Atomic Force Microscopy (AFM)
Imaging and applications

Dr. Sudip Ray

AFM Manager

Senior Research Fellow

MBIE Biocide Toolbox and Product Accelerator Programme

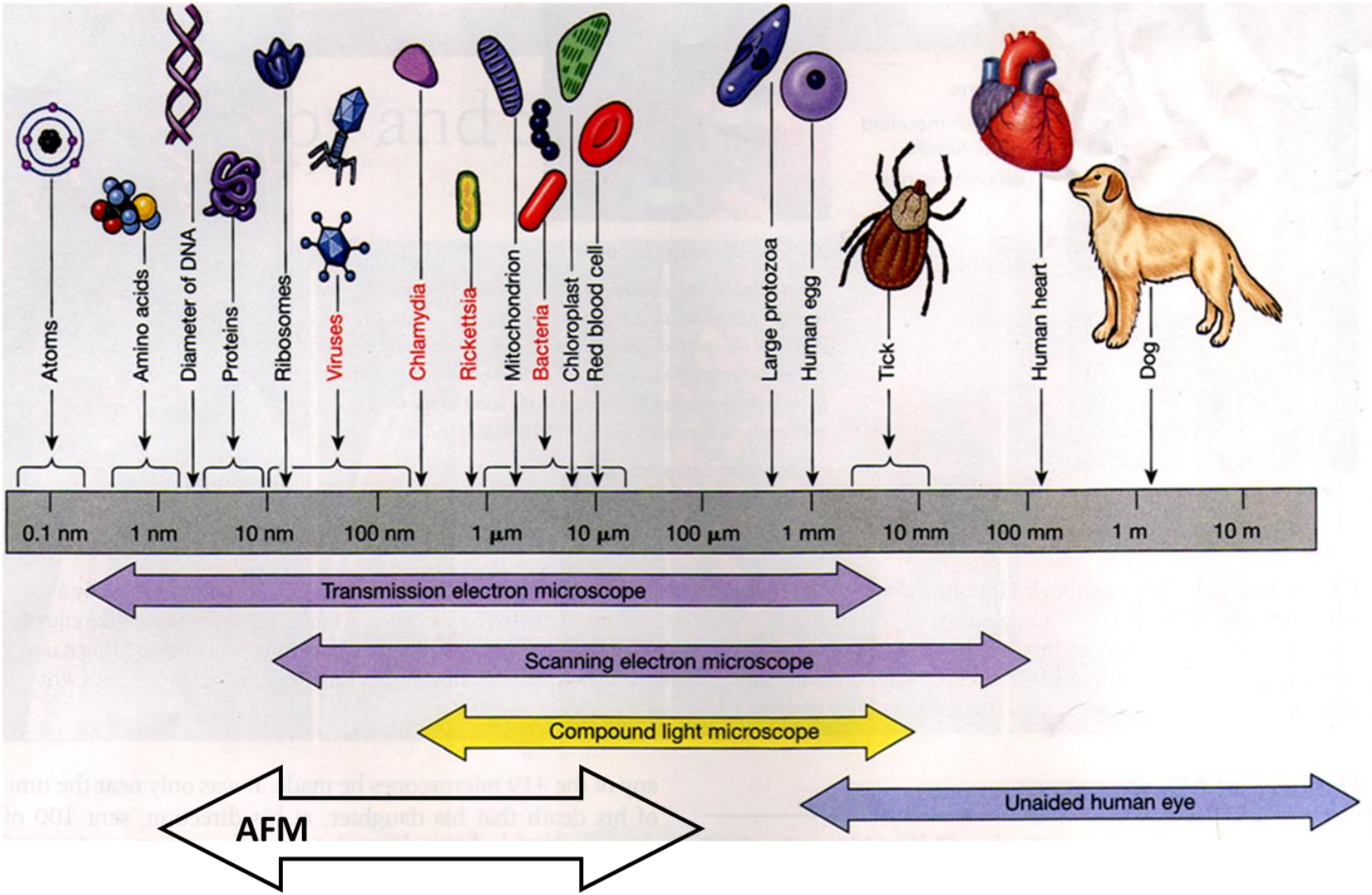
School of Chemical Sciences

The University of Auckland, Newmarket Campus

Content

- **Introduction**
  - **Comparison of AFM with other microscopes**
- **Measuring images**
  - **Sample preparation**
  - **Setting up the instrument**
  - **Scanning images**
- **Image processing and analysis**
- **Image artefacts**
- **Applications**
- **Concluding remarks**

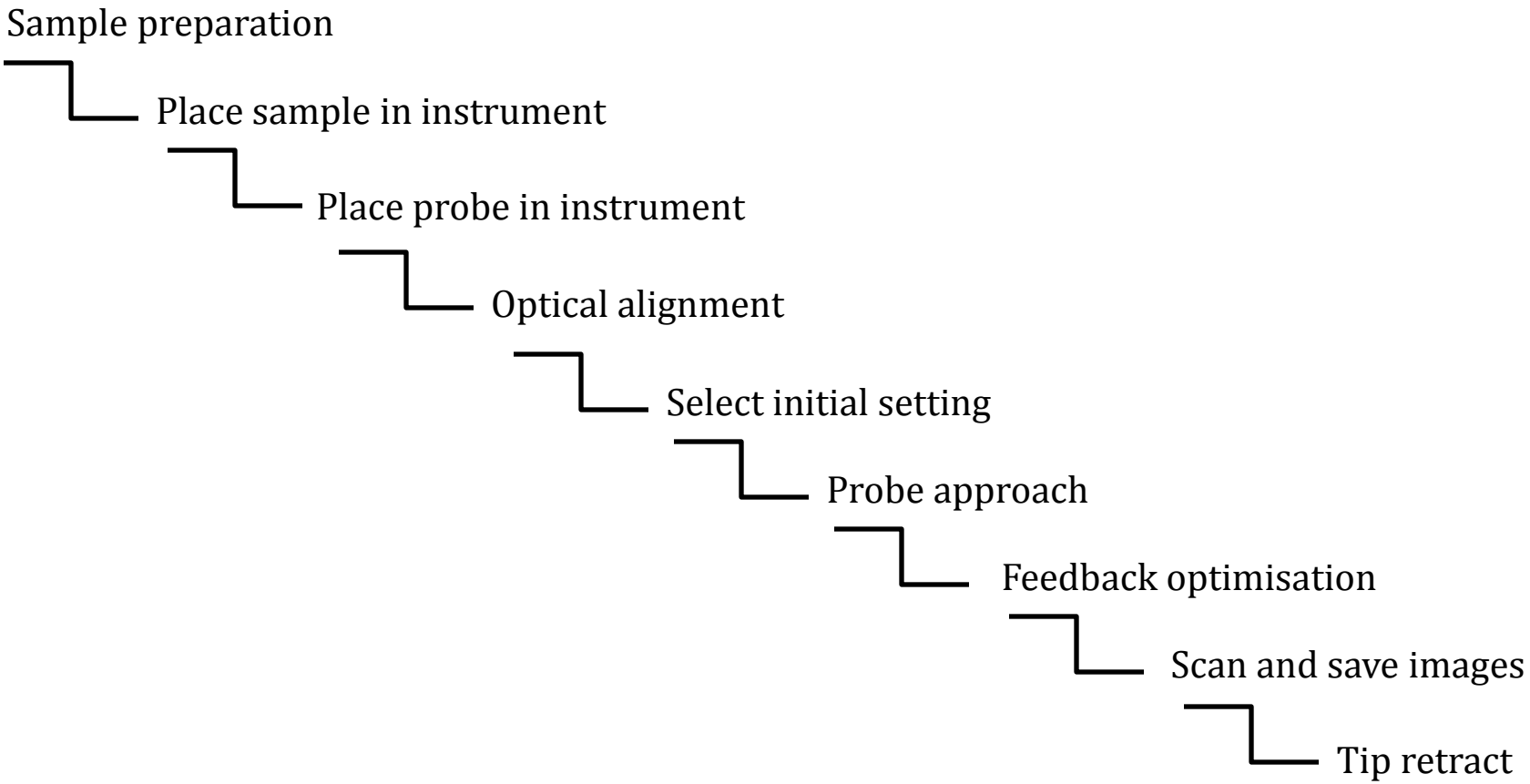
Introduction



Comparison of AFM with other microscopes

Image courtesy: Asylum Research

Measuring images: major steps

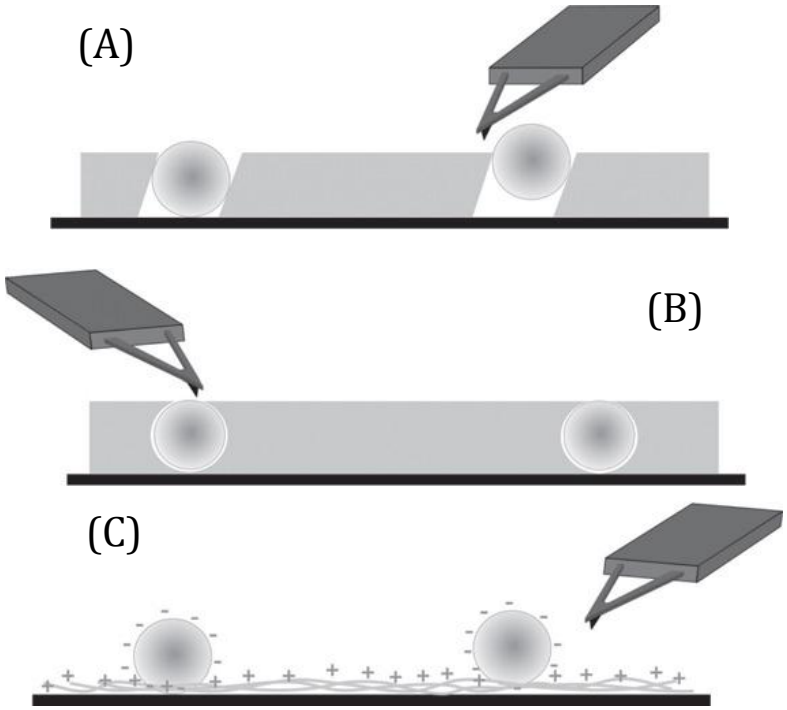


Measuring images: sample preparation

Specific sample preparation techniques

- Biomolecules – DNA and proteins
- Cell cultures
- Bacteria
- Particulate samples
- Polymers
- Nanotubes

Substrates for AFM



Cell immobilization procedures
(A) physical entrapment
(B) in an agar gel matrix
(C) chemical fixation

Image courtesy: http://www.ncbi.nlm.nih.gov/pubmed/22341216

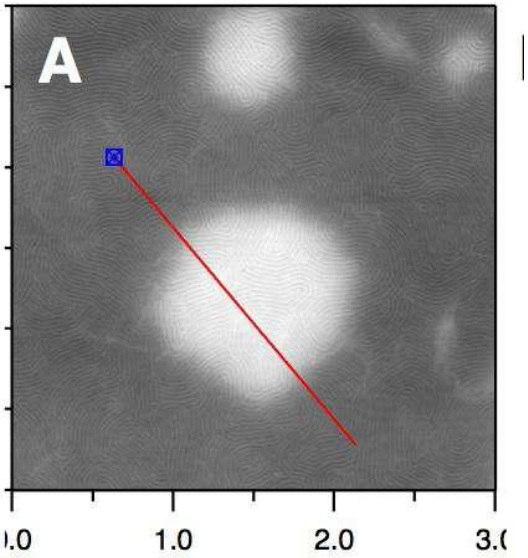
Measuring images: setting up the instrument and scanning images

- Measuring AFM images in contact mode
- Measuring AFM images in oscillating modes
- Selecting initial settings and probe approach
- Optimizing scan conditions

Image processing and analysis

Processing AFM images

- Levelling - Polynomial fitting
- Filtering
- Rotation, cropping, and scaling
- Displaying AFM images
- Histogram adjust
- Colour palettes
- Shading
- Three-dimensional views



Analysing AFM images

- Line profiles
- Roughness
- Particle and grain analysis

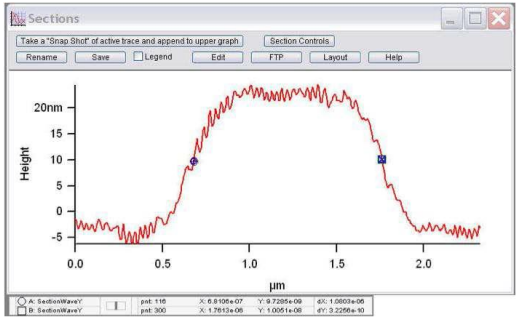
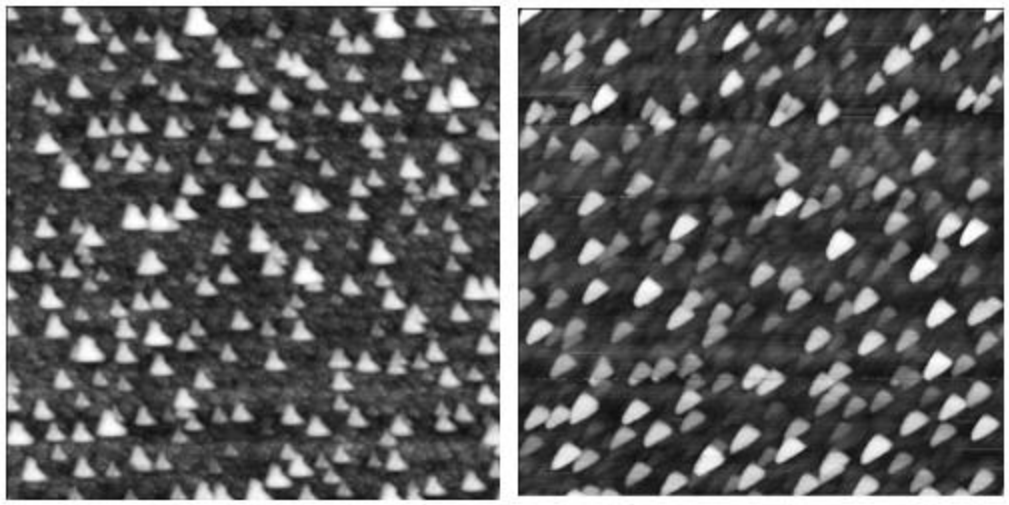


Image courtesy: Asylum Research

Image artefacts



Example: Formation of repeating patterns in the images by using broken or dirty probes

Image courtesy: http://www.nanophys.kth.se/nanophys/facilities/nfl/afm/fast-scan/bruker-help/Content/Resources/Graphics/ServiceApps/Troubleshooting/dull_dirty_tip.png

Applications of AFM: surface science and engineering

Biology

- Biomolecule imaging
- Bacterial cell measurements
- Lipid membrane imaging
- Mammalian cell imaging
- Biological force spectroscopy
- Protein unfolding

Physical and materials sciences

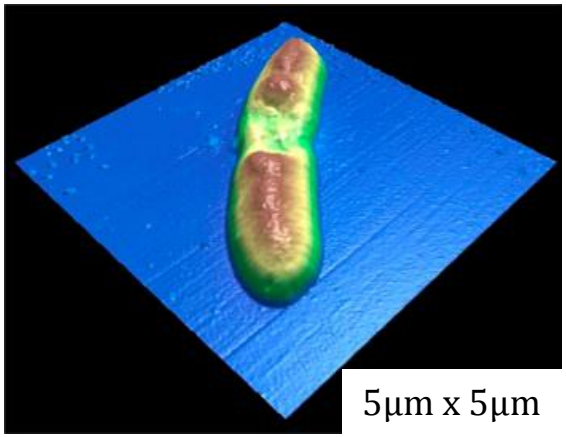
- Roughness measurements of high-performance materials
- Hardness measurement of polymer films
- Atomic-resolution imaging of crystal structures
- Friction measurement with AFM
- Phase imaging to identify surface features

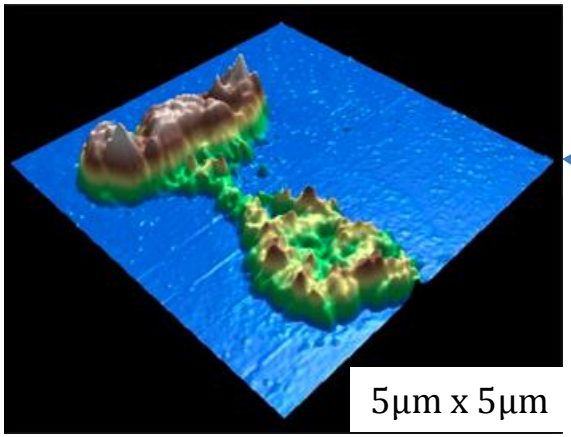
Nanotechnology

- Nanoparticle measurement
- Mechanical measurement of nanotubes
- Nanodevice construction with the AFM
- Nanoparticle–DNA interactions
- Electrical measurements of nanostructures with AFM

Applications of AFM: biology

Example: Effect of antibiotics on bacteria



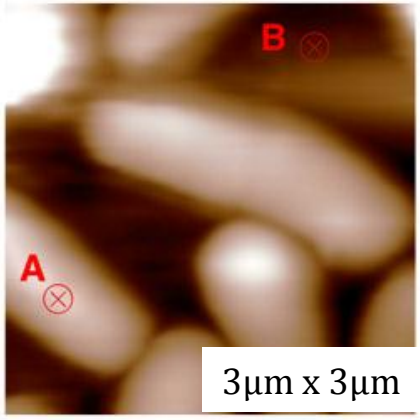


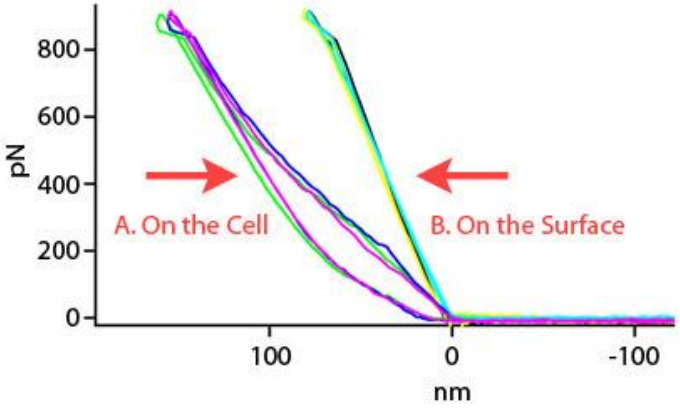
Bacteria: *Salmonella enterica*

Antibiotic: Polymyxin B

After the incubation with antibiotic for 30 min.

Example: Elasticity measurements on *E. coli* bacteria



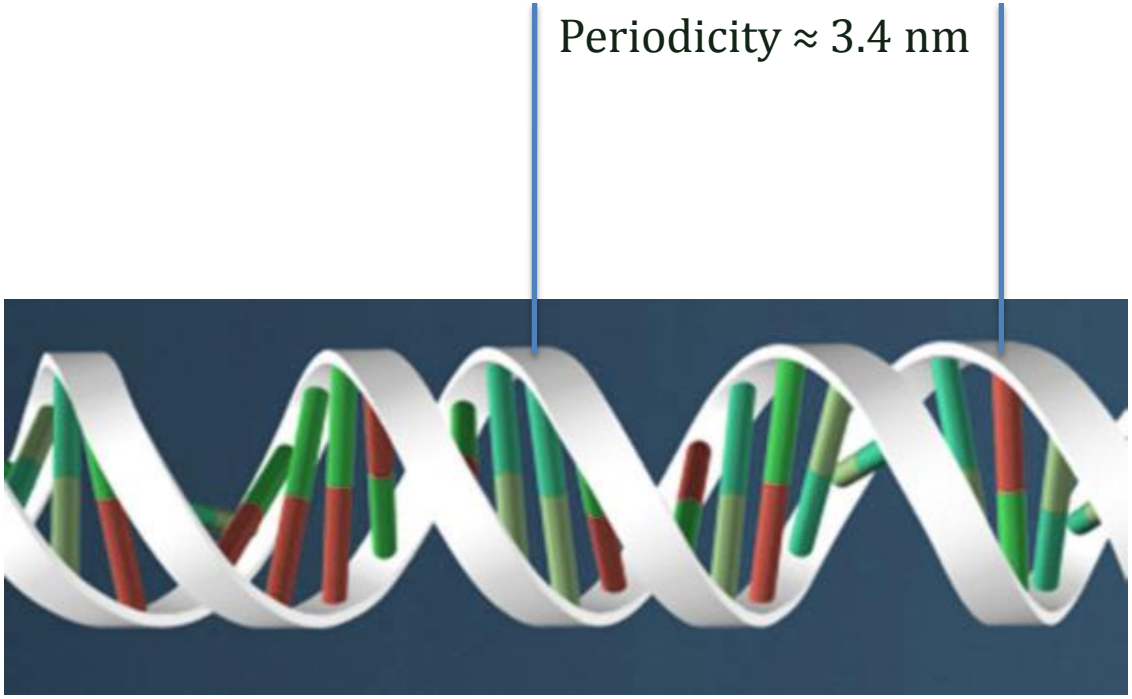


On the cell:
smaller slope and large hysteresis

Image courtesy: Asylum Research

Applications of AFM: biology

Example: Determination of DNA Double Helix structure

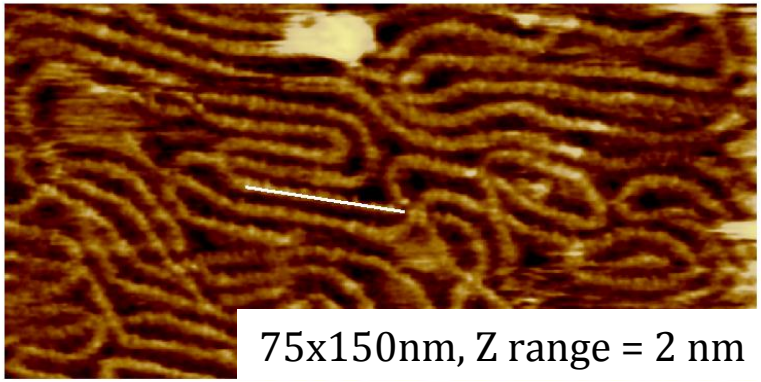


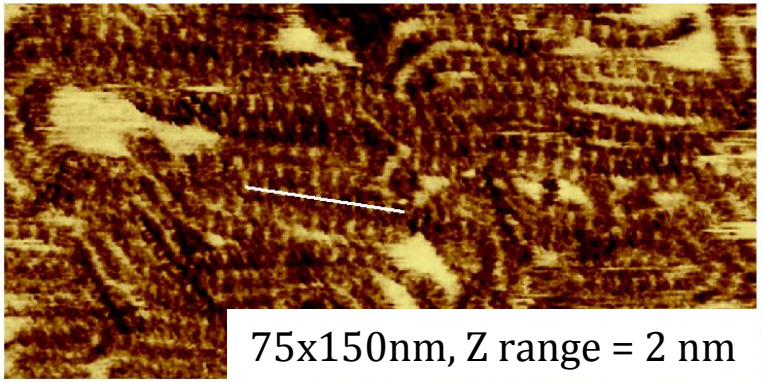
Minor Groove ≈ 1.2 nm
Major Groove ≈ 2.2 nm

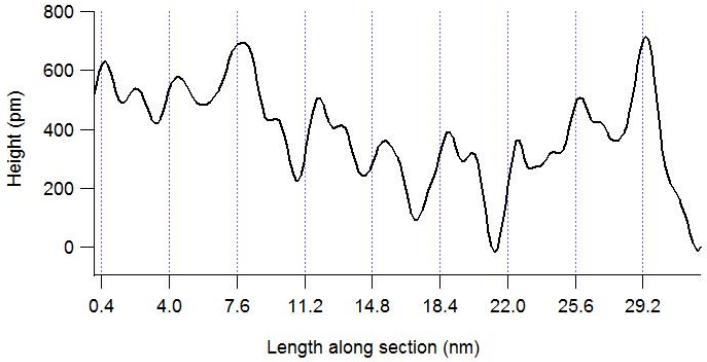
Image courtesy: Asylum Research

Applications of AFM: biology

Example: Determination of DNA Double Helix structure







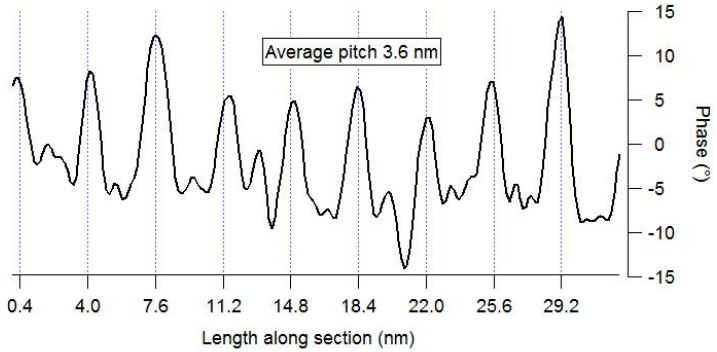
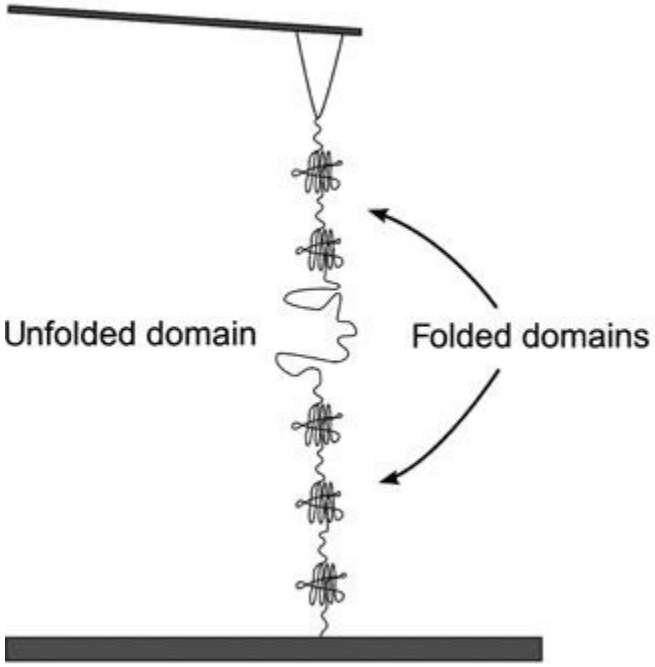


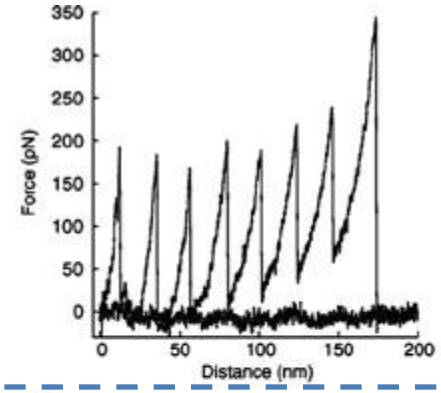
Image courtesy: Asylum Research

Applications of AFM: biology

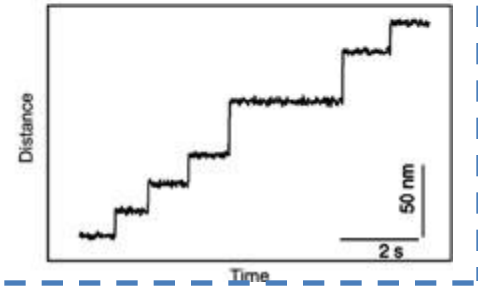
Example: Determination of Protein unfolding



Schematic of typical experiment, showing protein being stretched between AFM tip and a surface to which it is covalently bound



Typical force curve measured on a protein sample in constant velocity mode

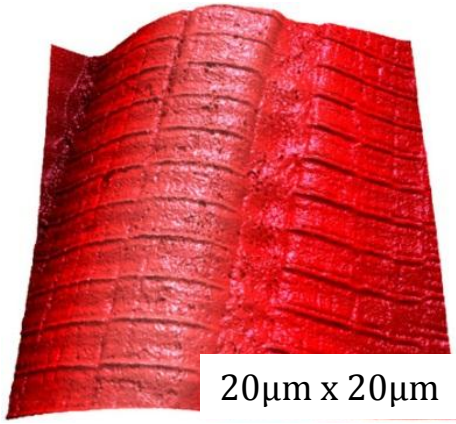


Typical result from the same sample in constant-force mode

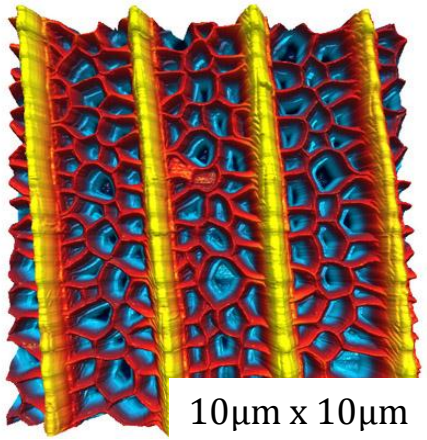
Image courtesy: Forman, J. R.; Clarke, J., Current Opinion in Structural Biology 2007, 17 (1), 58–66.

Applications of AFM: biology

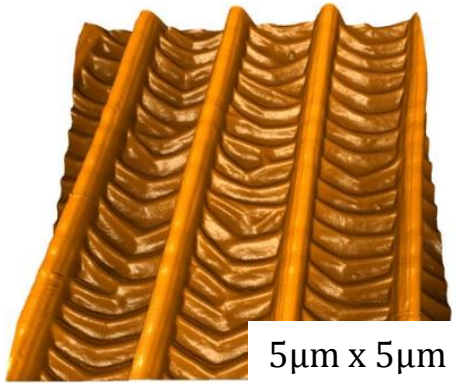
Example: Determination of Tissue structures



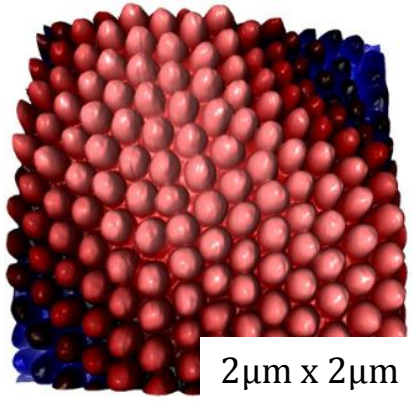
Mouse Skeletal Muscle Fiber



Moth Wing



Mosquito Leg

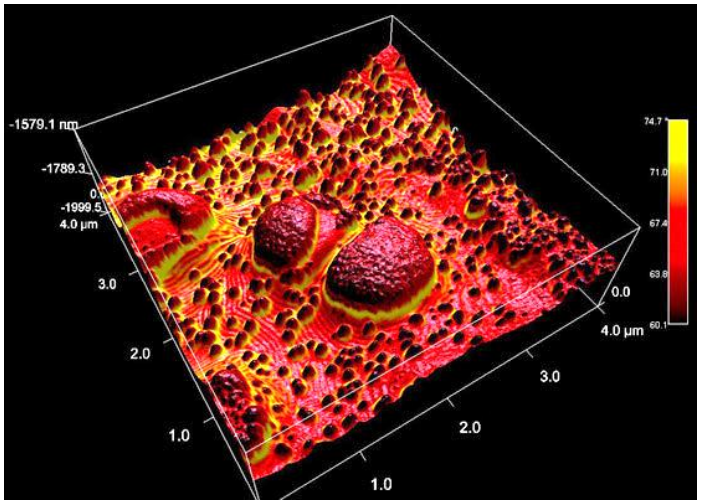


Mosquito Eye

Image courtesy: Asylum Research

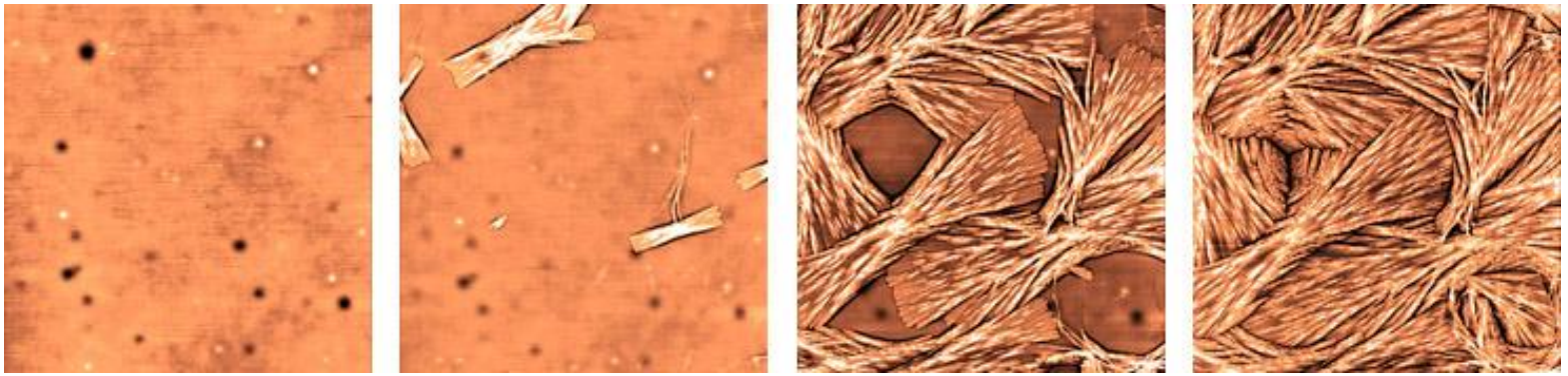
Applications of AFM: Polymer

Example: Drug delivery



Phase image of drug particles embedded in a block copolymer

Example: Crystallization studies

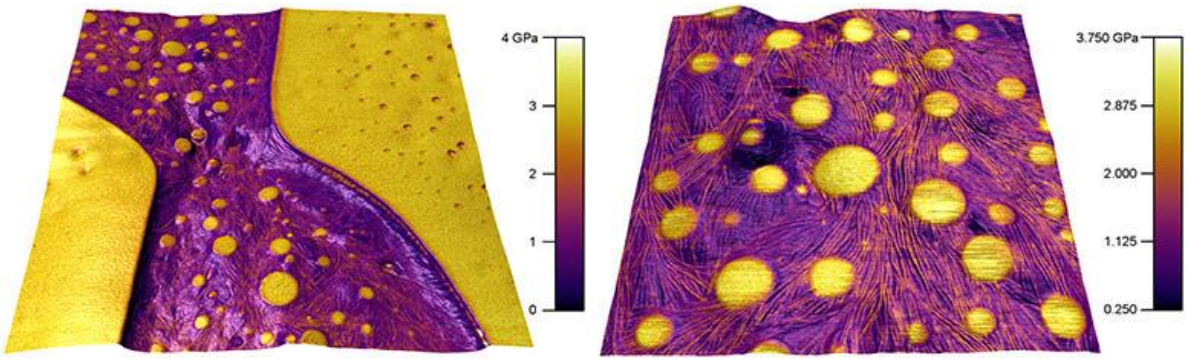


Syndiotactic polypropylene melted to 160°C, and left to crystallize at 105°C

Image courtesy: Asylum Research

Applications of AFM: Polymer

Example: Determination of phase distribution in polymer blends



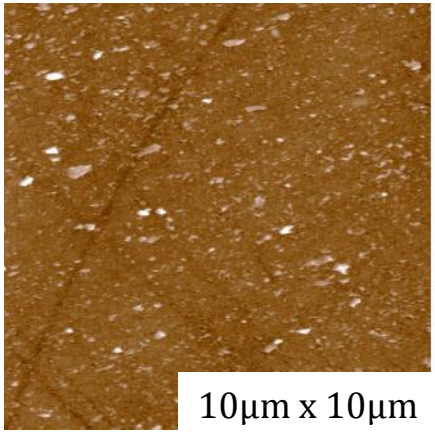
AM-FM modulus mapping on PS-PCL polymer blend

Image courtesy: Asylum Research

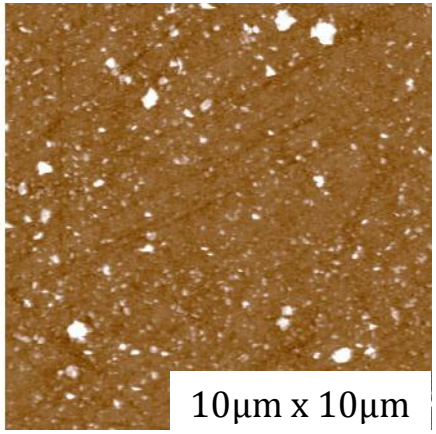
Applications of AFM: Commercial products

Contact Lens:

quality inspection - scratches and adsorbed proteins



Exterior surface, exposed to the air



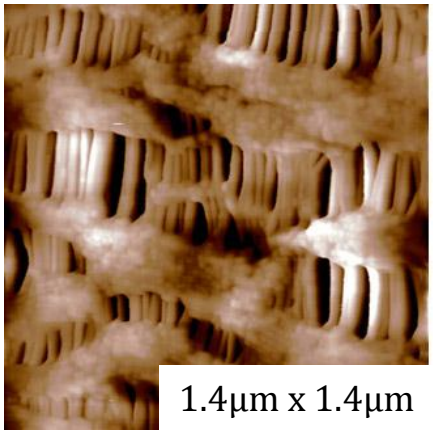
Interior surface, in contact with the eye

Medical devices: oxygenators

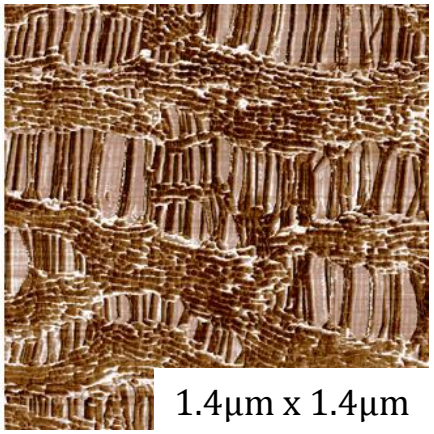
Celgard®: high burst/tensile strengths, excellent gas transfer

Microporous hydrophobic PP membrane consists of alternating fibers and lamellae

quality inspection – pore structures



Height image



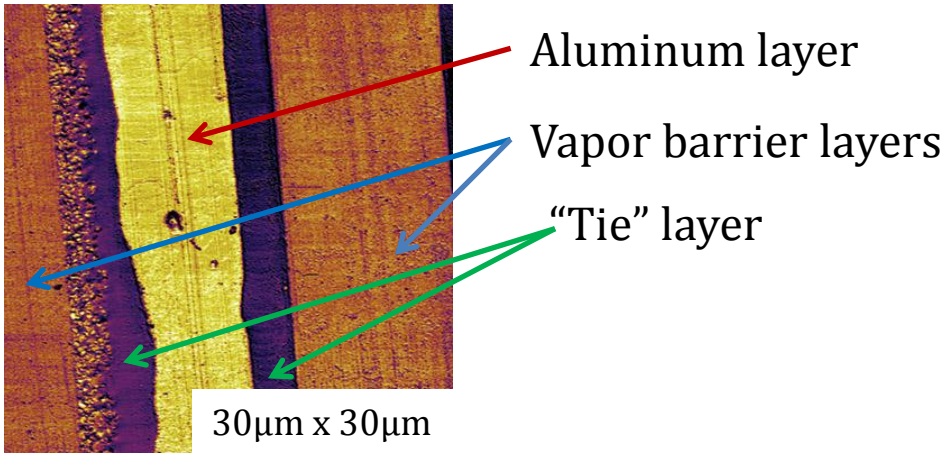
Phase image

Image courtesy: Asylum Research

Applications of AFM: Commercial products

Commercial coffee packaging bag

quality inspection - differentiating materials



Dark Chocolate

quality inspection - compositional differences

Chocolate is a complex material consisting primarily of a finely crystallized continuous fatty lipid matrix (cocoa butter) in which cocoa powder and sugar particles are dispersed.

With time, the lipid crystals tend to merge to form larger crystals on a micron scale, significantly effecting the texture and taste of the chocolate.

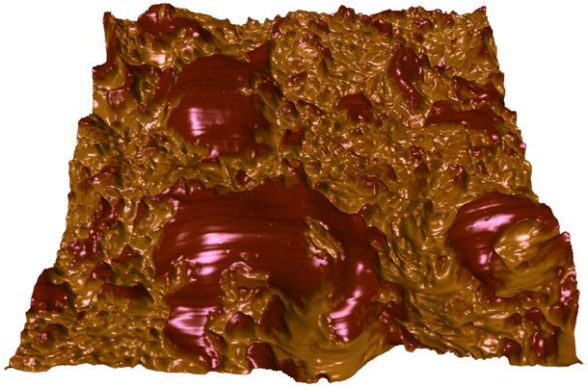


Image courtesy: Asylum Research

Applications of AFM: Fundamental research

Atomic resolution by Non-contact AFM

A : C_{60} model

B to E : AFM showing frequency shift Δf at differing tip heights

F : image used for measure of bond length

- L_h = 1.38Å
- L_p = 1.454 Å

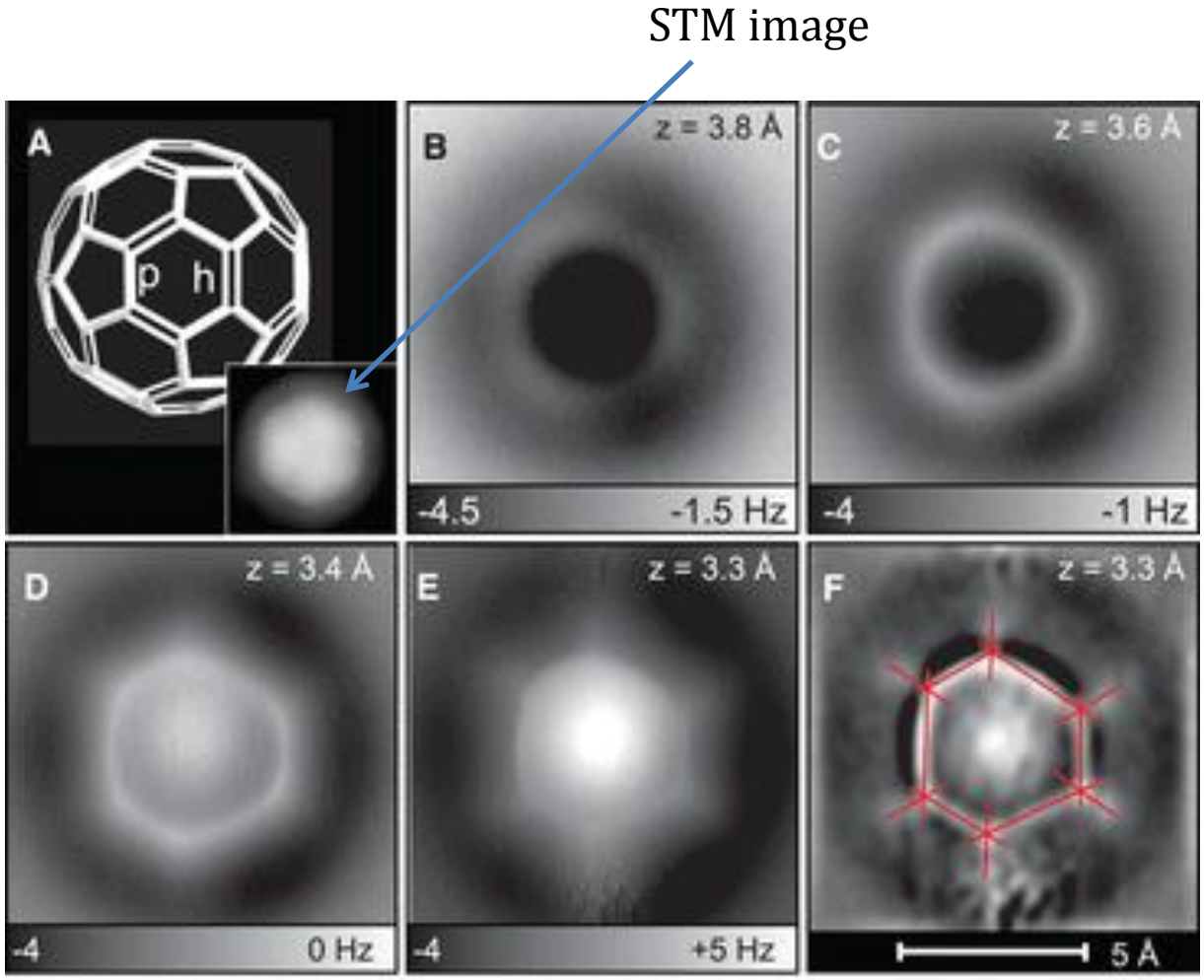


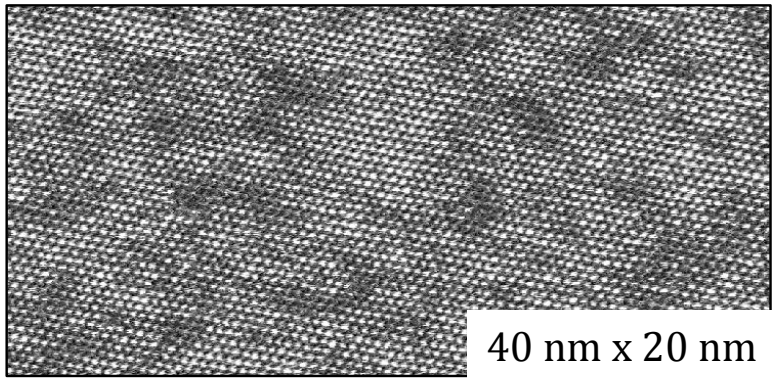
Image courtesy: http://www.sciencemag.org/content/337/6100/1326.figures-only

Applications of AFM: Fundamental research

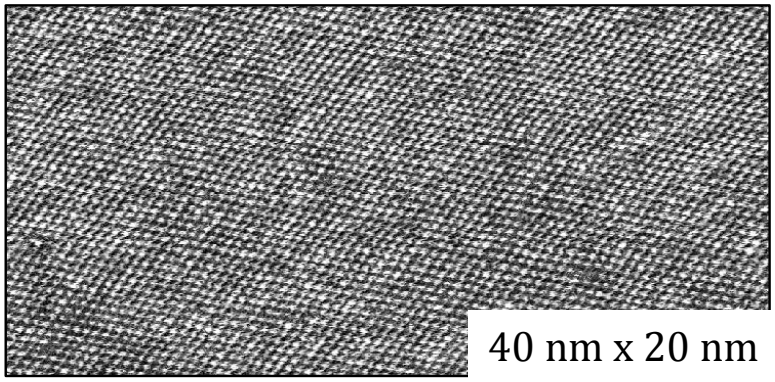
Atomic-resolution images of mica

Overnight experiment: mica in 1 M CsCl solution on Cypher ES

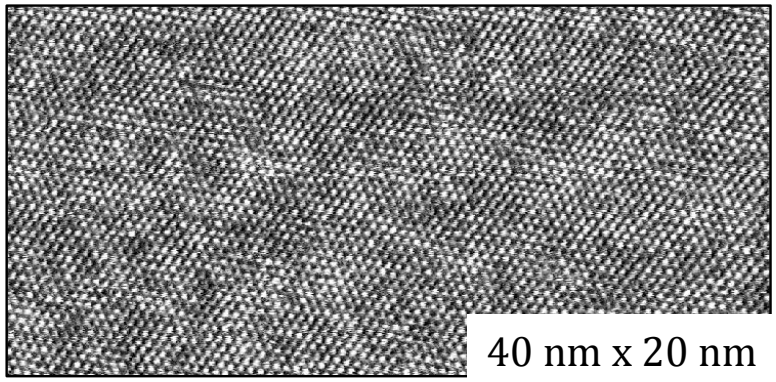
9PM



1AM



5AM



9AM

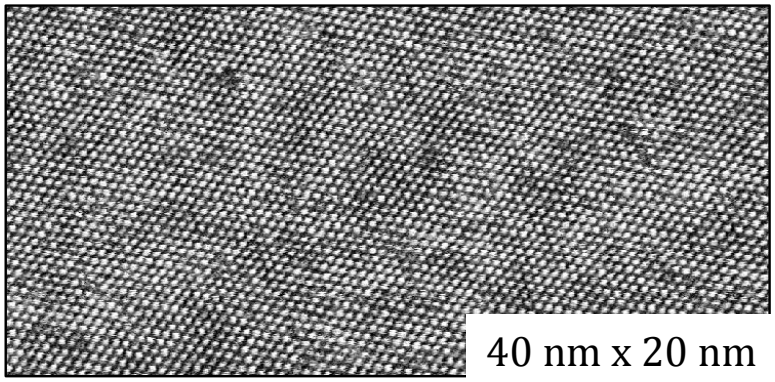


Image courtesy: Asylum Research

Concluding remarks

- The probe tip must be clean and particularly sharp
- Sources of external noise and the vibration isolation must be optimized
- The sample must be well fixed to the substrate, which should not be moving
- The instrument must be at thermal equilibrium and without drift
- Scanning parameters must be optimized