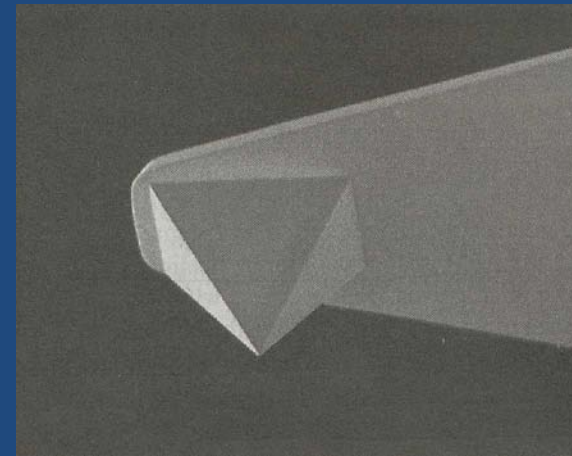
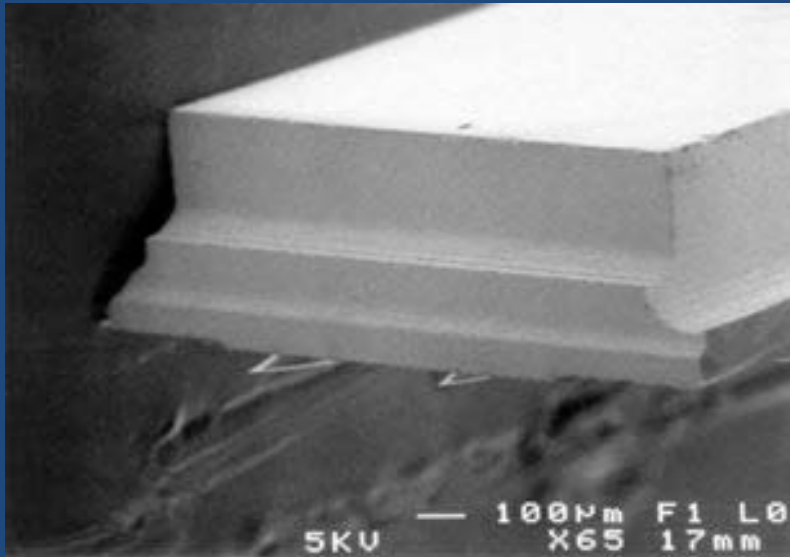


AFM Cantilever/Tip Styles



DNP Silicon Nitride Probes

spring constants: 0.58, 0.32, 0.12, 0.06 N/m

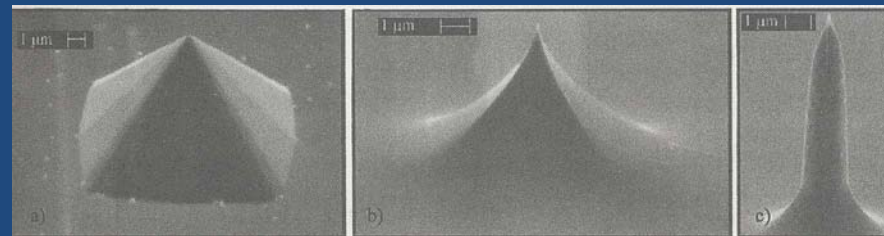
tip radius of curvature: 20-60nm

cantilever length: 100 & 200µm

reflective coating: gold

shape of tip: square pyramidal

tip half angle: 35



Carbon nanotubes as AFM tips?

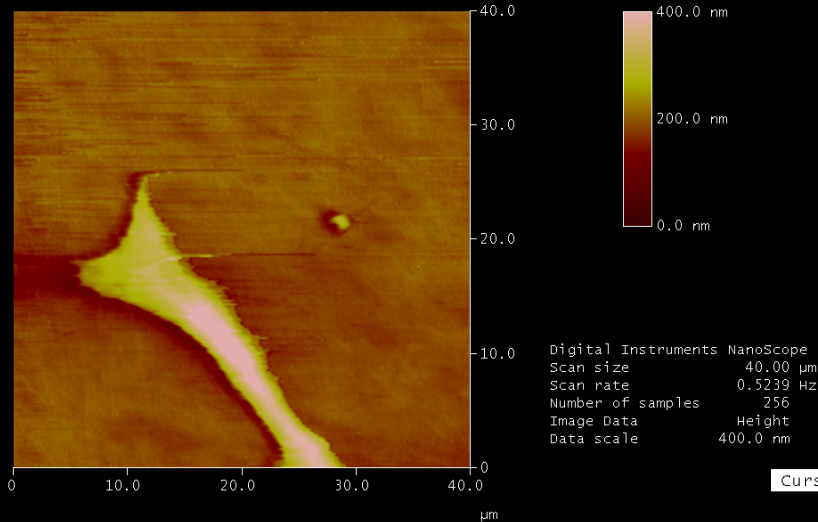
AFM Tip Manufacturing Process



AFM Image Acquisition and Analysis

Clear Execute Undo

Flatten

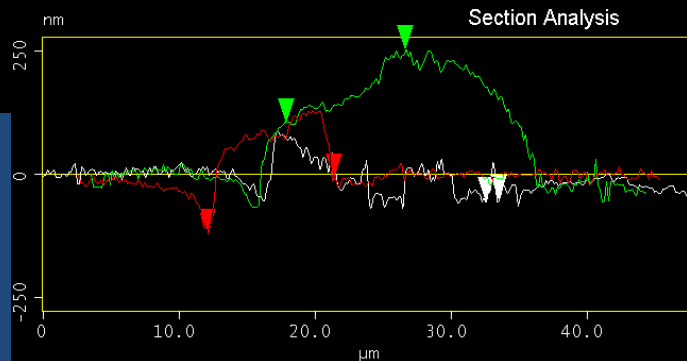


Original image

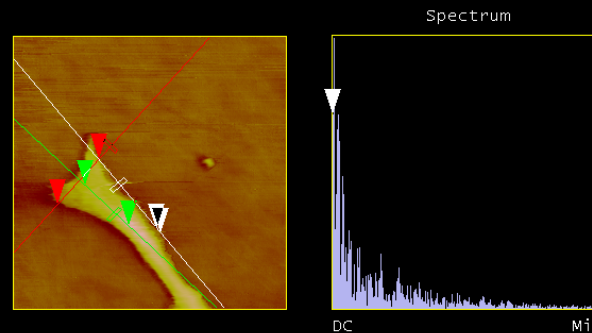
- 40X40 μm (variable)
- scale bar (variable)
- image parameters –
 - ✓ P&I gains
 - ✓ scan rate
 - ✓ set point
 - ✓ # samples/line
 - ✓ scan angle

Cursor Marker Spectrum Zoom Center Line Offset Clear

Section analysis
 height and width
 measurements of
 interesting features



L	937.50 nm
RMS	31.978 nm
lc	DC
Ra(lc)	24.217 nm
Rmax	81.451 nm
Rz	81.451 nm
Rz Cnt	2
Radius	55.390 μm
Sigma	41.436 nm



Surface distance	9.264 μm
Horiz distance(L)	9.219 μm
Vert distance	112.80 nm
Angle	0.701 $^\circ$
Surface distance	8.763 μm
Horiz distance	8.750 μm
Vert distance	153.33 nm
Angle	1.004 $^\circ$
Surface distance	966.66 nm
Horiz distance	937.50 nm
Vert distance	4.133 nm
Angle	0.253 $^\circ$
Spectral period	DC
Spectral freq	0 / μm
Spectral RMS amp	10.095 nm

axon4

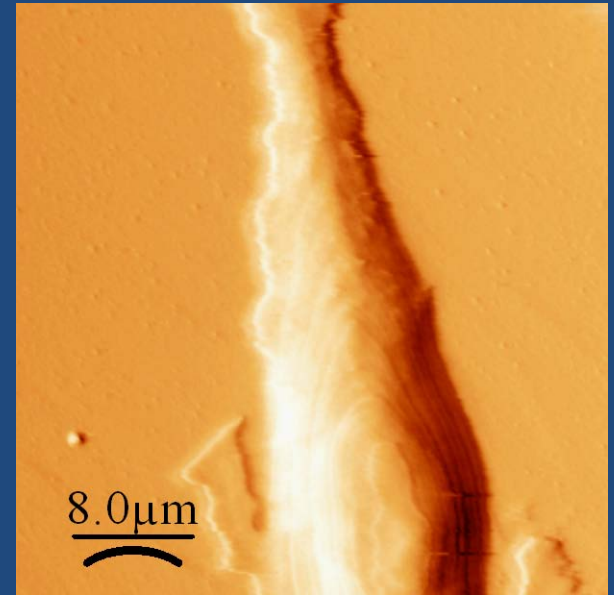
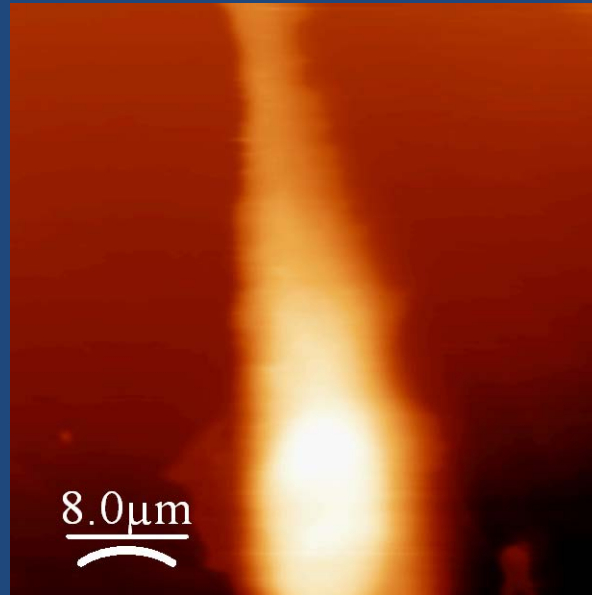
Cursor: fixed 3 Zoom: 1:1 Cen line: Off Offset: Off

AFM Image Acquisition and Analysis

Channel selection

Height Mode

Error Mode

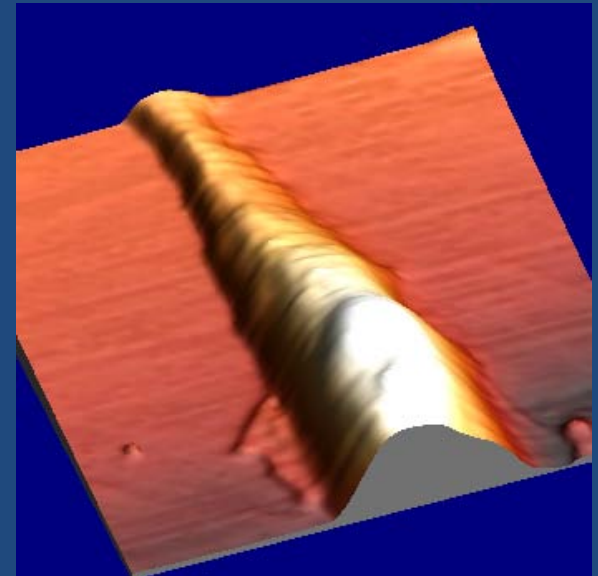


Offline Analysis

http://www.nanotec.es/index_noticias.html

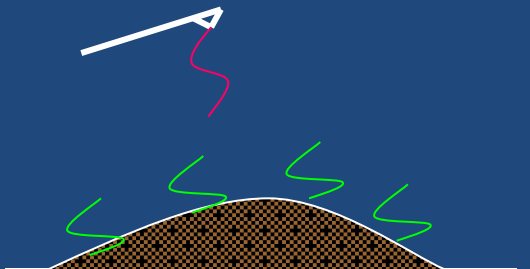
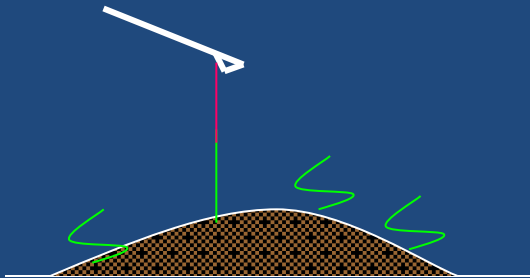
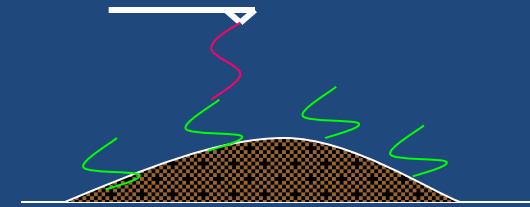
<http://nanoprobenetwork.org/>

Free WSxM software

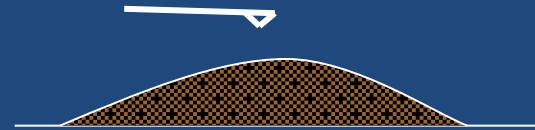


AFM Force Measurements

Pulling



Pushing



Sample Preparation

Flat Substrate

Free of Contaminants

Securely attached to the surface (or not?)

Size

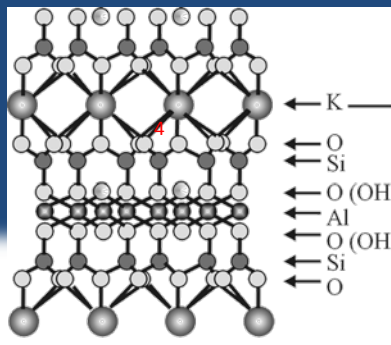
Separation of particles/dilution

Vacuum, dry, fluids

Other Environmental conditions

What is your sample and what are it's characteristics?



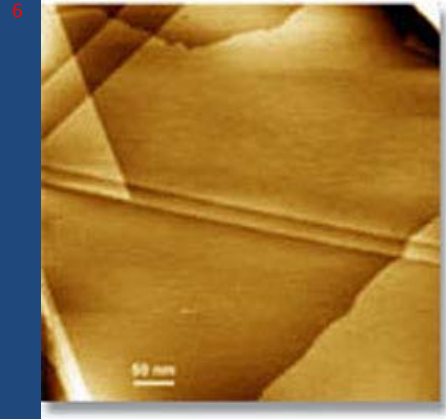


Mica

- An example of an atomically flat surface is Mica.
- Mica has flat layers surface that comprises of Aluminum, Silicon, Oxygen and Potassium.
- Mica has highly perfect basal cleavage.
 - Cleavage - tendency of crystalline materials to split along definite planes, creating smooth surfaces
 - Basal cleavage: cleavage parallel to the base of a crystal, or to the plane of the lateral axes
- Cleaved easily with the help of adhesive tape
- The area around the two planes have not been exposed to air making it extremely clean.
- Can be modified with silanes either to promote absorption or covalent bonding of bio molecules

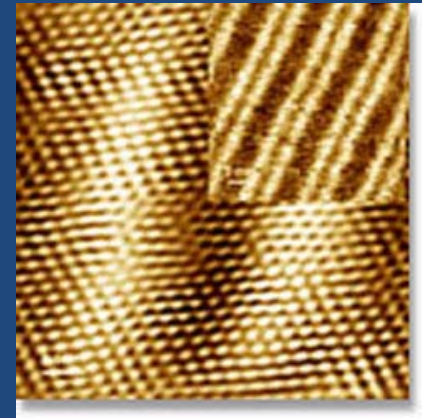
Evaporated Gold (Au 111)

- Used to immobilize samples because the metal surface can attract different molecules.
- Drawback is that gold can be exposed to the air and get contaminated.
- However using a flame based on hydrogen gas can clean the gold surface making it clean and flatter



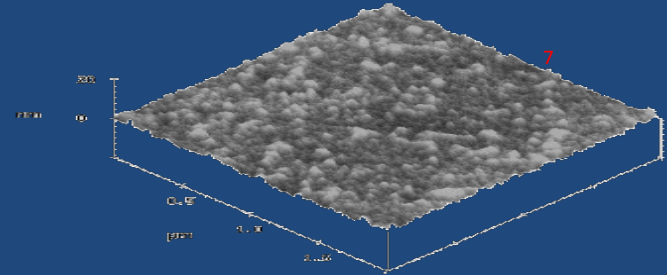
Low resolution scan of Au (111) surface, showing atomic steps.
Scan size: 500 nm

<http://mnelab.com/p.htm>



High resolution scan of Au (111) surface, with reconstruction strips (inset) hexagonal atomic structure.
Scan size: 5 nm; inset: 20 nm

Glass



- Before use the glass is cleaned by washing with concentrated Hydrochloric Acid or Nitric Acid about 5 times for 1 min with Millipore water in an ultrasonic bath running at 50 kHz. This process makes the glass slides clean and smooth.
- Can also be modified with silane molecules bringing new chemical functions at the surface for further covalent modification
- Good for imaging cells and other large structures, but too rough to image of absorbed molecules

Immobilization



- If the mechanical touch force is larger than the immobilization force then the AFM tip can simply sweep away the sample as observed with the dust particles in AFM lab.
- Ideas to keep the sample firmly on the substrate.
 - Biological Immobilization
 - Tape the edges of the sample
 - Use a small drop of glue or wax to fix the sample on.
 - Avoid using a material that would cause drifting, contamination or peeling.

SPM Manufacturers



Agilent Technologies



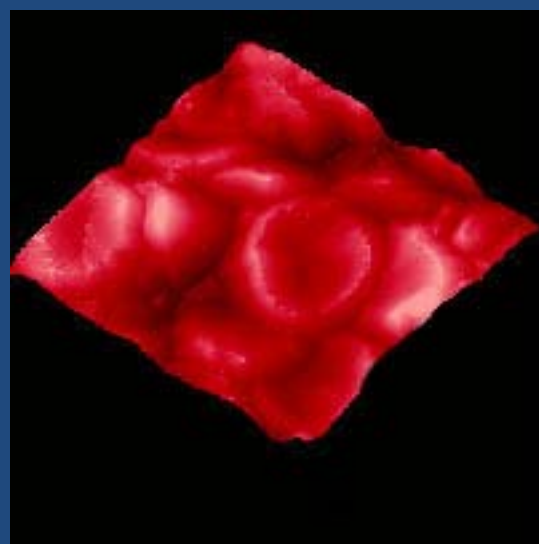
References

Ron Reifenberger, **A Primer on Scanning Tunneling Microscopy (STM)**,
<http://www.nanohub.org/resources/1185/>

Arvind Raman, Atomic Force Microscopy
http://www.nanohub.org/resource_files/2005/11/00522/2005.11.28-raman.pdf

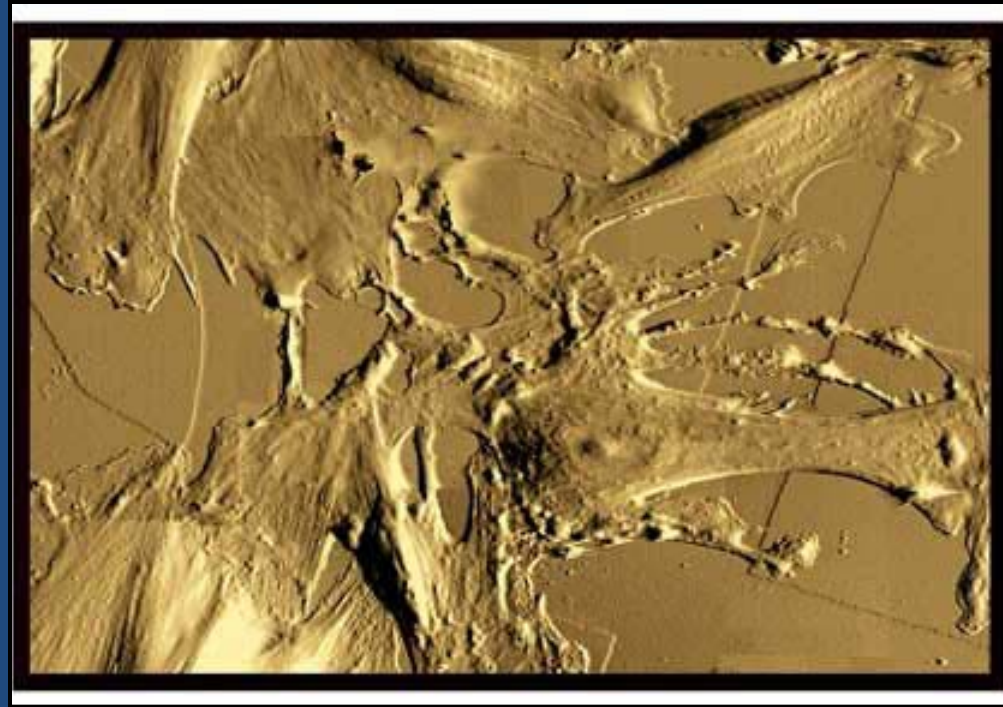
**Ron Reifenberger, Nanometrology Room Design:
The Performance and Characterization of the Kevin G. Hall Nanometrology Laboratory,**
<http://www.nanohub.org/resources/3779/>

VEDA as a simulation resource

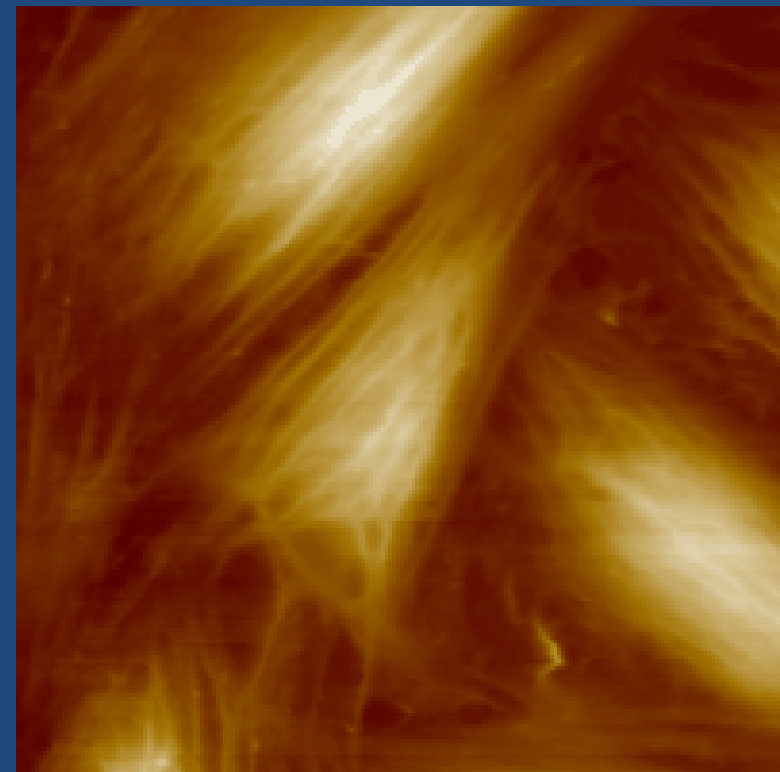


Contact mode image of human red blood cells
15 μ m scan
courtesy M. Miles and J. Ashmore, University of Bristol, U.K.

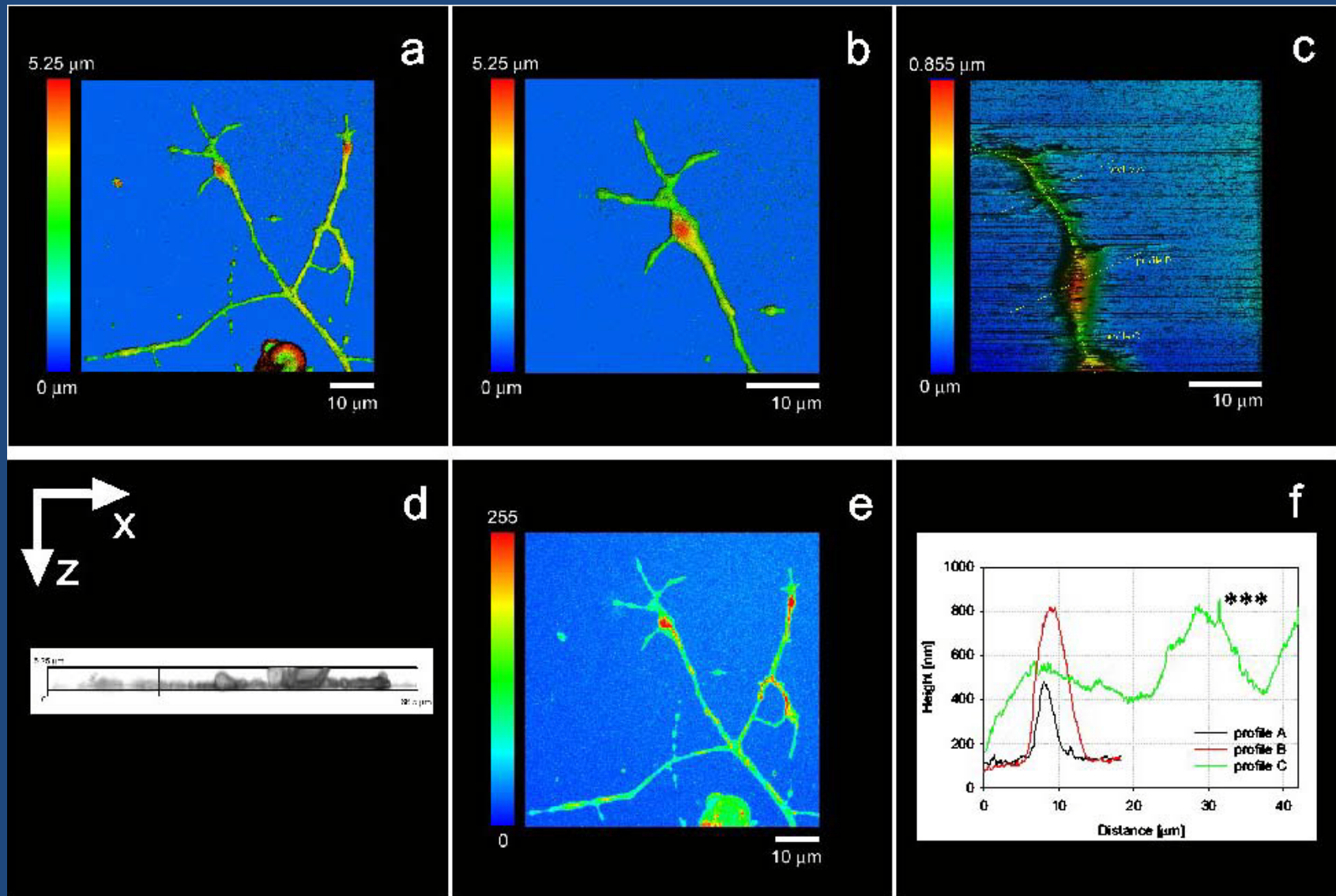
Field of view Mosaic of 10 Images taken each at 100 μ m x 100 μ m
Liquid AFM image of fibroblast-like cultured cells chemically fixed with glutaraldehyde on a glass cover slip. From this image one can see the cell-to-cell contacts, cell division, and the formation of stress fibers.
Image Courtesy of M. Drechler, LS Pharm Tech - FSU Jena, Germany



Living endothelial cells grown directly on a petri dish and imaged by AFM on a Digital Instruments BioScopeTM using contact mode in liquid. The image shows the interaction between multiple cells and between the cells and the substrate. Scan time was 35 min and scan size = 65 μ m. Imaged by I. Revenko, M.D., Applications Scientist, Digital Instruments. Sample courtesy of Georges Primbs, Miravant Inc.



AFM Compared to Confocal Microscopy

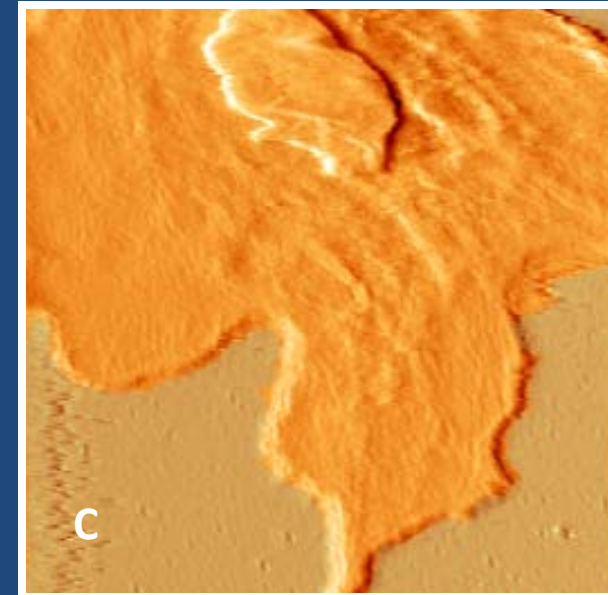
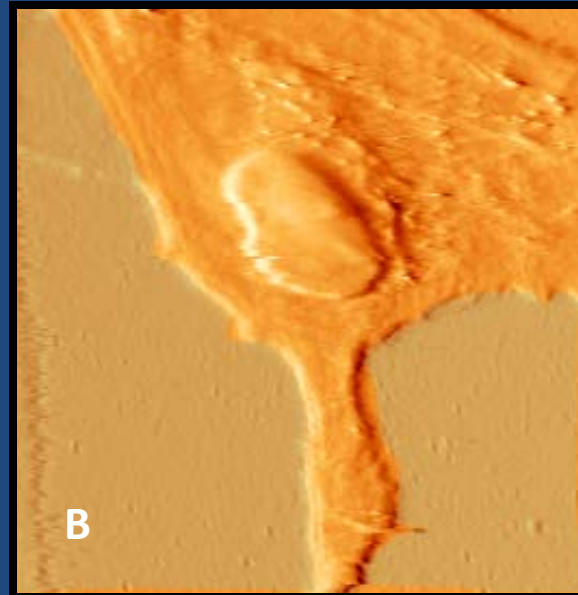
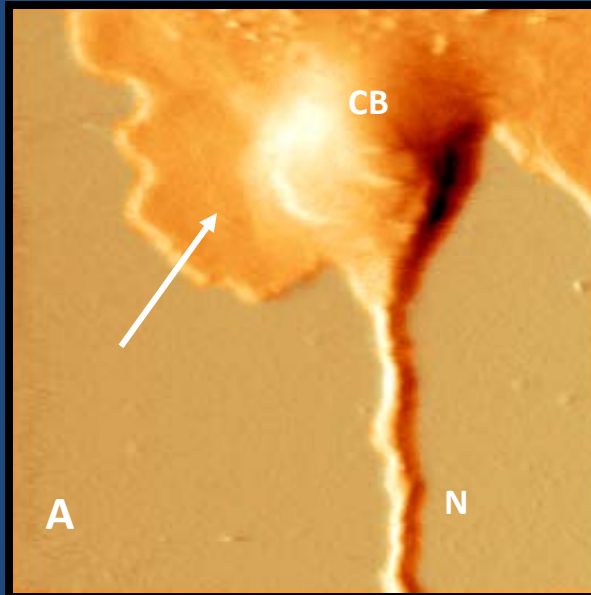


H.McNally, B. Rajwa, and J.P. Robinson, accepted for publication
in the Journal of Neuroscience Methods, April 2003



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Cell Death by AFM Probe



time →

