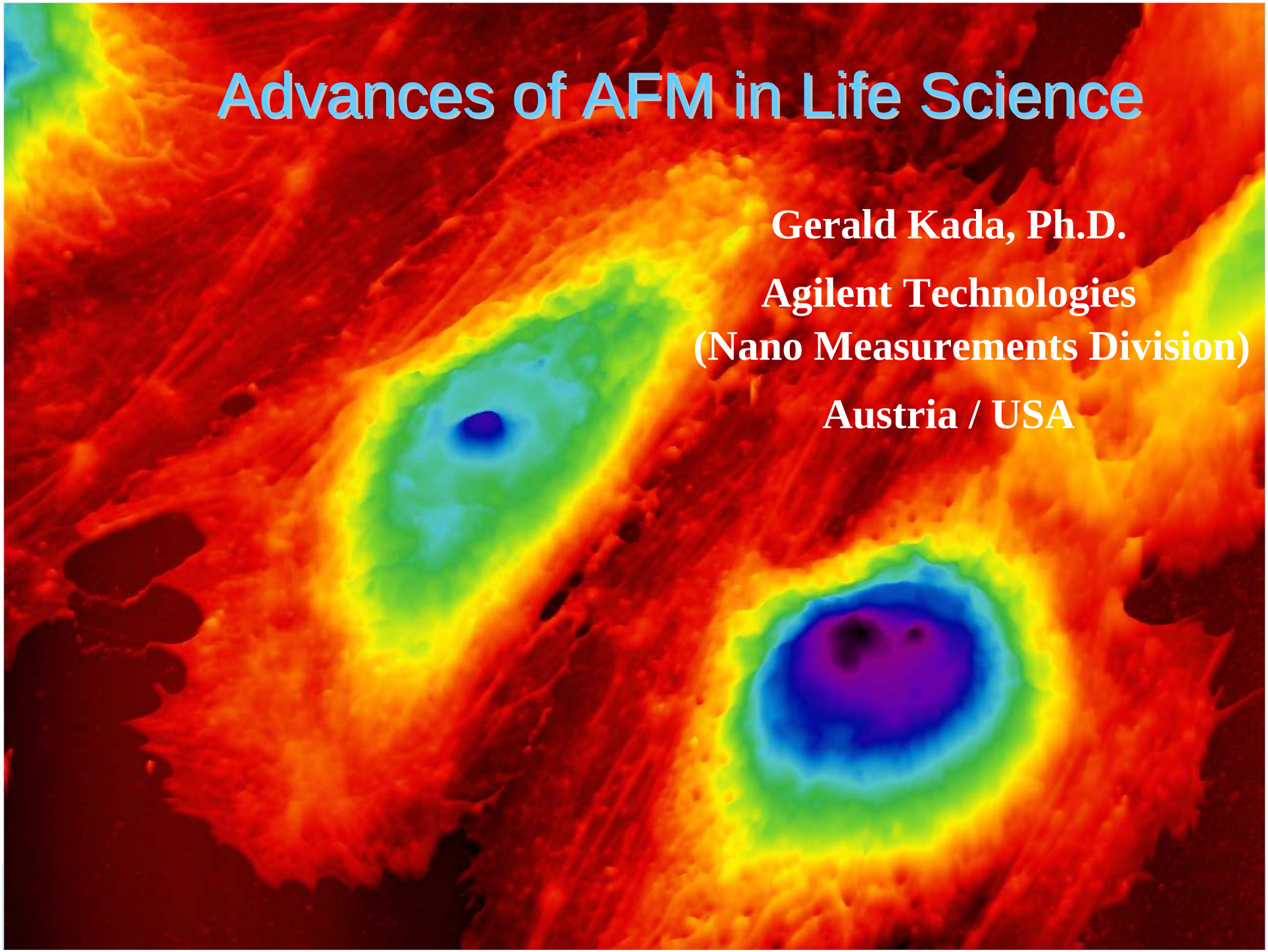


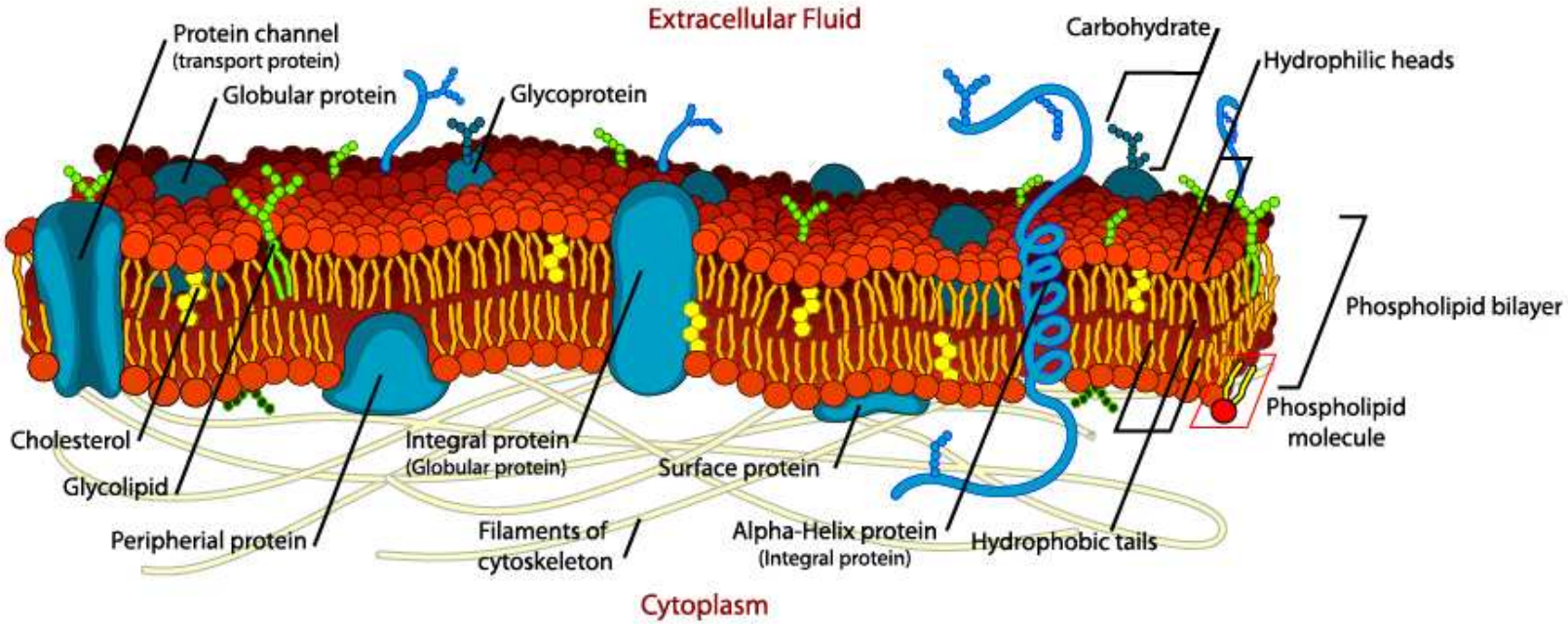
Advances of AFM in Life Science

Gerald Kada, Ph.D.

Agilent Technologies
(Nano Measurements Division)

Austria / USA

The cell membrane – the main interest for AFM



60% of prescription drugs target membrane proteins!

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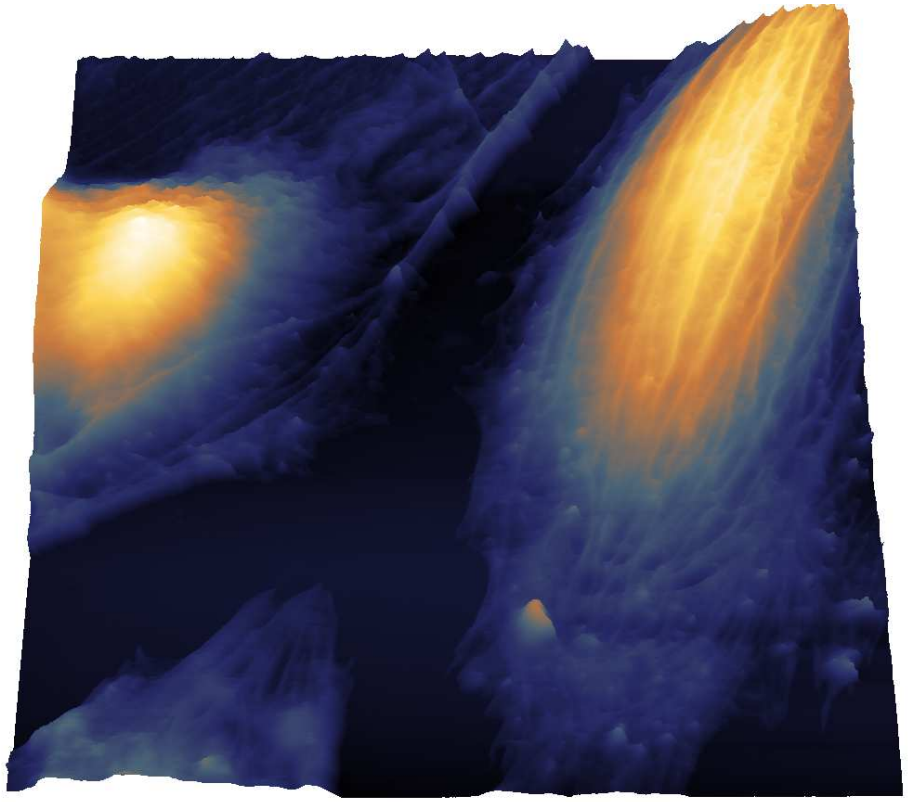
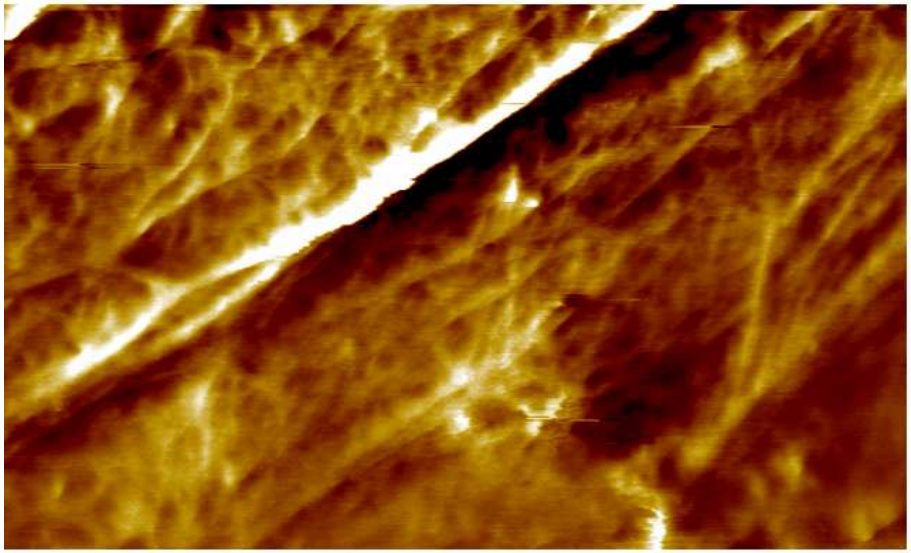
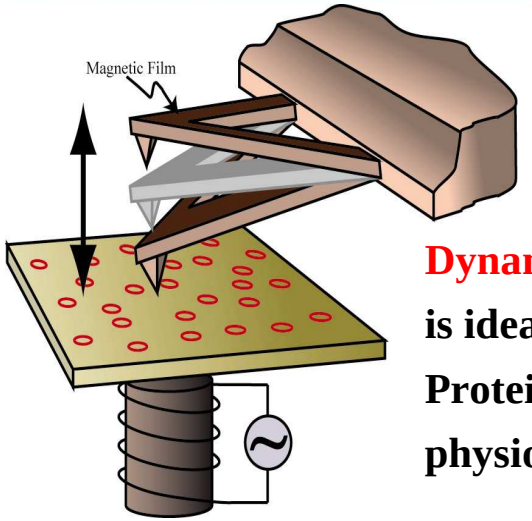
Dr. Gerald Kada
Nano-measurements Division

… on Top of a Living Cell in dynamic force mode







Live endothelial cells, in PBS buffer solution

(Left) 70 µm scan, 5 µm height

(courtesy of K. Van Vliet, MIT)

(Right) 13x20 µm scan, 150 nm height

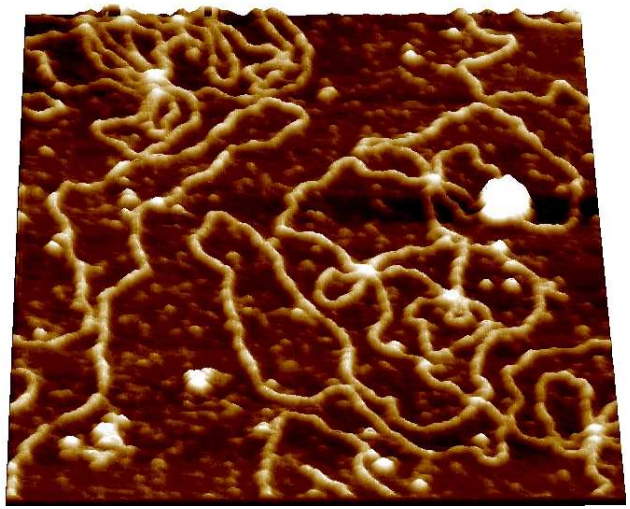
(courtesy of Ch. Riethmüller, Uni Münster)

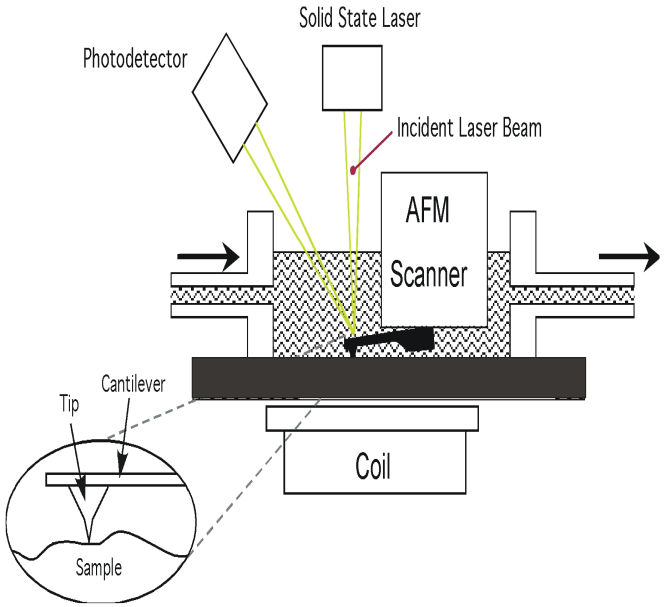
Dynamic Force Mode (MAC)

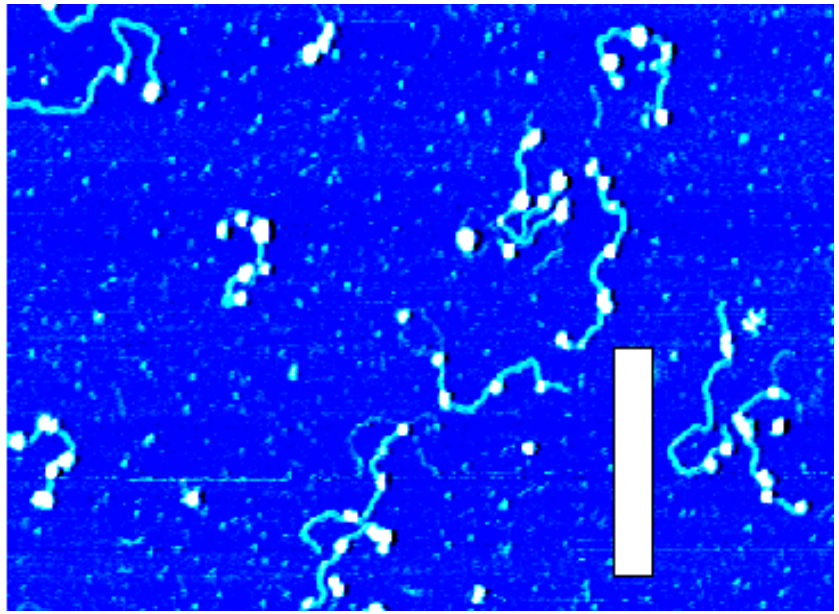
is ideal for imaging DNA, Proteins or Cells under physiological conditions

Salt-induced ‘melting’ of DNA-protein complexes

Plasmid DNA in buffer solution
450 nm scan size







−1.4M NaCl

− 0M

0.8 x 1 µm scans

Wang, Lindsay et al, Biophys J (2002)

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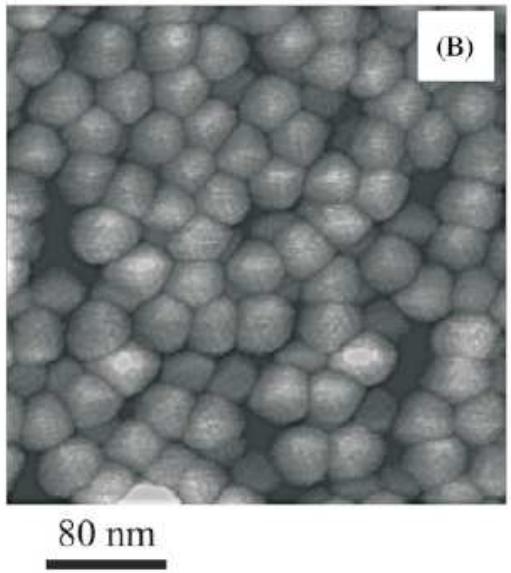
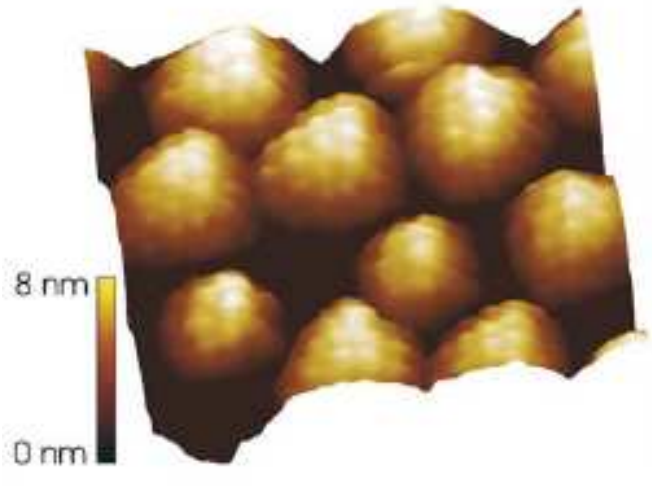
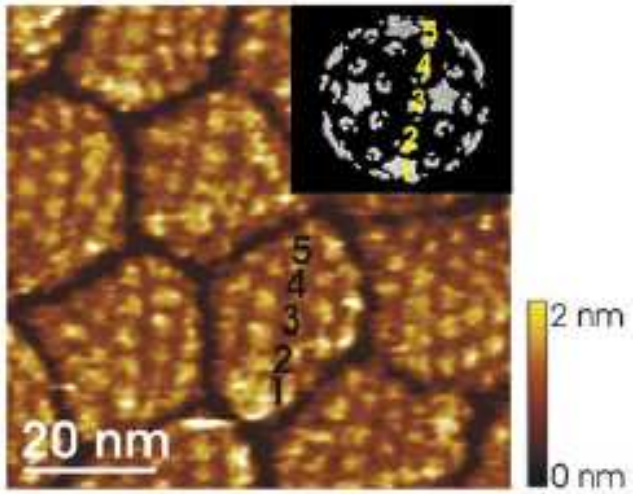
Nano-measurements Division
Nanobioeurope June 9-13, 2008

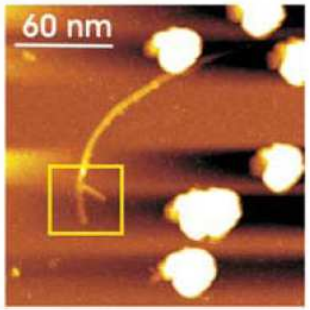
Live Human Rhinovirus (MAC mode in liquid)

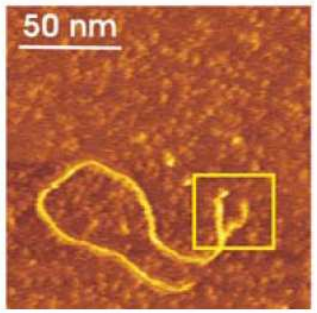




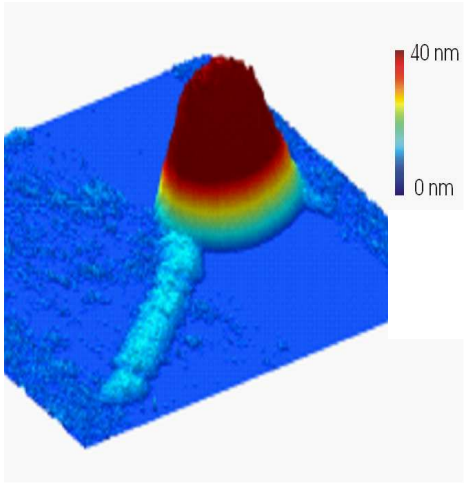


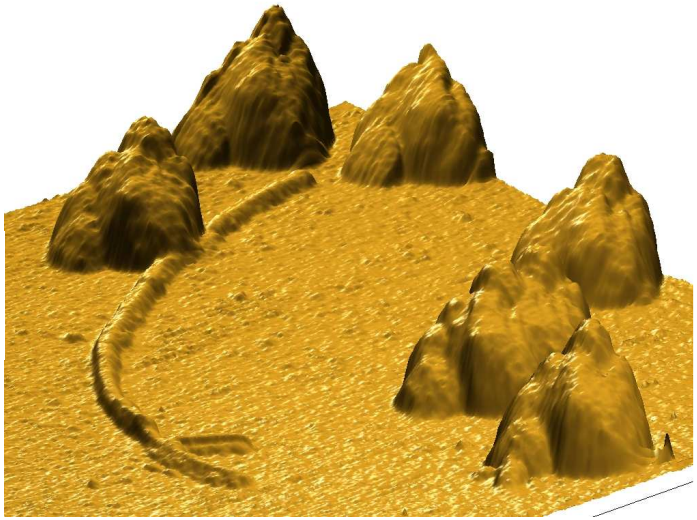
RNA release from virus by decreasing pH





Kienberger, Hinterdorfer et al, J Virology (2004) & Structure (2005)





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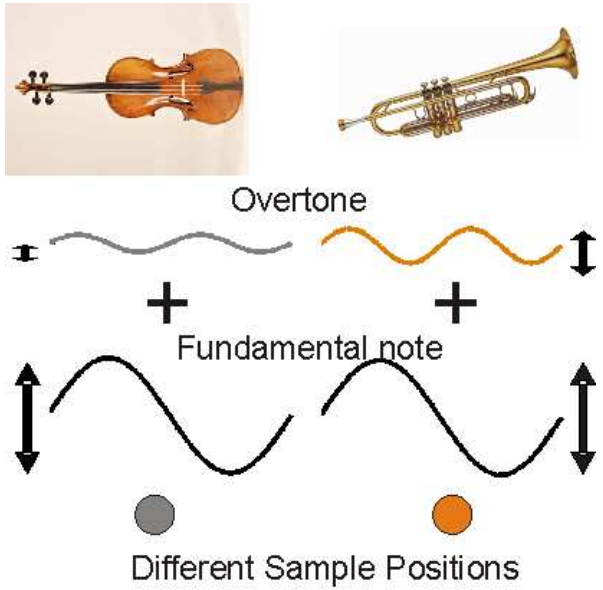
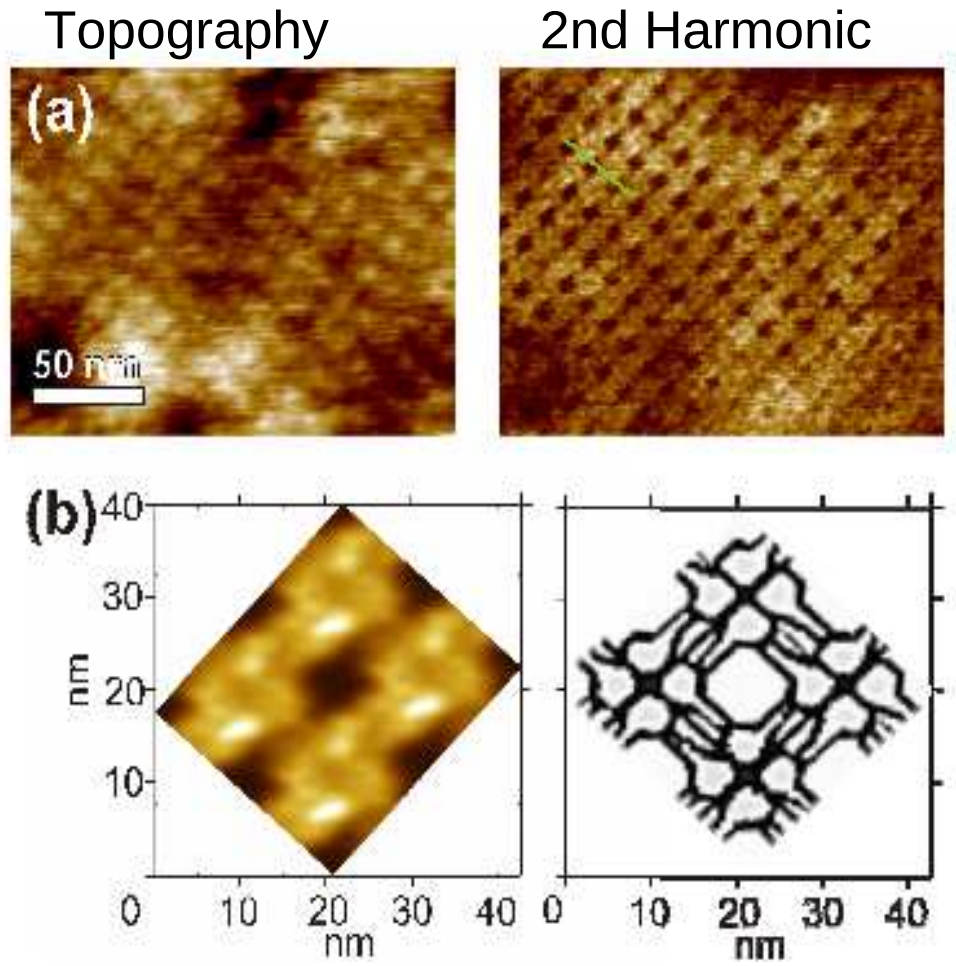
Higher Harmonics imaging **with Triple Lock-In Box**
Bacterial S-Layer



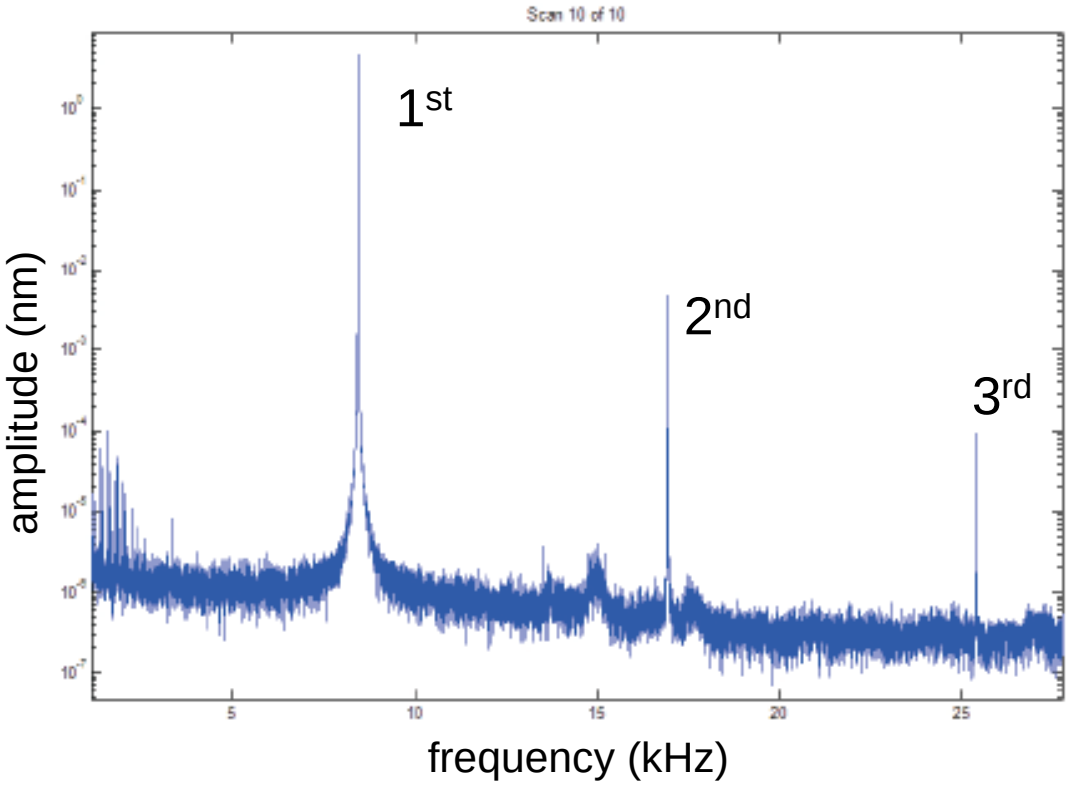


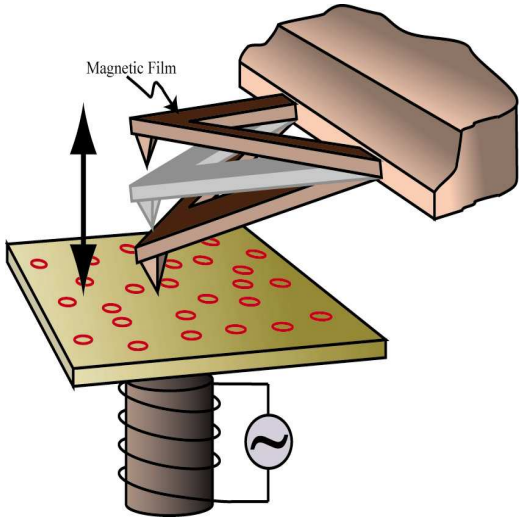
(b) Averaged image of 55 unit cells

<1 nm resolution on
protein crystal in liquid (MAC)
3 pm amplitude sensitivity in 2nd harmonics

Preiner et al, PRL 2007

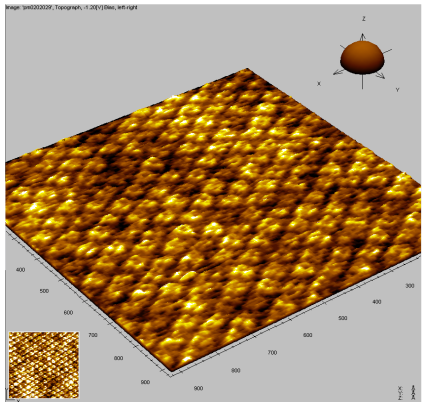
Frequency spectrum (MAC mode in liquid)
Glass surface



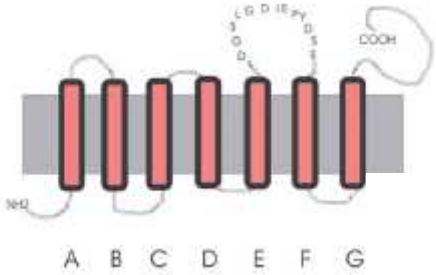


Magnetic AC (MAC) mode

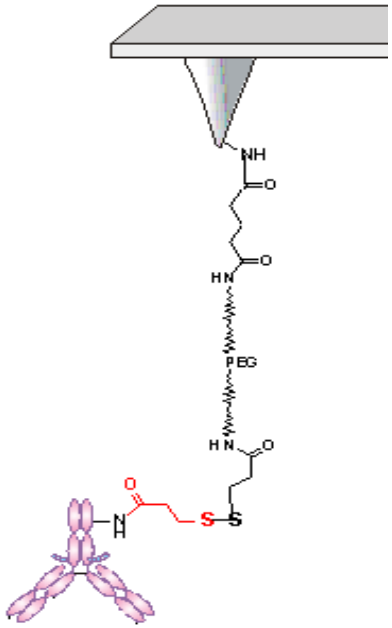
Single Molecule Studies of Antibody–Antigen Interaction Strength Versus Intra-molecular Antigen Stability

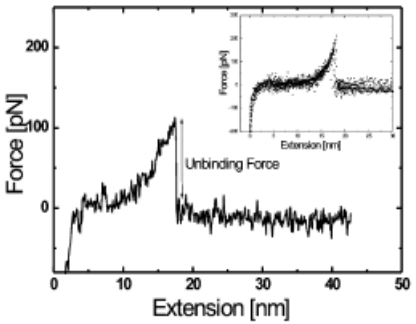


Sendai-loop



Anti-Sendai Antibody on the Tip,
Bacteriorhodopsin Surface
(“Purple Membrane”)

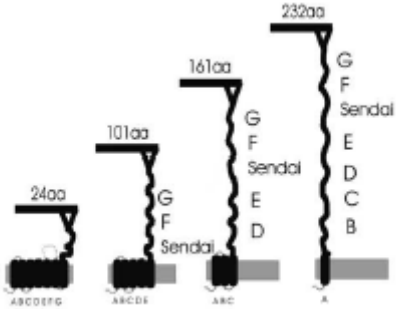


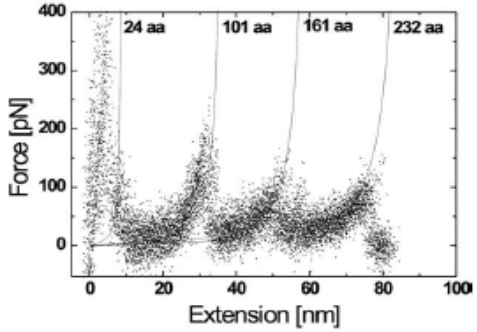


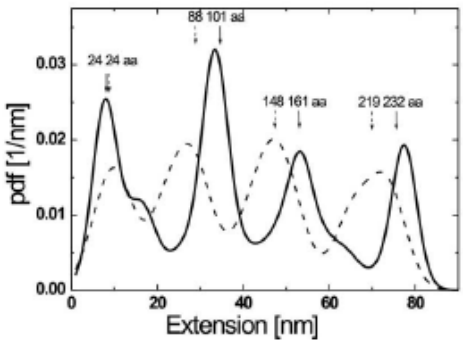
(d)

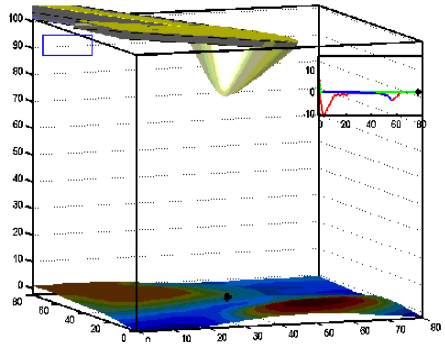
Kienberger, Kada et al, JMB 2005
Preiner et al, BJ 2007

Unravelling single Bacteriorhodopsin









Sequence-dependent pulling pattern –
with and without Sendai-loop

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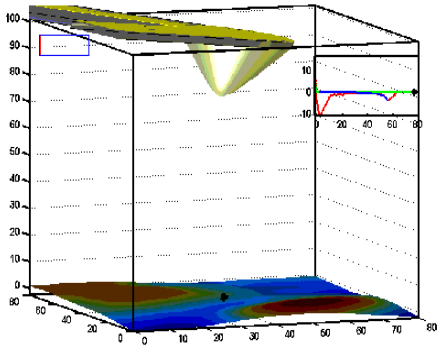
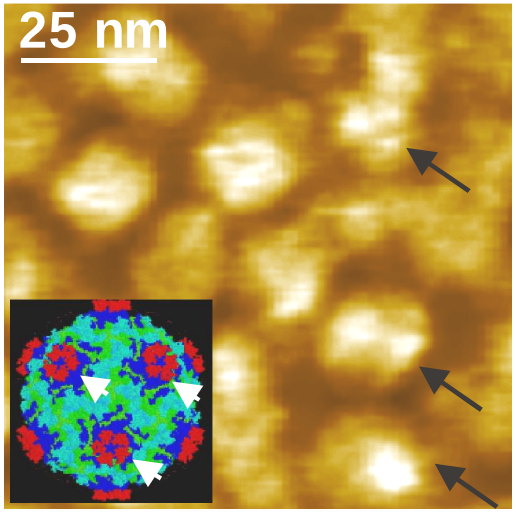
Nanobioeurope June 9-13, 2008

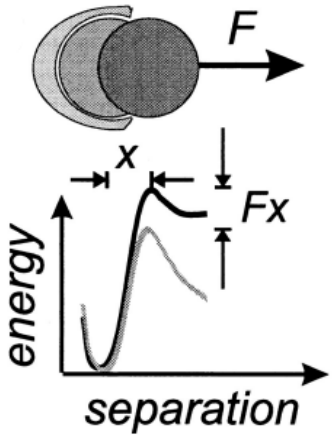
Varying the loading rate for x_β (binding pocket size), k_{off} (kinetic off-rate) and ΔG (Energy)

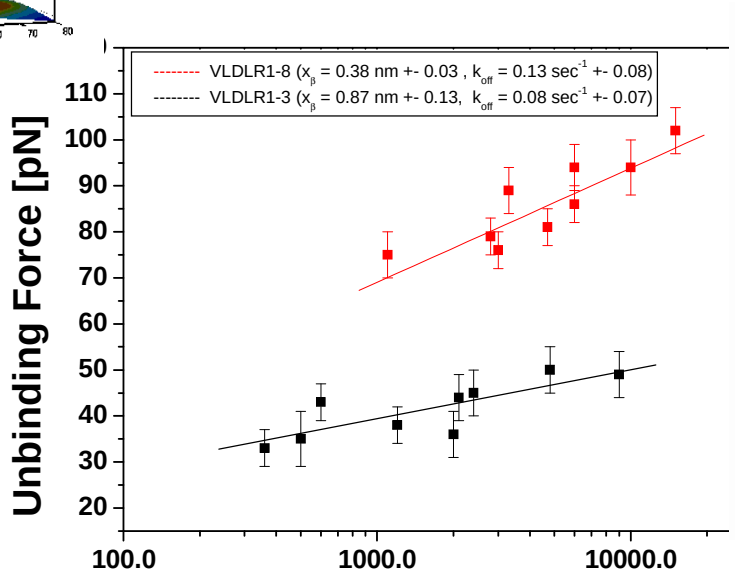


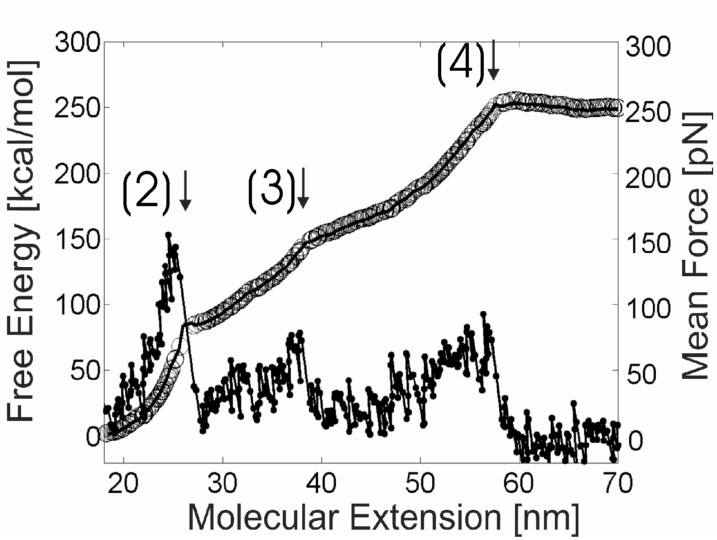


Kinetics ⇔ Thermodynamics

$\Delta G = - RTlnK_D$







$F^*(r) = k_BT/\mathbf{x_\beta} \bullet \ln(r \bullet \mathbf{x_\beta}/\mathbf{k_{off}} \bullet k_BT)$

Bell, Evans, Jarzynski, Hummer '78,'93,'98,'06

Rankl, Hinterdorfer, Preiner, Univ Linz

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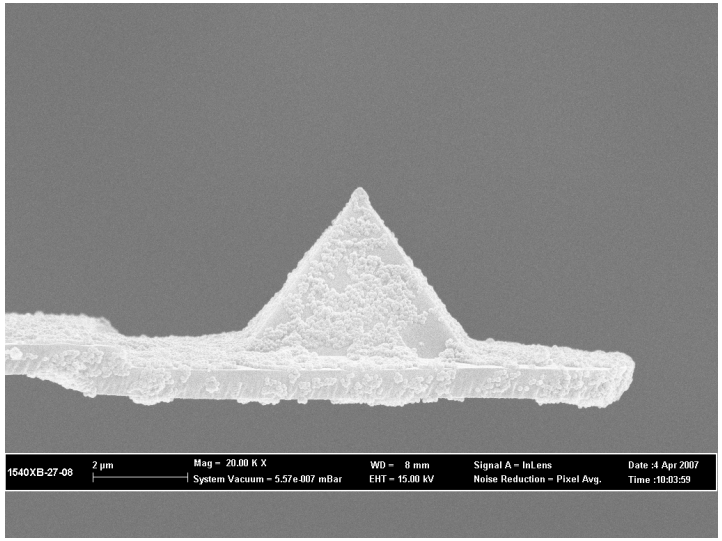
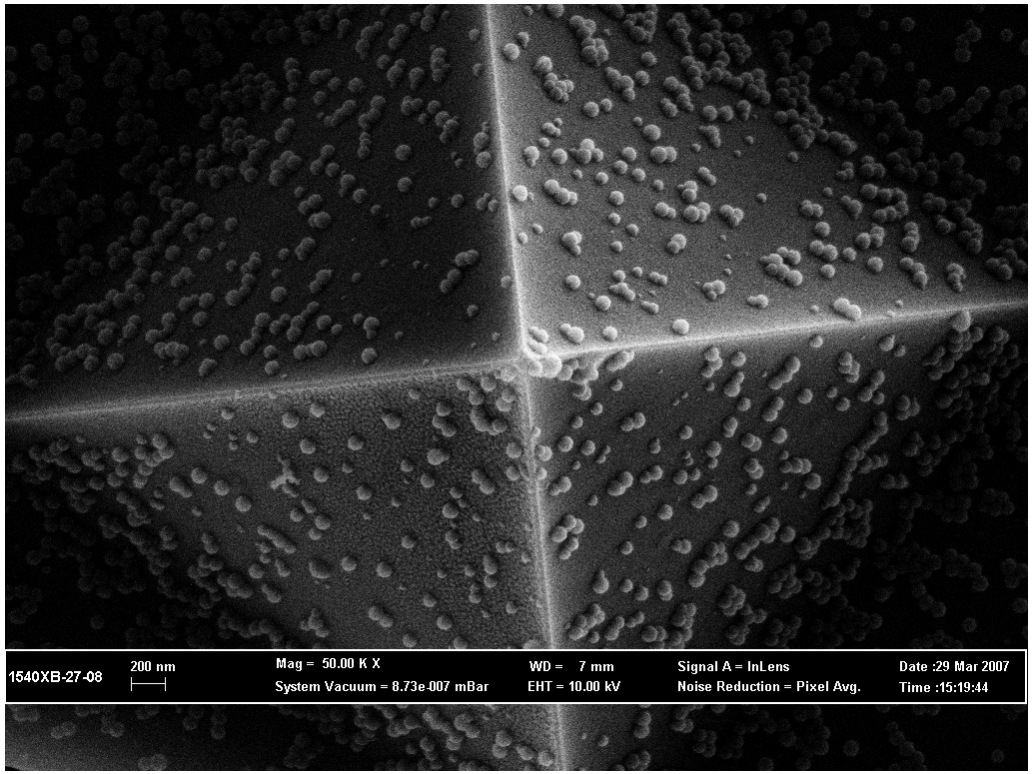
Close look on modified AFM tip



Fully covered AFM tip



Single Molecule tethering to AFM tip

Näreoja, Kienberger, Ebner, Hinterdorfer, Uni Linz
Kada et al, Nanotoday 2008

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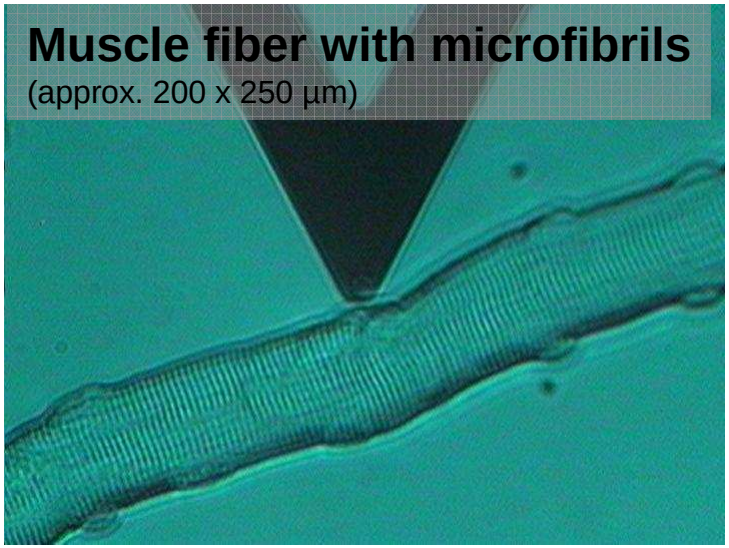
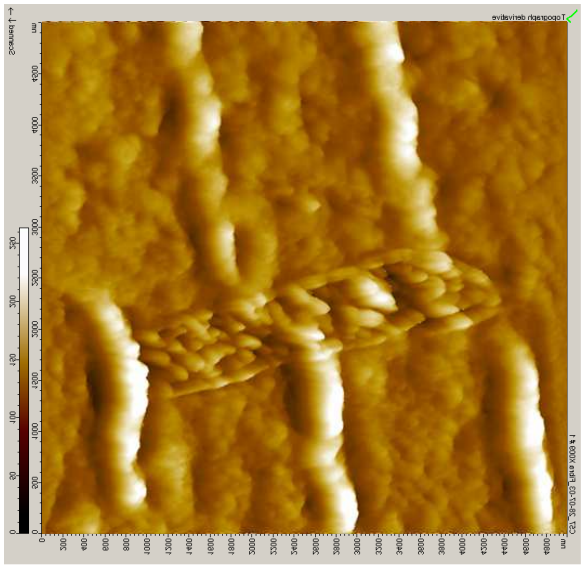
Nano-measurements Division
Nanobioeurope June 9-13, 2008

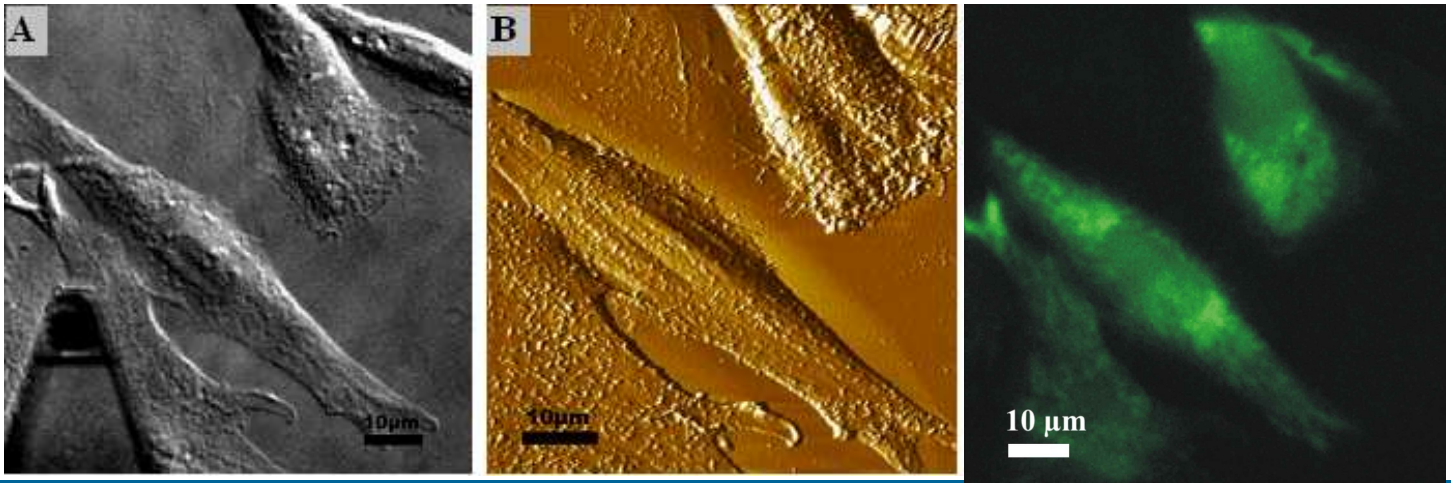
Cell imaging: **Optical Microscopy combined with AFM imaging**





Mitochondrion lying between two muscle microfibrils (5 x 5 µm scan)

Defranchi et al, Micr.Res.Tech. 2005



DIC (Differential interference contrast) **simultanous** with **AFM** imaging and **Fluorescence**

Madl et al., Ultramicroscopy 2006

Immunofluorescence
(Muscle Fibers)

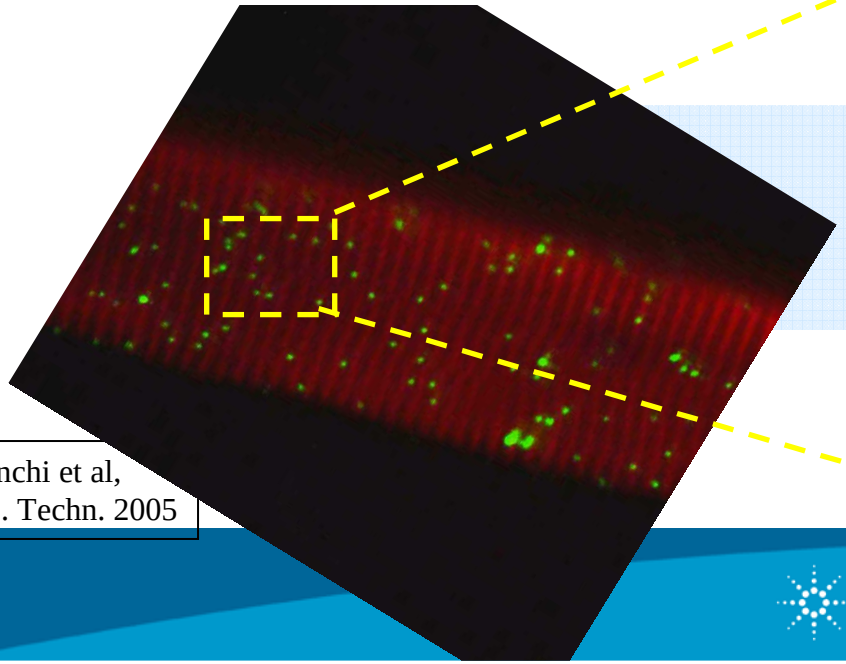
Bottom: **Immunofluorescence Images**

- Staining the sarcomeric protein **alpha-actinin (red)** using a monoclonal antibody;

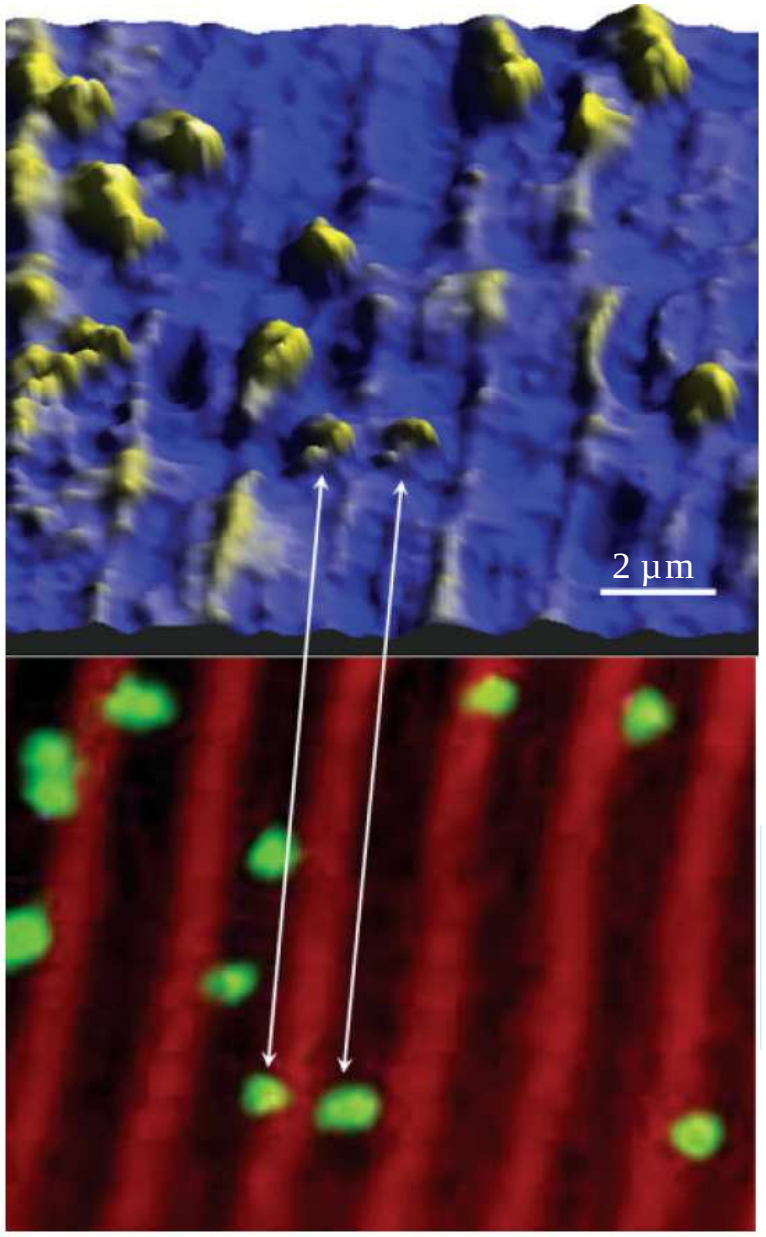
- Adding 500nm fluorescent **microspheres (green)**

Top: AFM Topography Image of the same area

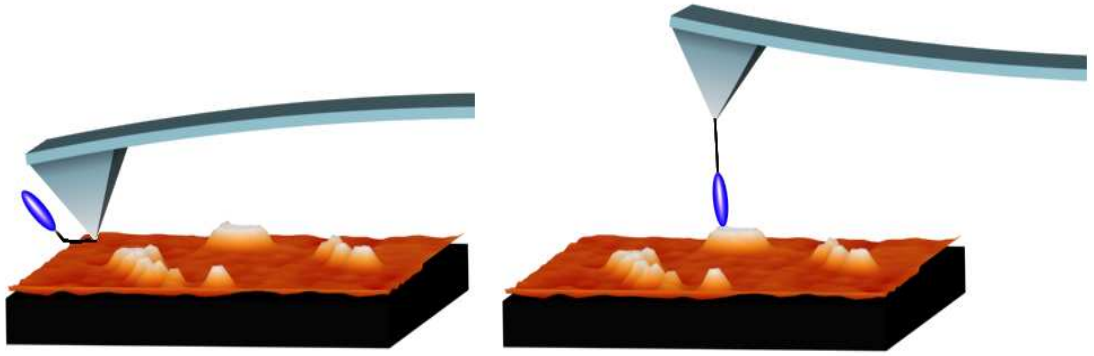
The pattern allows unambiguous identification of individual beads and their positions relative to sarcolemma folds at the top and to Z lines (positive for anti alpha-actinin antibody, bottom).

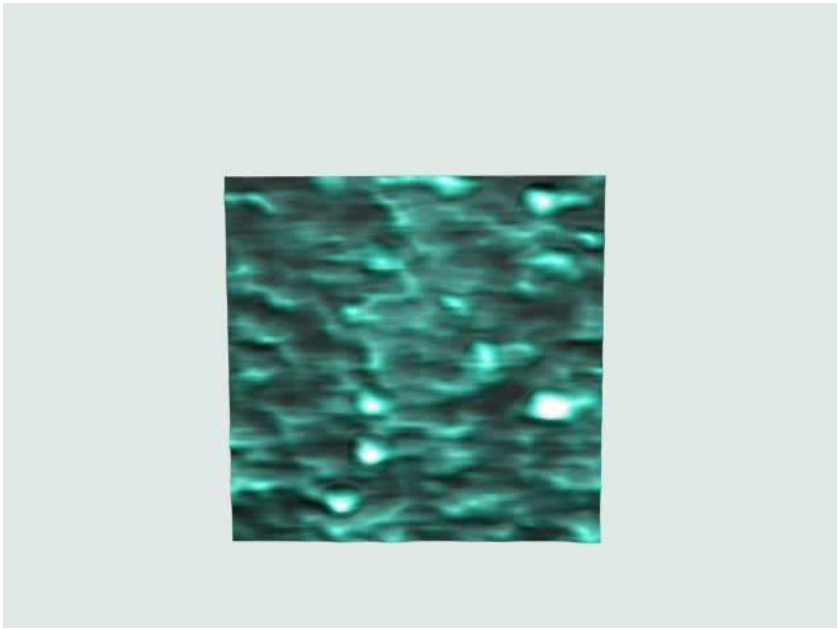


Defranchi et al,
Micr. Res. Techn. 2005

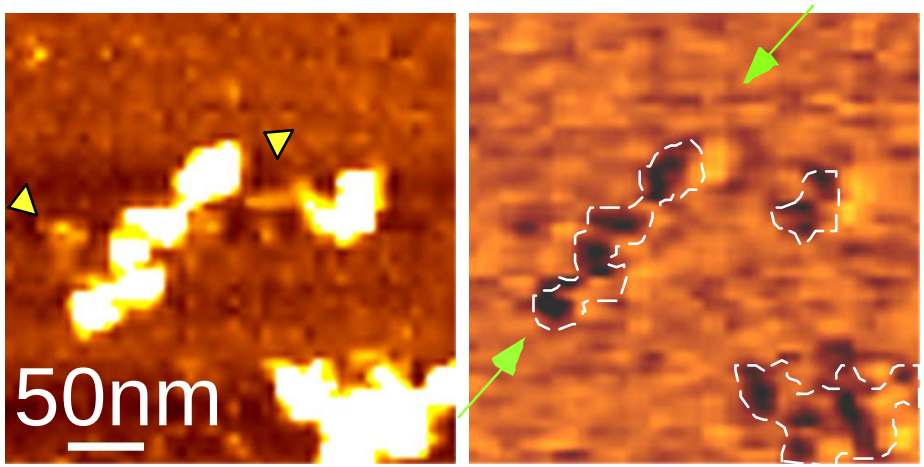


Topography and Recognition Imaging (TREC)





Topography Recognition



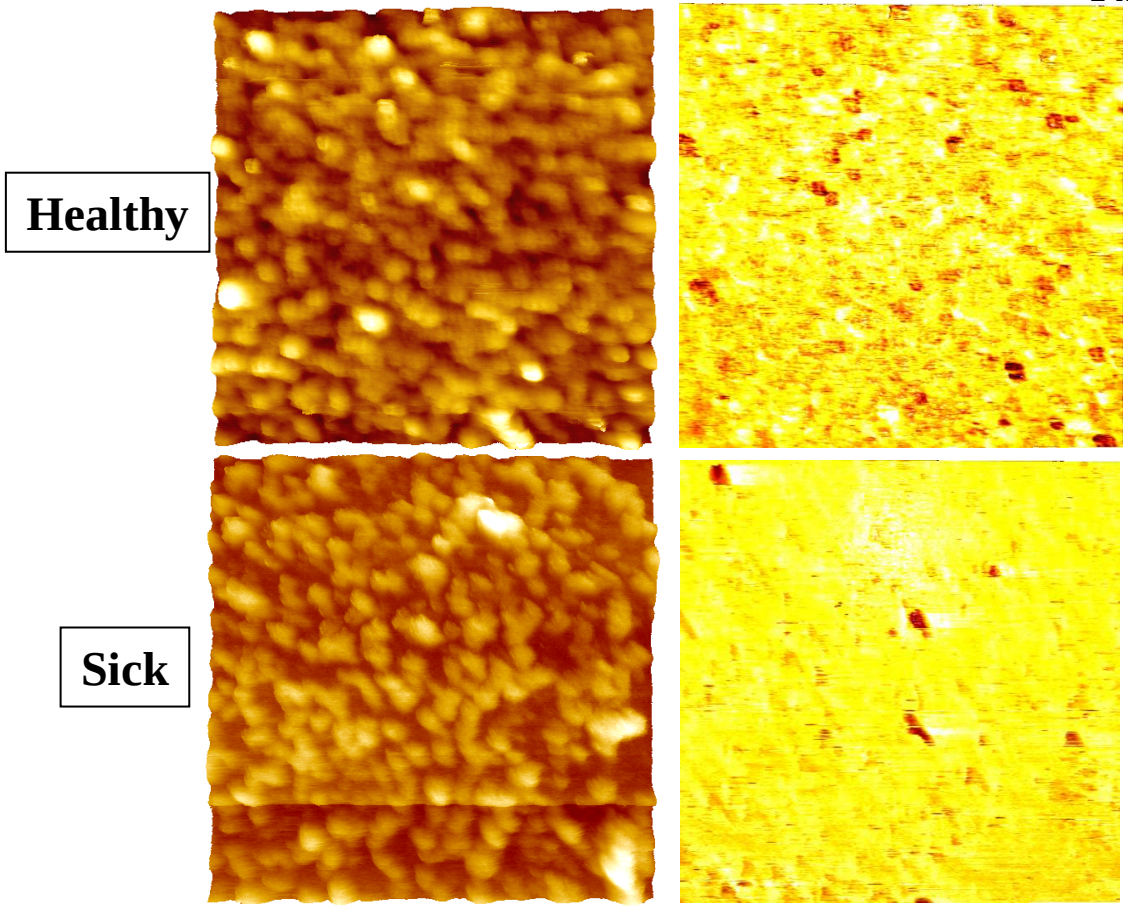
DNA-protein complexes as stored in the chromosomes

H. Wang, ASU

Medicinal Diagnostics with TREC:
Patients with Cystic Fibrosis

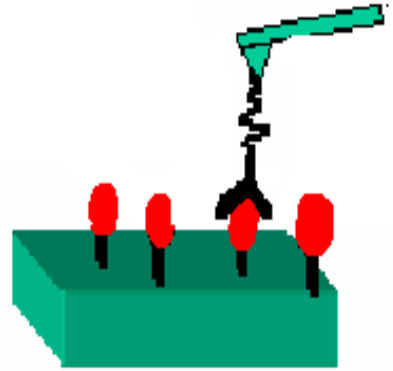
Topography Recognition

1 x 1 um scans



Healthy

Sick

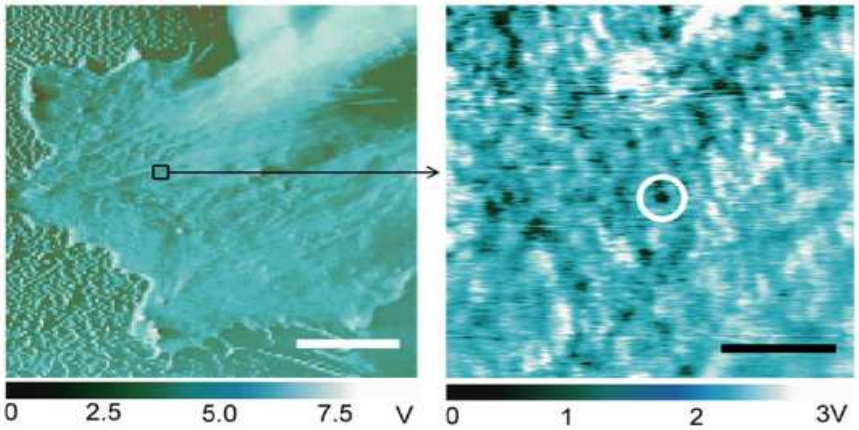


Sample:
Erythrocyte (red blood cell) membranes on mica - taken from human blood of patients
Tip:
Antibody against CFTR membrane protein

Top: healthy person
Bottom: patient with Cystic Fibrosis (degenerative CFTR [i.e. **C**ystic **F**ibrosis **T**ransmembrane conductance **R**egulator - a chloride channel] proteins in erythrocyte membrane)

Nikova et al.,
Appl. Scanning Probe Meth. III (2006)

Recognition imaging examples:

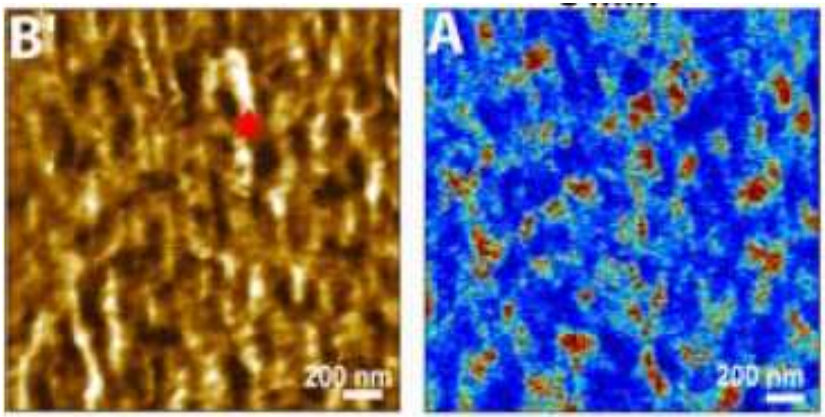


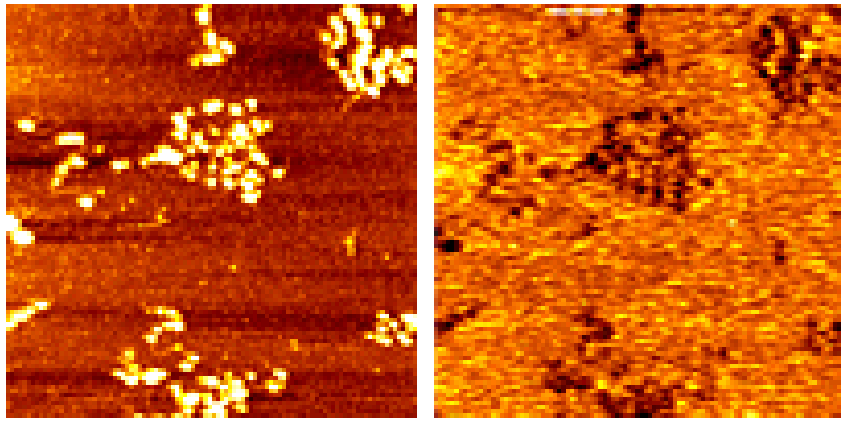
VEGFR2 receptors on fixed HUVEC cells
(scale bar 10 µm, 500 nm resp.)

Van Vliet et al, PNAS 2007

VE-cadherin domains on MyEnd cells

Chtcheglova et al, BJ 2007





MMTV chromatin (DNA/protein complexes)
(1.5 µm scan)

Stroh et al, PNAS 2004

Finally…

Thanks for your attention !

Acknowledgements

- **University of Linz**, Biophysics Institute
  Prof. Peter Hinterdorfer
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  Prof. Gerhard Schütz
  Josef Madl
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  Lilia Chtcheglova
  Johannes Preiner
  Cordula Stroh
  Andreas Ebner

- **Arizona State University**, Biodesign Institute
  Prof. Stuart Lindsay
  Hongda Wang

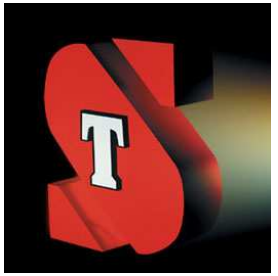
- **Massachusetts Institute of Technology**
  Prof. Krystyn van Vliet

Agilent Technologies Inc.
Nano Measurements Division
Tianwei Jing
W. Travis Johnson
Wenhai Han
Sergei Magonov
Maozi Liu (Agilent Labs)

Contact:

Gerald Kada: gerald_kada@agilent.com

www.agilent.com/find/AFM



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NanoToday review
publ. March 08

Atomic force microscopy in bionanotechnology

Atomic force microscopy (AFM) is extensively used for imaging surfaces ranging from micro- to nanometer scales, with the objective of visualizing and characterizing surface textures and shapes. It is the only technique that provides subnanometer resolution under physiological conditions, needed for imaging biological species like proteins and living cells. Measurements of molecular recognition forces provide insights into the function and structure of biomolecular assemblies. Furthermore, in combination with fluorescence microscopy, AFM can identify biomolecules based on fluorescence labeling and high-resolution topography imaging. This review summarizes recently developed techniques for advanced topographical imaging and sensitive force measurements.

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Within the field of scanning probe microscopy, atomic force microscopy[1] (AFM) is extensively used in a wide range of disciplines such as life science, solid-state physics and materials science. The AFM has evolved into an imaging method that yields structural details of biological samples such as proteins, nucleic acids, membranes, and cells in their native environment[2–5]. AFM is a unique technique for providing subnanometer resolution at a reasonable signal-to-noise ratio under physiological conditions. As a result of continuous developments in sample preparation, imaging techniques, and instrumentation, AFM is now a companion technique of X-ray crystallography and electron microscopy (EM) for the determination of protein structures[6], for example. It complements EM by allowing visualization of biological samples in buffers that preserve their native structure over extended time periods. AFM does not rely on symmetry averaging and crystallization, therefore revealing defects and structural anomalies not observable in classical ensemble measurements[7]. Unlike EM, AFM yields three-dimensional maps with an exceptionally good vertical resolution (less than a nanometer).

In addition to high-resolution imaging of proteins, nucleotides, membranes, and living cells[2], the measurement of mechanical forces at the molecular level provides detailed insights into the function and structure of biomolecular systems[5]. Inter- and intramolecular interactions can be studied directly at the molecular level, as

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