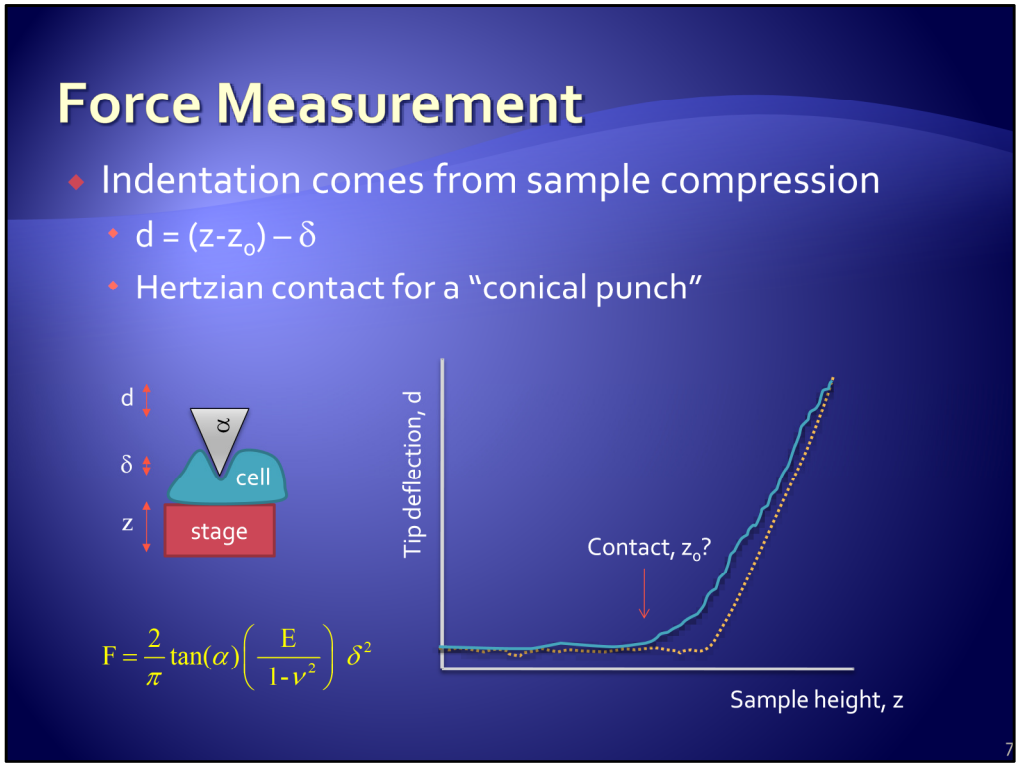
For soft samples that are easily damaged by large forces, AFM imaging is possible through feedback between the tip deflection and the position of the stage so that the cantilever deflection (and loading force) is held constant.

However due to limited responsiveness of the feedback (mechanical side), some residual fluctuations in the deflection signal are still present. The output of the feedback, which corresponds to the height signal shows the overall topology of the sample. The fine details in topography that are not compensated by the height feedback signal generate residual deflections of the cantilever are read by the error signal mode. These two signal channels correspond to the height signal (3-4 microns) and the deflection signal (100 nm). Adding these two images together gives you the true height.

The mode described is the DC mode ("contact mode") for AFM imaging. An alternative is the AC mode ("tapping mode") where the tip is resonated above the sample and when the tip approaches the sample the amplitude of the cantilever motion is suppressed which can be used as the imput signal for the distance feedback. This minimizing lateral forces on the sample since the tip is not dragged across the sample.



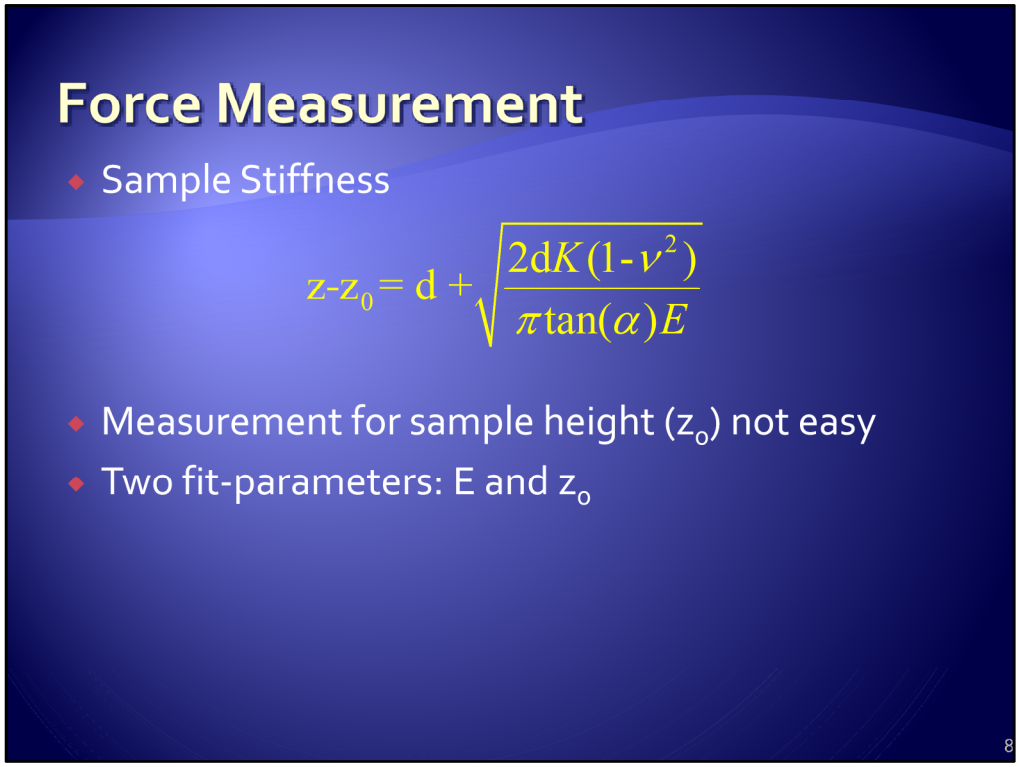
In a force curve, the lateral position of the tip is not changed (it is not scanning across the surface of the cell) while the height of the stage is ramped such that the tip is brought in and out of contact and the deflection of the cantilever is monitored. Before contact, the cantilever will fluctuate due to the brownian motion of the fluid environment. Once contact is reached, a sharp kink in the deflection curve is easily detected.



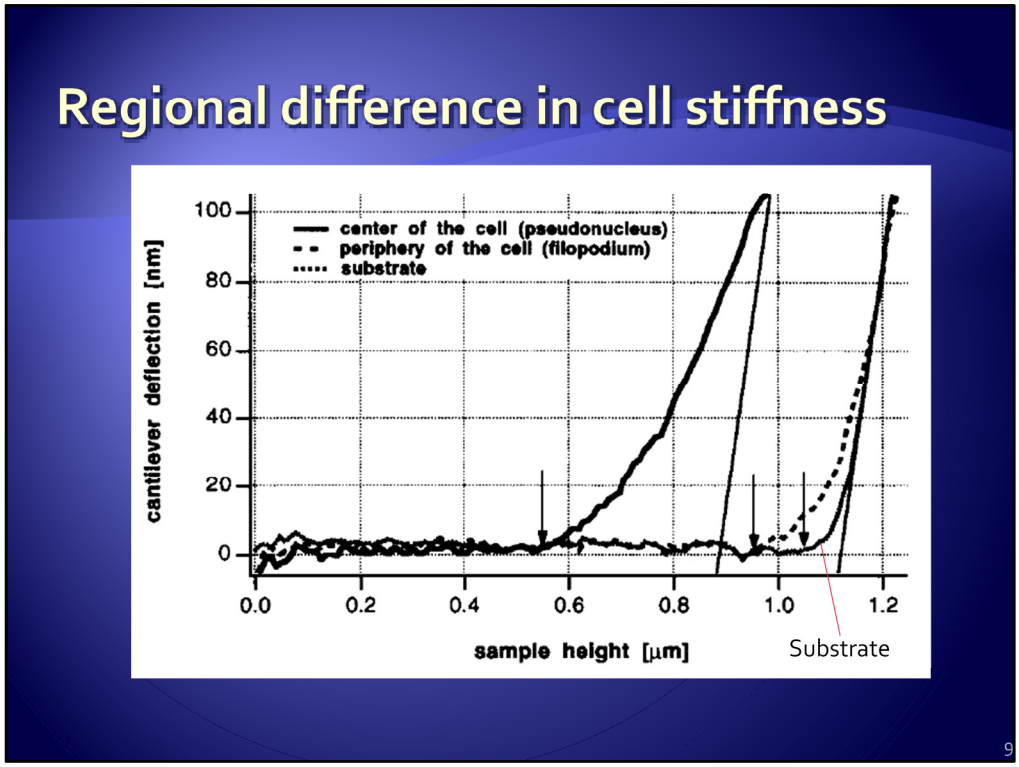
On a soft sample the z movement of the sample will cause a deflection of the cantilever and an indentation (δ) of the sample. The deflection of the cantilever will not be proportional to the sample height (z) but deviates by the sample indentation (δ). As a result the deflection curve will not have a sharp kink as seen for a stiff substrate like the stage.

The more indentation you have, the more contact area between the sample and the tip leading to a nonlinear relationship between loadinf force (F) and indentation (δ).

In the case of a conical tip, the loading force (F) can be calculated from the material properties of the sample (E, ν), the opening angle of the conical punch (α), and the indentation (δ). For cells, since they have a large water content, we assume that they are incompressible (ν=0.5).



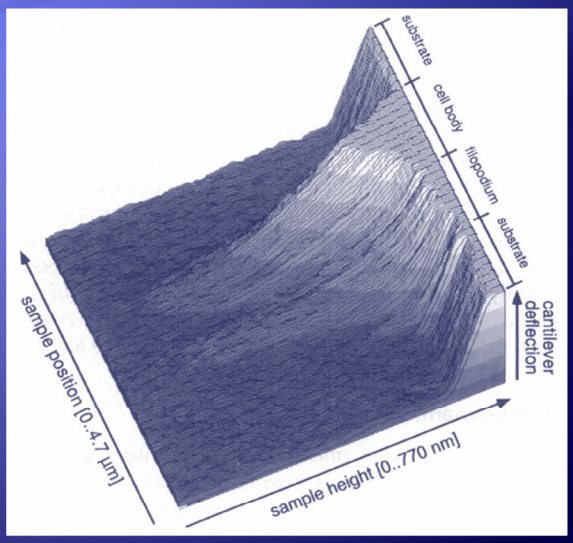
In Manfred Radmacher's work, they took two measurements from the force curve (z, d) and solved for the two unknowns.



Force curve taken at different locations on the platelet. The substrate curve is the farthest to the right. The arrows denote the point of contact. The filipodium is stiffer than the pseuodonucleus of the platelet where there are a lot of granules containing clotting agents.

Line Scan

- Stacked force curves
- Shows height of cell along scan
- Cell body: more compressible
- Periphery: less compressible



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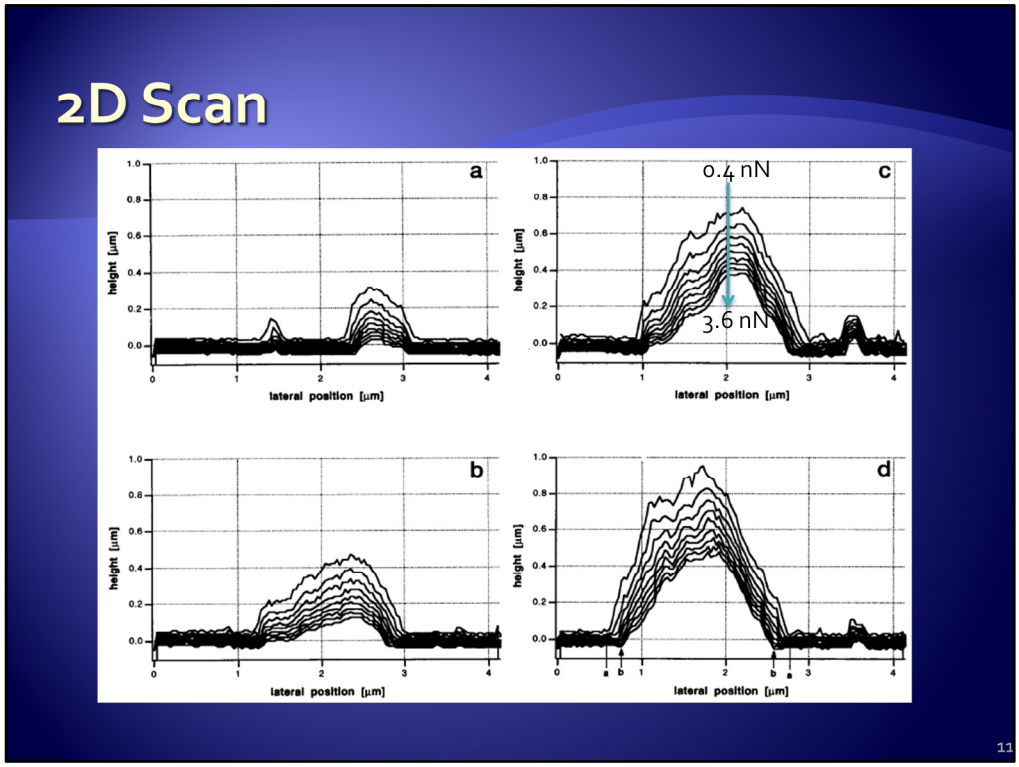
This graph shows all the force curves taken while scanning along one line over a platelet. The force curves are stacked one behind each other to show a force map. From these, several regions can be observed. At the front of the map is the force curves for the bare substrate and show the proportionality with sample height. Behind this is the force curves for when the tip is on the periphery of the platelet, which is higher than the substrate. At high loading forces, the periphery has a proportionality close to what is seen for the bare substrate measurement. Next is the cell body which is softer.

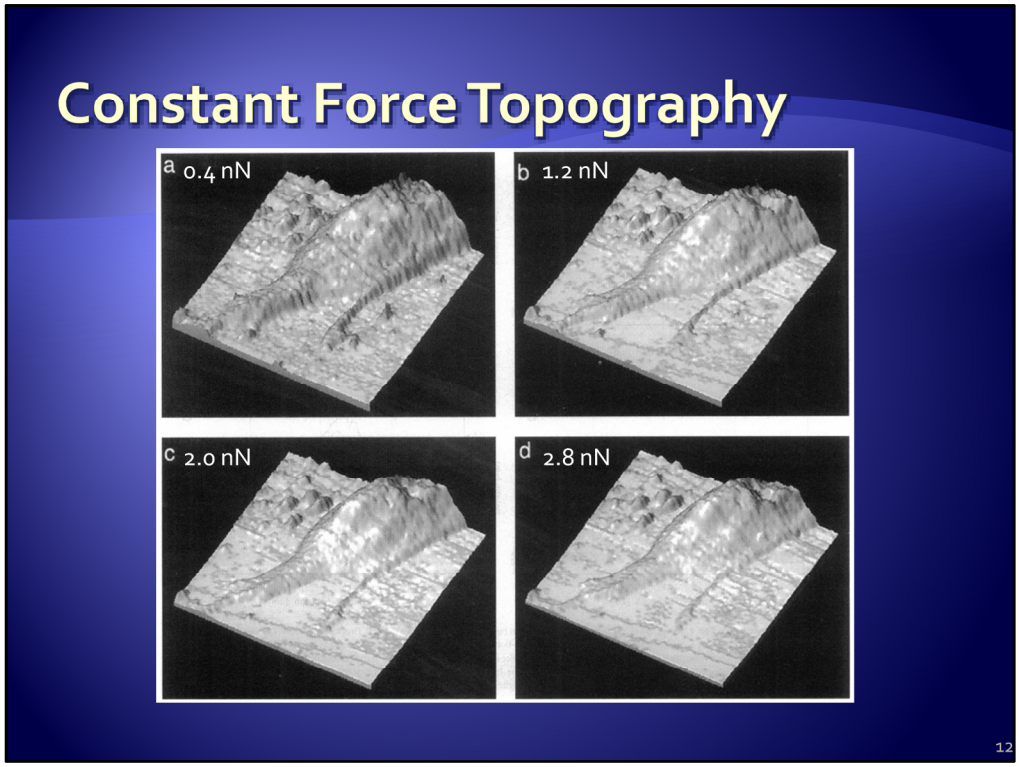
The shades denote regions of constant loading force (based upon tip deflection).



These are topology traces at different locations on the cell. Each trace in the four graphs corresponds to a higher constant force, starting at 0.4 nN and increasing by 0.4 nN, up to a maximum of 3.6 nN of force.

From the higher force, there is substantial compression of the cell such that at most points it gets reduced to ½ of its height. It also shows that there are different regions of elasticity in the cell.

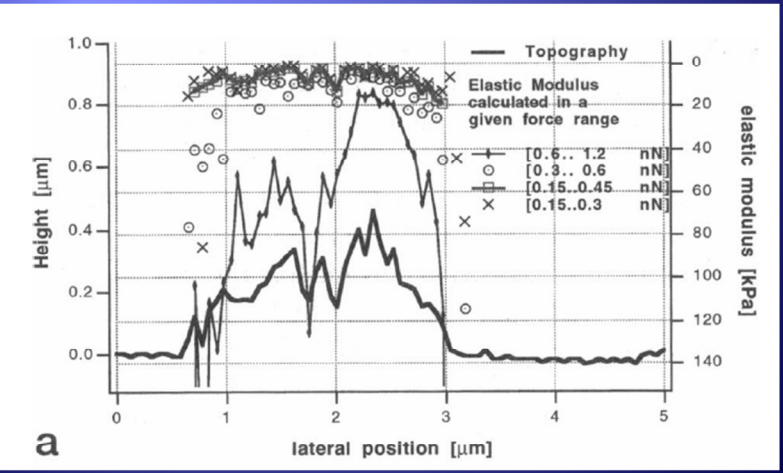
The arrows in panel d show the apparent width of the cell. At high forces, the cell appears narrower (b arrows) than at lower tip force (arrows a). This is due to the compression.



Multiple line scans are combined to generate a topology map of the cell. At higher forces, the cell is compressed and looks reduced in area.

Feel the substrate

- Low loads – surface High loads – CSK



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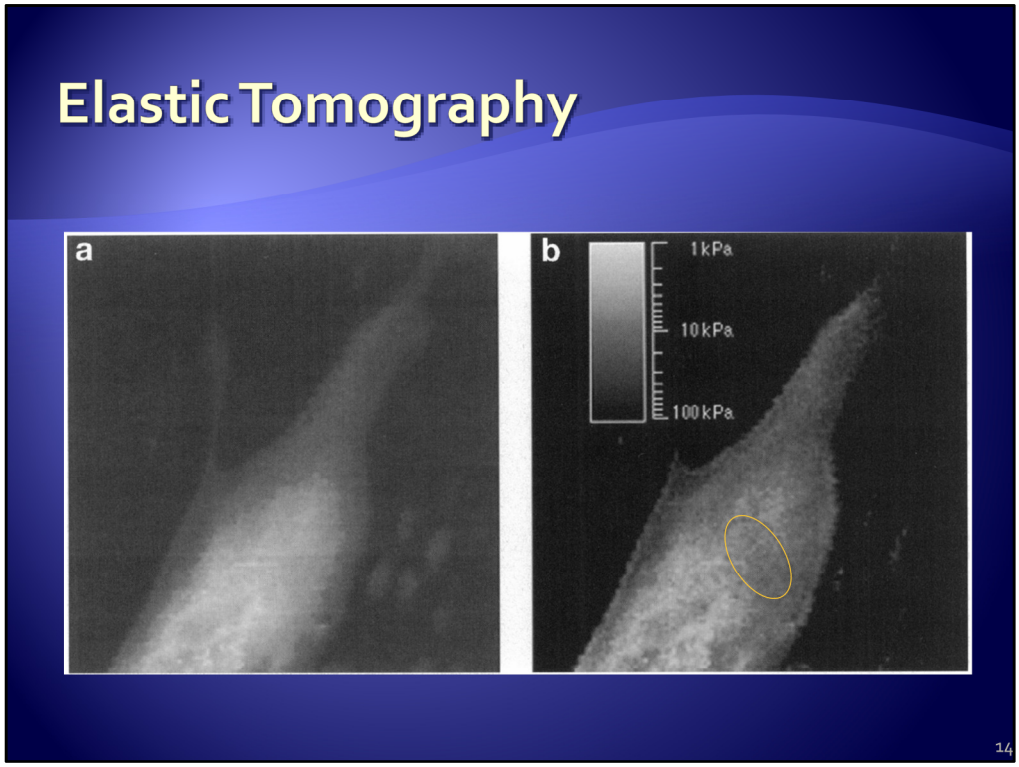
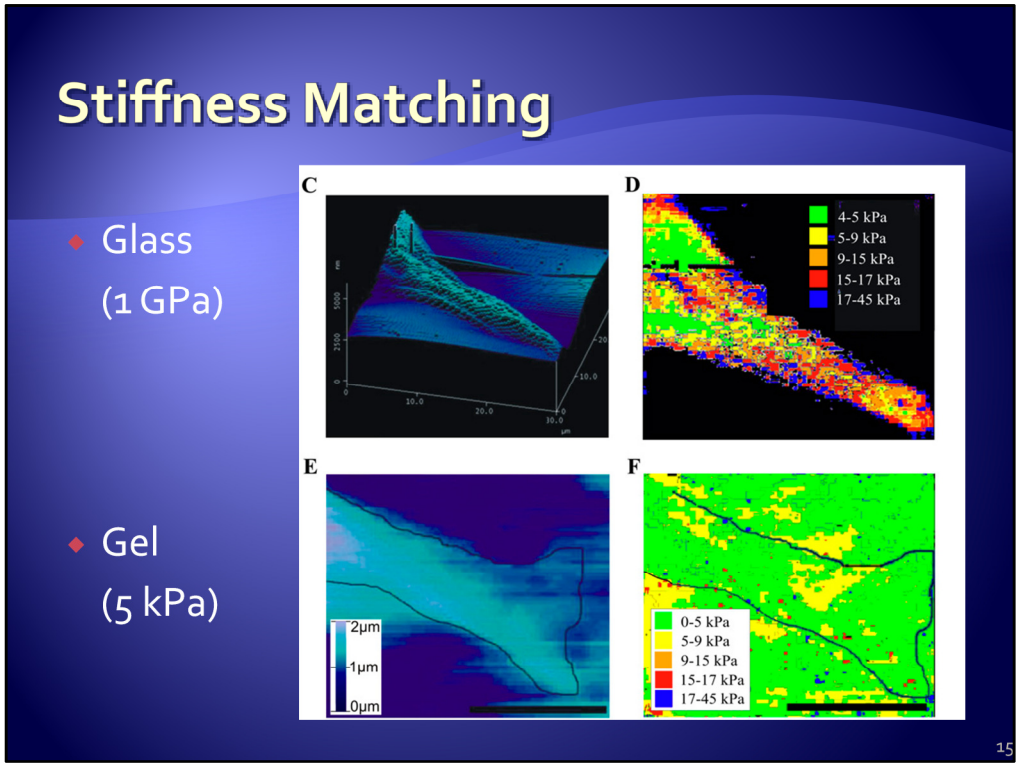
If the sample is substantially compressed, then the force curve will be affect by the stiffness of the underlying substrate. This is an artifact because for large forces (diamond, solid-line curve) the measured elastic modulus resembles the topography. Using smaller forces and little indentation, the elastic modulus softer (10-20 kPa) and the measurement is independent of the loading forces.



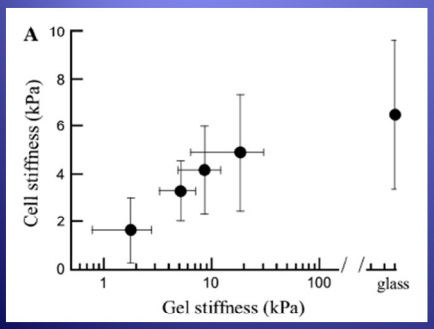
Using this approach, different regions of stiffness are seen inside the cell. The cell is stiff at the boundary, possible due to the stiffness of the substrate. However, there are also stiff regions observed in the interior of the cell (circles)

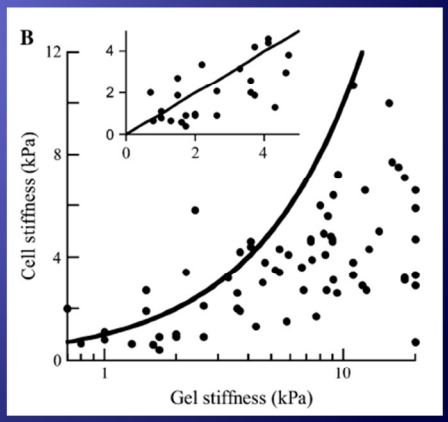


10 years later, AFM is used to measure the stiffness of fibroblasts on substrates with different stiffness. They use polyacrylamide gel that a controllable elasticity based upon the amount of cross-linker added. When the stiffness of cells were measured on these substrates, they are much softer than what is seen on glass. According to the color scale, cells on glass have regions of high stiffness, e.g. orange, red, and some blue around the periphery, but some in the interior too. On gels, the cells are very soft, having mostly uniform regions of elasticity (mostly green with a little yellow).

Stiffness regimes

- For 1-5 kPa, cell stiffness matches substrate
- For >5 kPa, cells are softer than substrate





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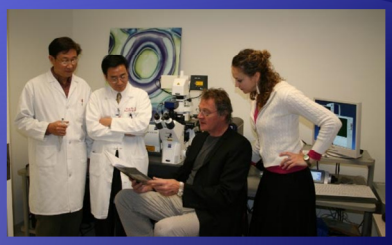
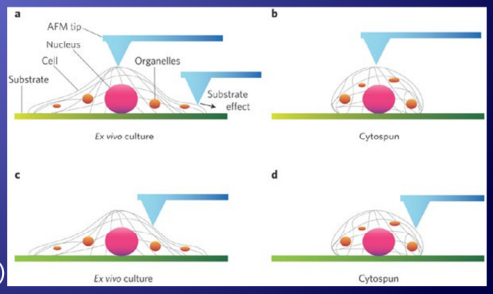
Each data point in panel B is the measurement of a single cell. The dark line indicates the line of matching between cell and substrate stiffness (y=x). From the inset image, at low substrate stiffness, there is a close match between cell and substrate stiffness. At higher stiffness of substates, cells are more often softer than their underlying substrate.

The softness of cancer

- Method to distinguish between metastatic cancer and normal cells
- Cancer needs to be flexible to migrate through tissue to get into blood stream



Jian Yu Rao, Yu-Sheng Jin, and James Gimzewski , and Sarah Cross (UCLA)



Nature Nanotech. **2**, 780–783 (2007)

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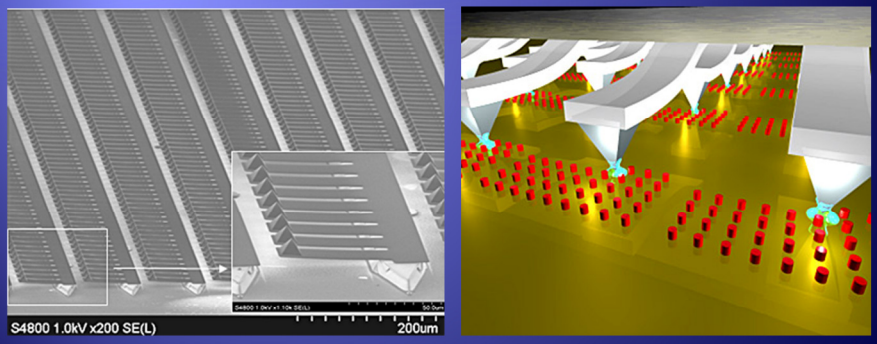
One application recent published is to use cell elasticity as a biomechanical marker for cancer cells that can be found in the body fluid in the pleural cavities surrounding the lungs of patients. These cells are collected and spun down onto a substrate for AFM measurements.

Figure 1. A 2D nano Print Array

The Future?

- Nanoink's 2D nano PrintArray for nanoscale patterns



- High throughput cell measurements?

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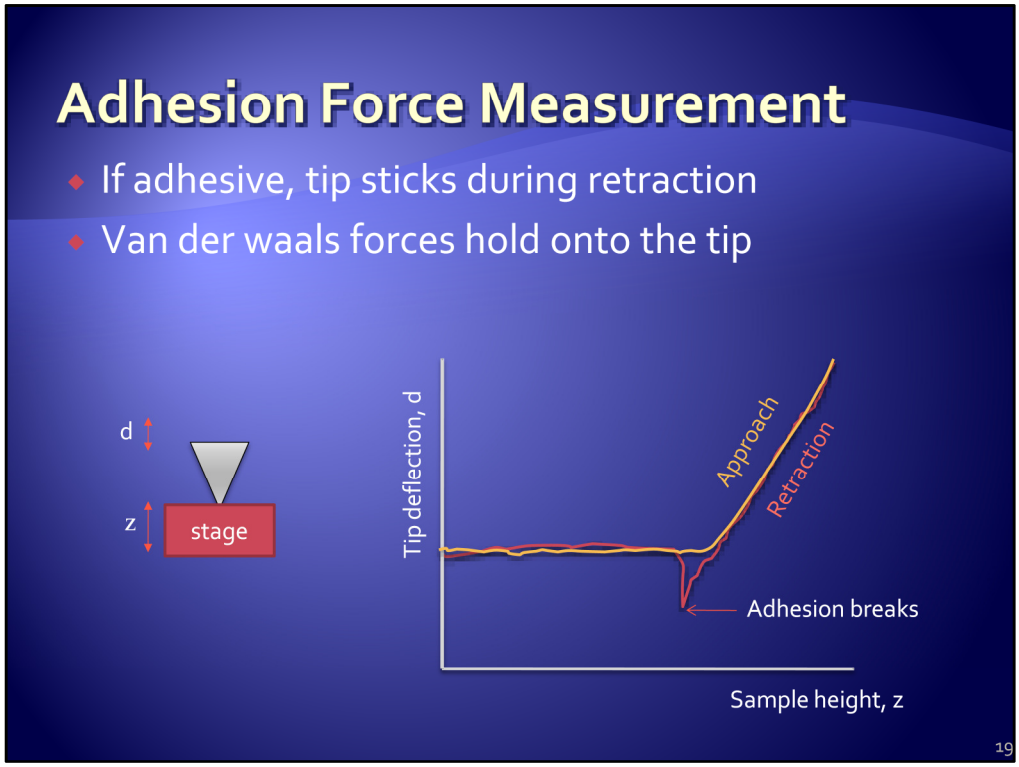
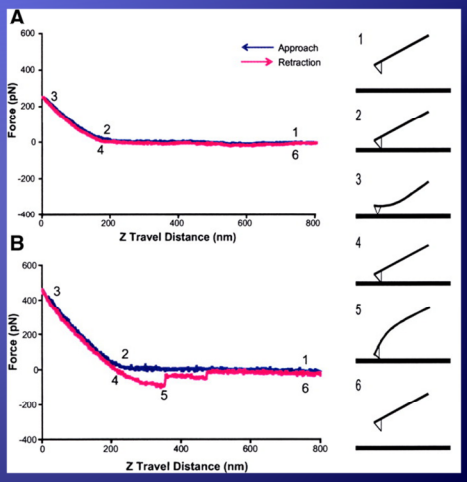
Large arrays of AFM tips have been made. This is intended for nano dip-pen lithography. Why not for cell mechanics?



In a force curve, the lateral position of the tip is not changed (it is not scanning across the surface of the cell) while the height of the stage is ramped such that the tip is brought in and out of contact and the deflection of the cantilever is monitored. Before contact, the cantilever will fluctuate due to the brownian motion of the fluid environment. Once contact is reached, a sharp kink in the deflection curve is easily detected.

Integrin Adhesion Forces

- Fibronectin and $\alpha_5\beta_1$-integrin on vascular smooth muscle cells
- Individual bond force is 39 ± 8 pN
- Cells have multiple bonds at each focal adhesion due to integrin clustering



Am J Physiol Heart Circ Physiol 289: H2526-H2535, 2005.

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Typical nonadhesion (*A*) and adhesion (*B*) force curves generated by using AFM. A schematic of the AFM probe movement during the process of force curve generation is shown at center. AFM fibronectin (FN)-coated probes were controlled to repeatedly contact and retract from the surfac eof vascular smooth muscle cells at a speed of 800 nm/s and 0.5-Hz frequency. During retraction, when a specific adhesion occurred, the rupture of this adhesion was detected as a sharp deflection shift in the retraction curve (*B*).

Based upon the binding probability, the individual force of the integrin-FN bond is 39 ± 8 pN