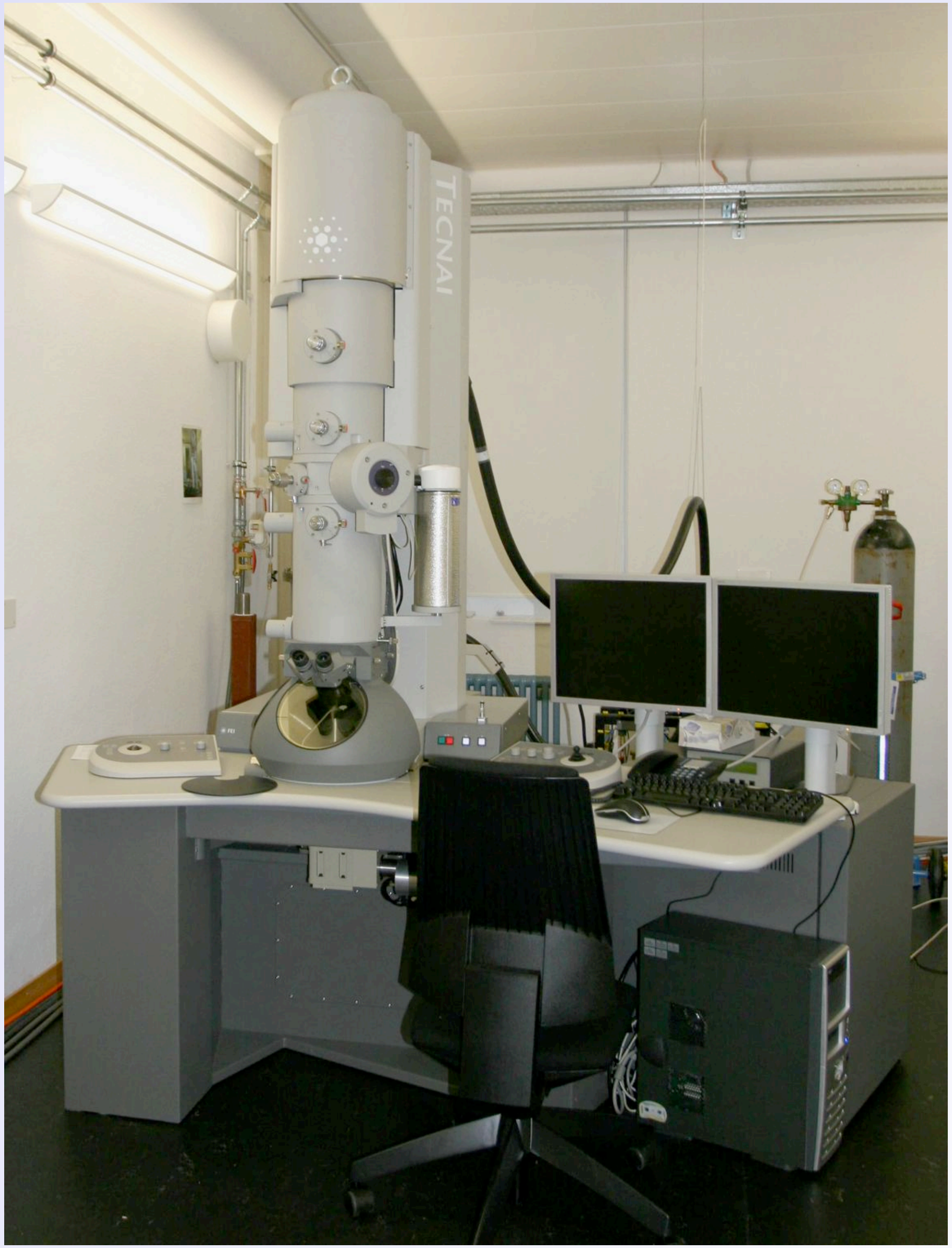
Atomic Force Microscopy for the Study of Membrane Proteins

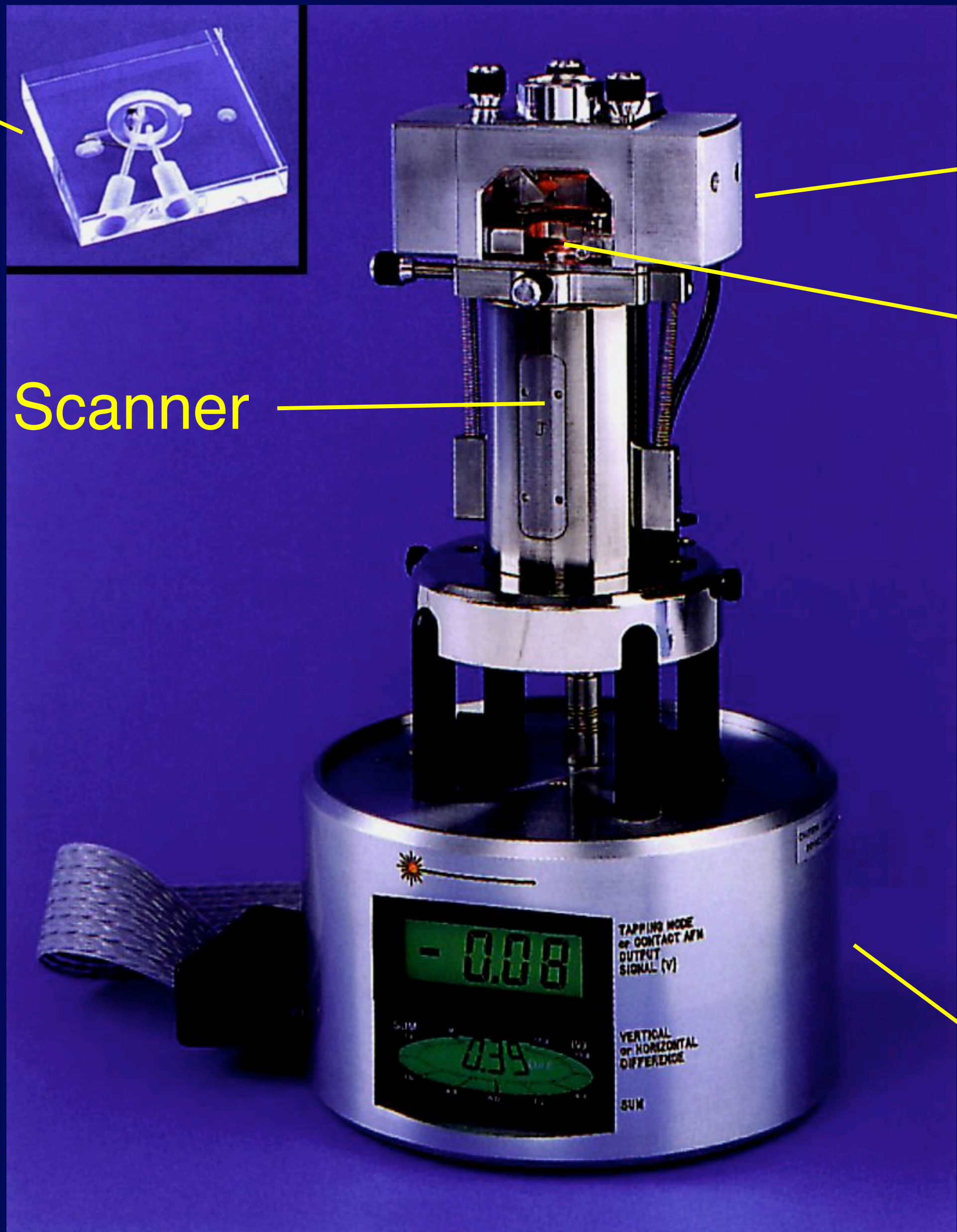
Paul Scherrer Institute
27th January 2015

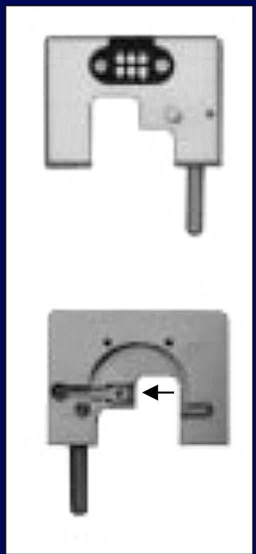
Dimitrios Fotiadis
Institute of Biochemistry and Molecular Medicine
University of Bern
dimitrios.fotiadis@ibmm.unibe.ch



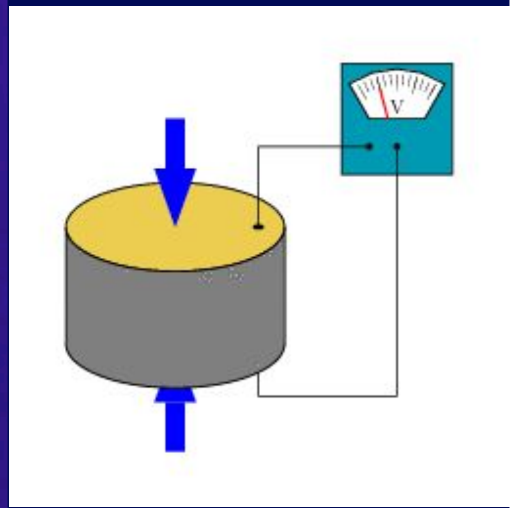
FEI Tecnai F20 (University of Bern, Institute of Anatomy)

Atomic Force Microscope





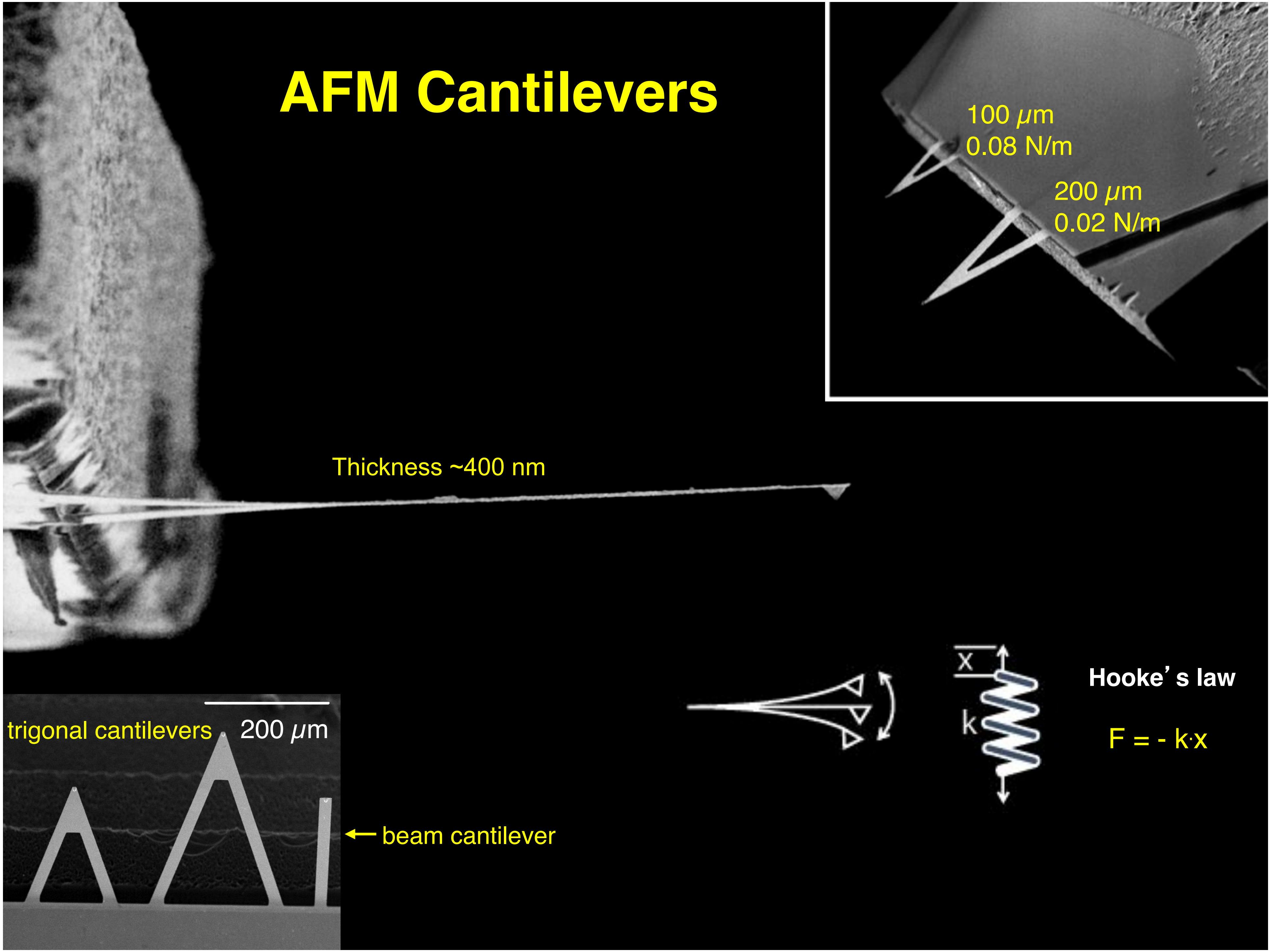
Dry cell



Atomic Force Microscope

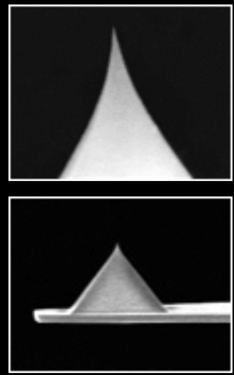
Main field of application:
Semiconductor industry

AFM Cantilevers

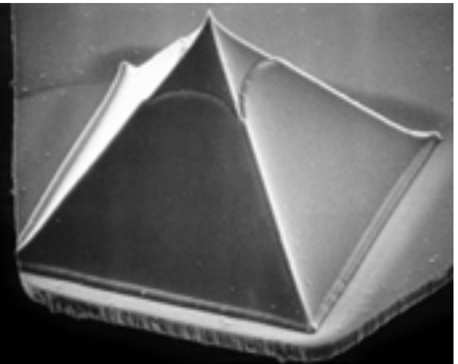


AFM Tips

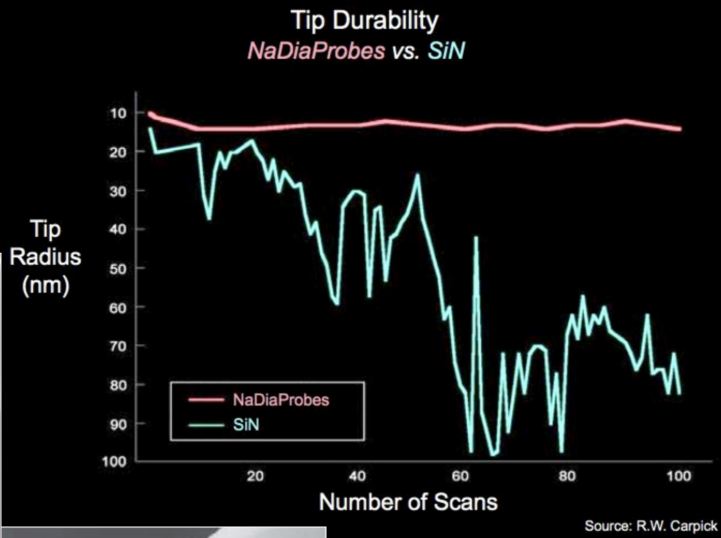
Tip radius ~15 nm

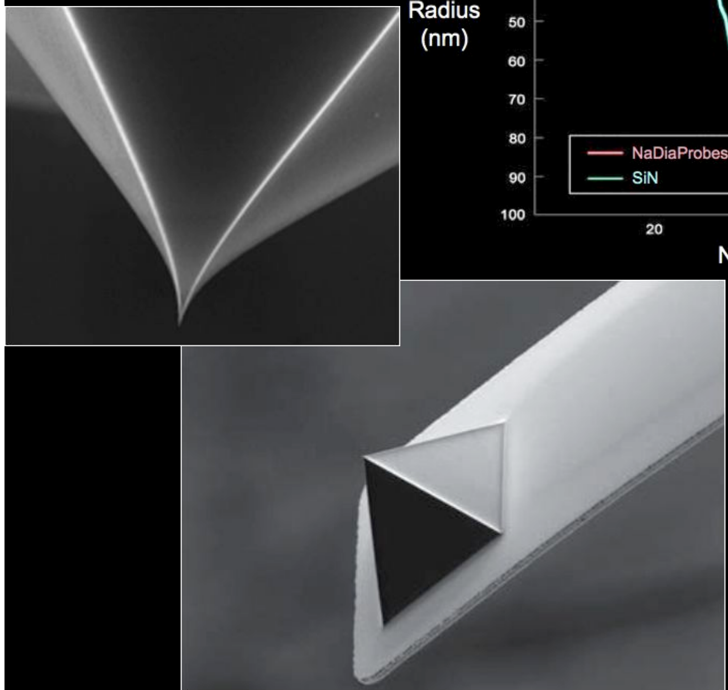


OMCL-TR400PSA - Olympus, Inc.



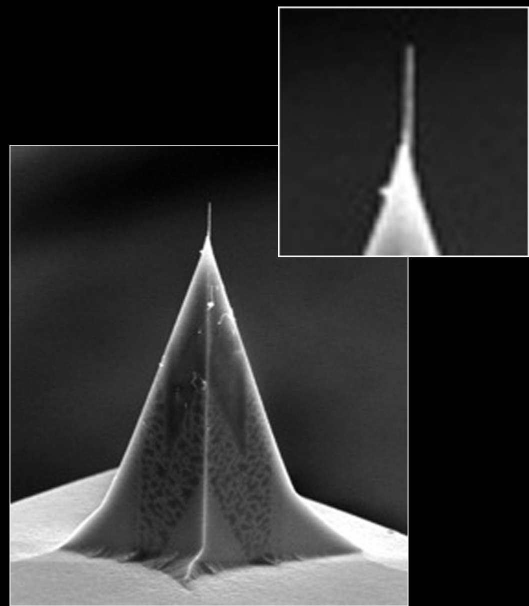
silicon nitride cantilever





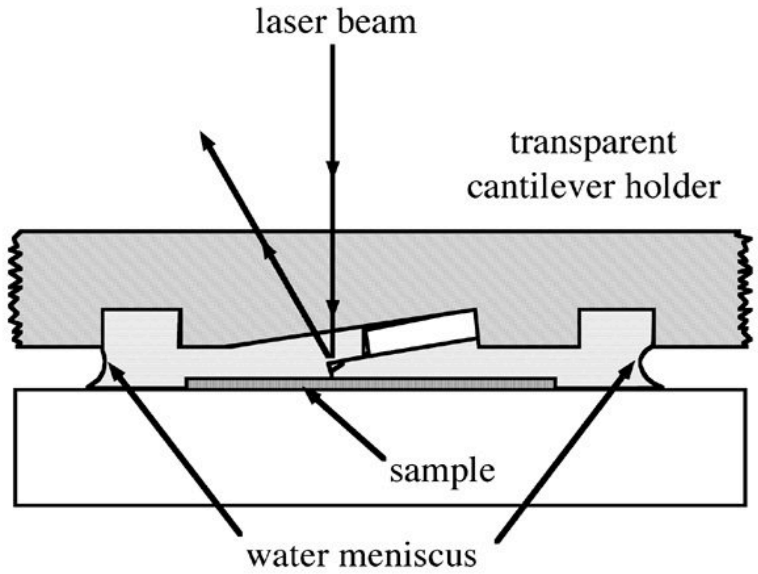
NaDiaProbes - Advanced Diamond Technologies, Inc.

carbon nanotube

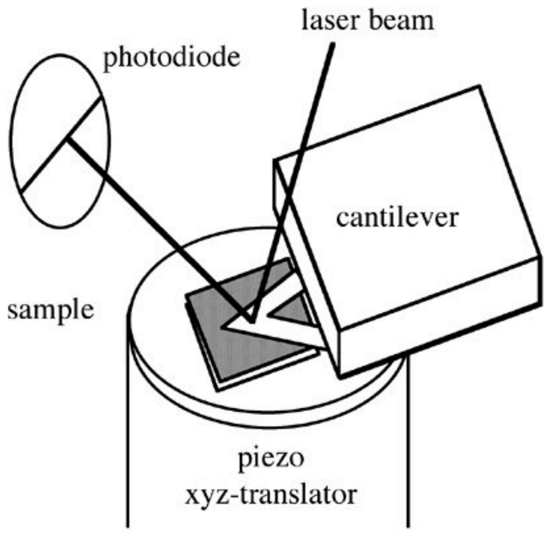


Carbon Design Innovations, Inc.

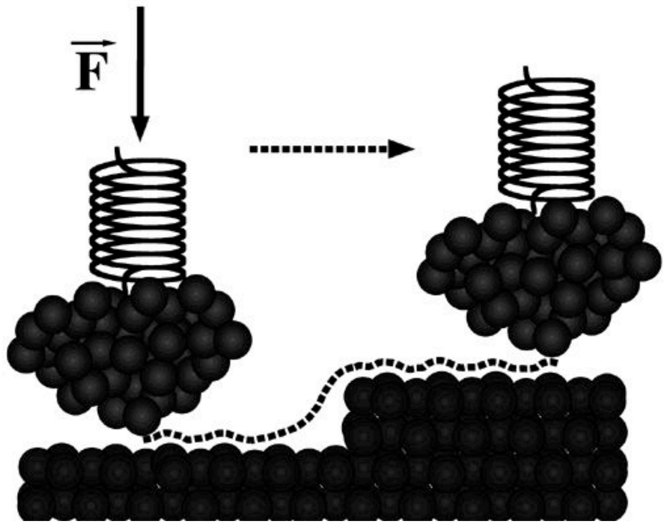
Principle of AFM



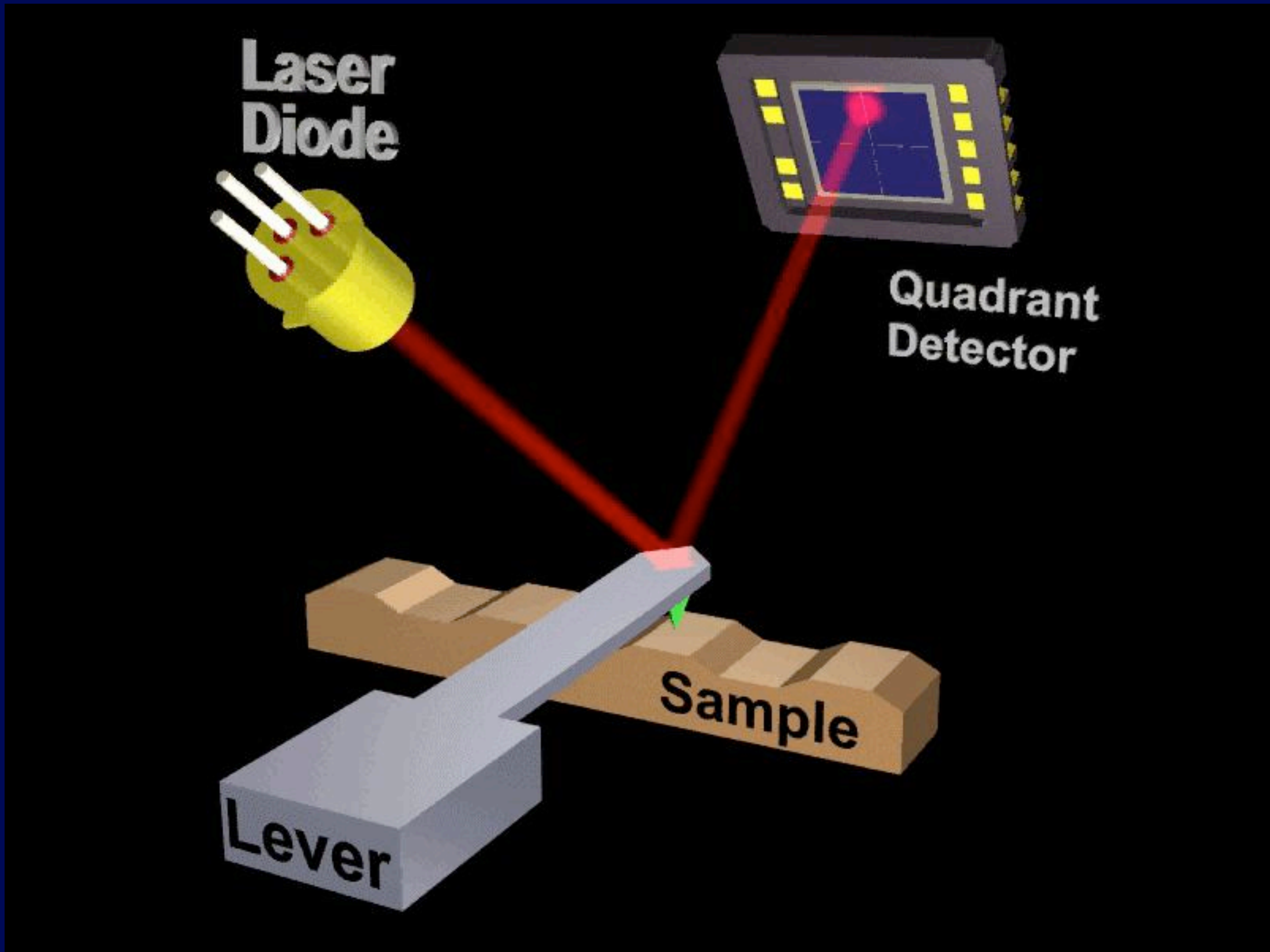
Backside of cantilever is reflective, e.g. gold coating.



With biological samples: 50-150 pN

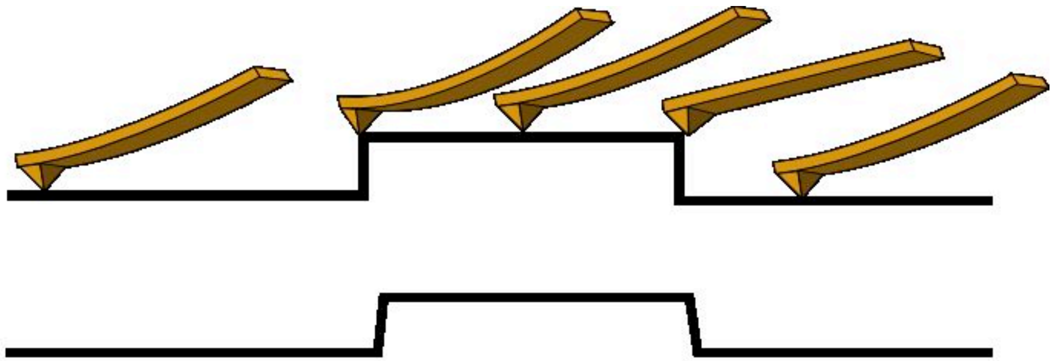


Principle of AFM



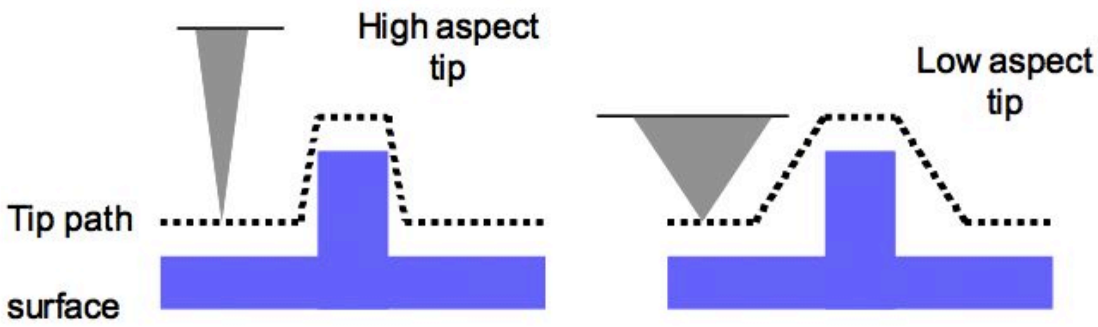
Courtesy H.-R. Hidber, Physics Institute, University of Basel

The two most commonly used AFM modes



Contact mode

+ high-resolution imaging
+ relatively fast
- friction / lateral forces



Influence of the tip geometry

AFM in Biology

- Relatively new imaging technique in biology

- Can look at surfaces and interactions of molecules

- Samples can be imaged under near-physiological conditions, e.g. RT or 37°C, normal pressure and aqueous solution

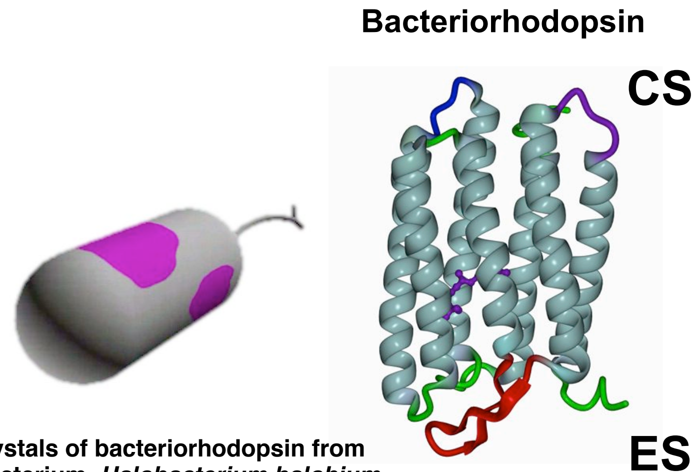
  → conformational changes using the same sample

  → determination of dynamic events

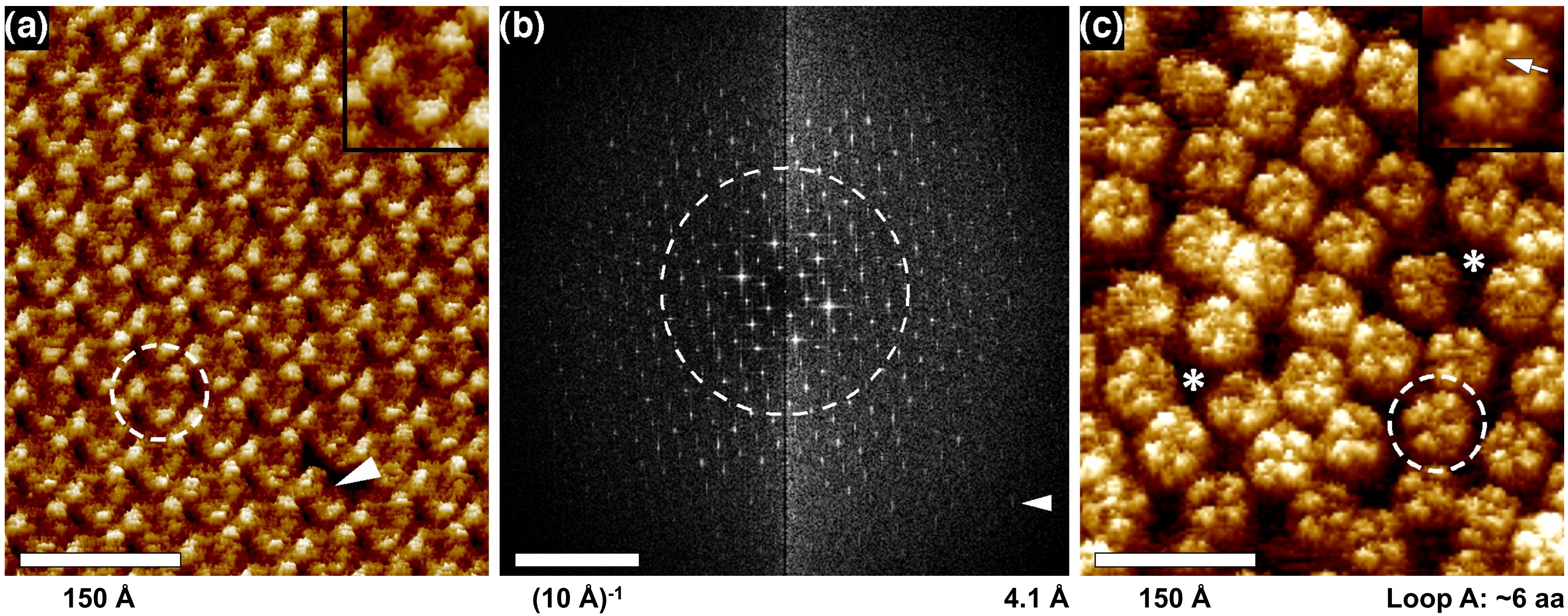
- Lateral resolution < 5 Å, vertical resolution ~1 Å

- Manipulation / dissection of the biological sample possible (nanoscalpel)

AFM topographs:
Outstanding signal-to-noise ratio and sub-nanometer resolution



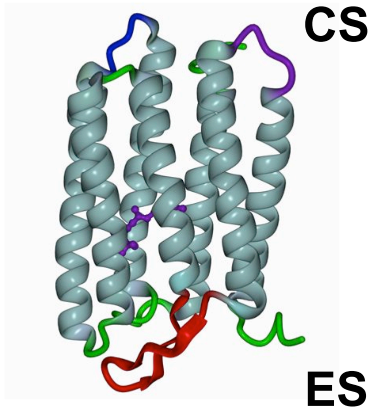
Native 2D crystals of bacteriorhodopsin from the archaebacterium, *Halobacterium halobium*.



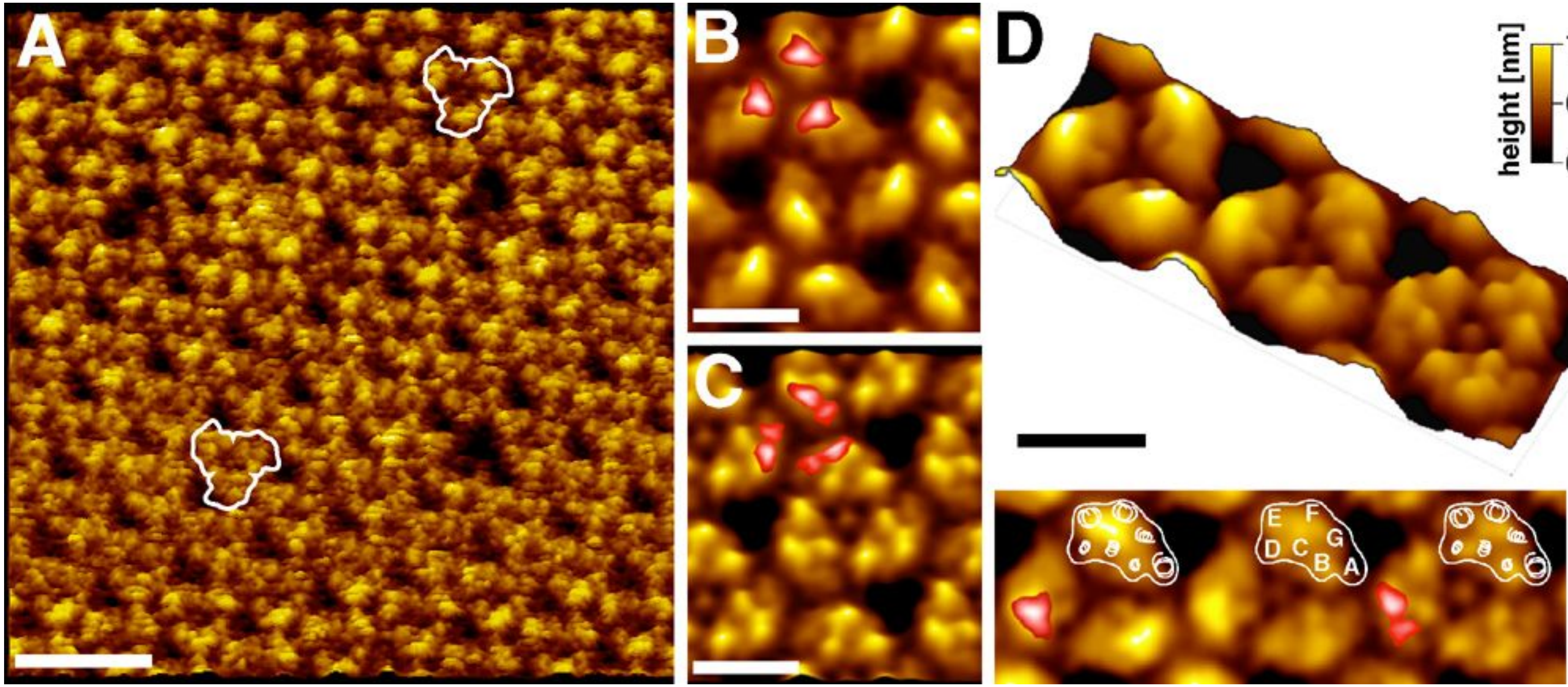
Fotiadis (2012) Curr. Opin. Biotech.

Force-induced conformational changes of bacteriorhodopsin

Bacteriorhodopsin



~100 pN



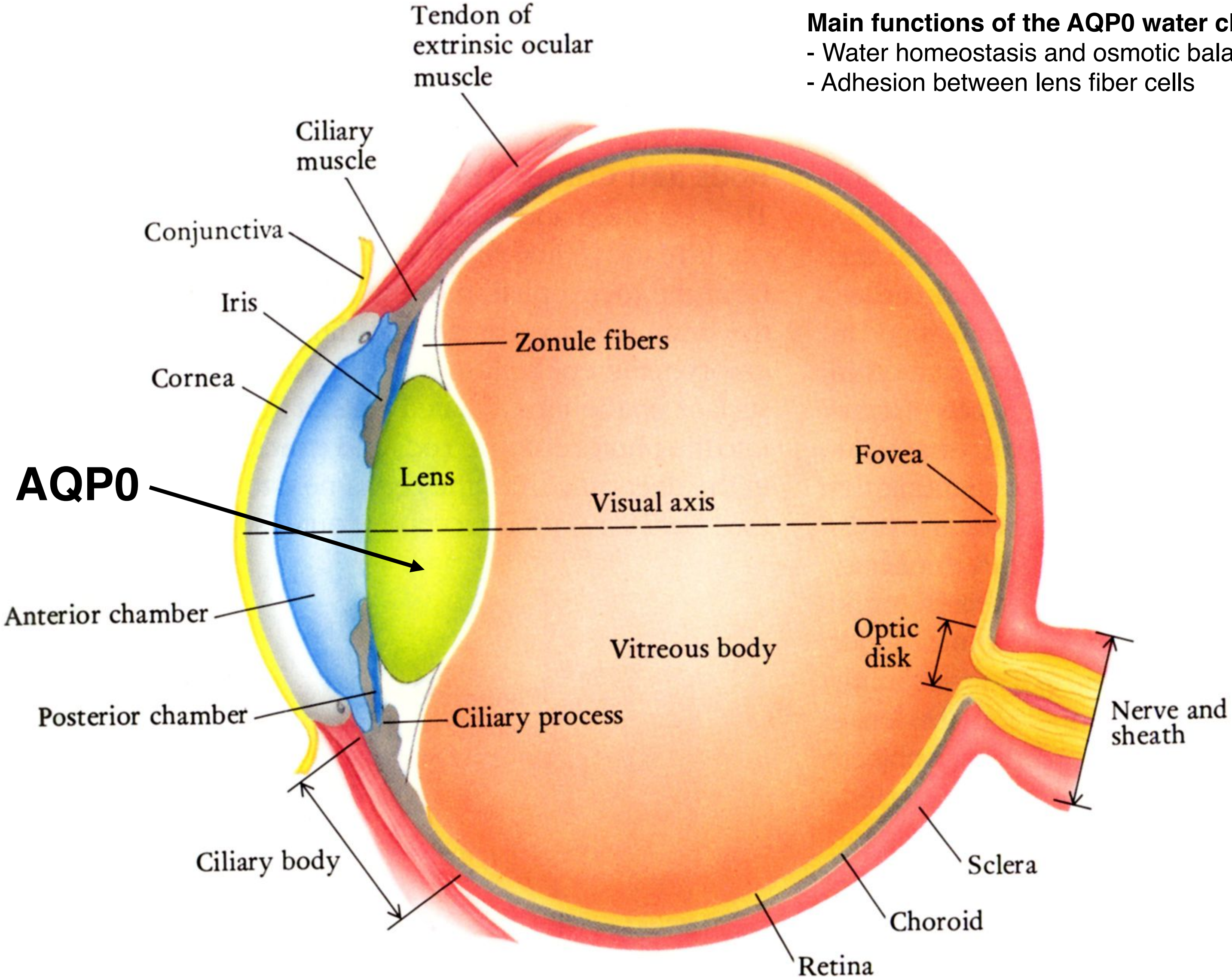
~200 pN

→ AB loop / CD loop / C-terminus / rest of compressed EF loop

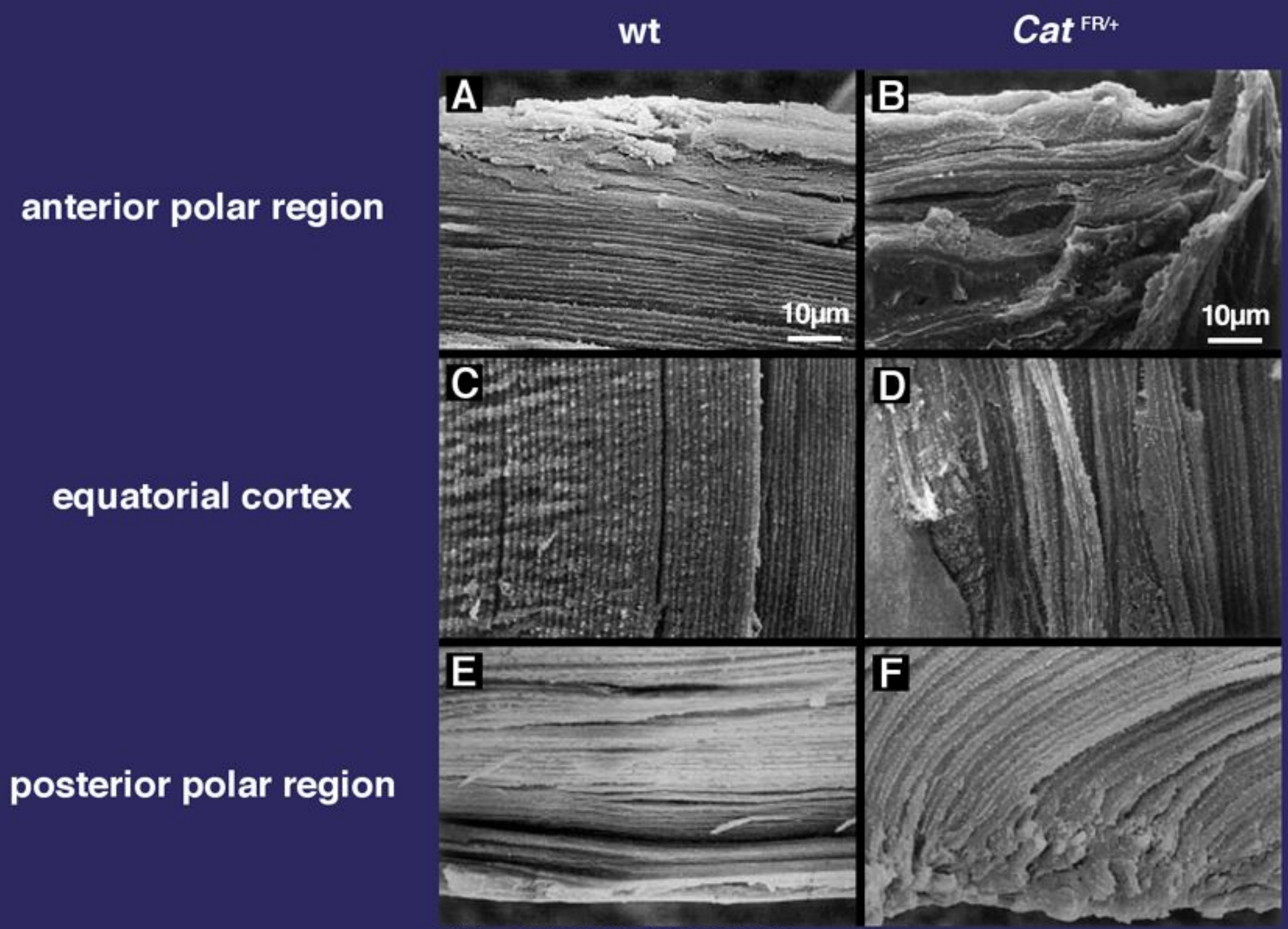
Müller *et al.* (1995), J. Mol. Biol.

Horizontal section of the vertebrate eye

Main functions of the AQP0 water channel:
- Water homeostasis and osmotic balance in the lens
- Adhesion between lens fiber cells

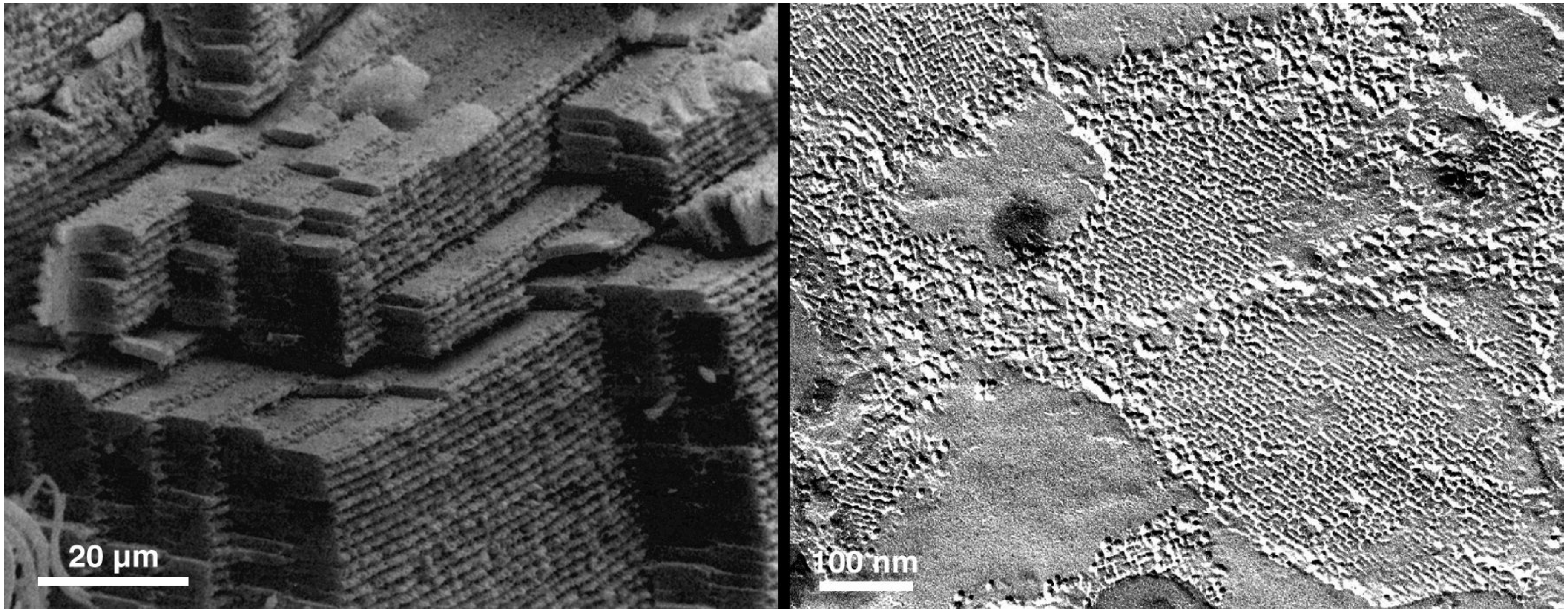


Lens fiber cells: Architecture in the eye

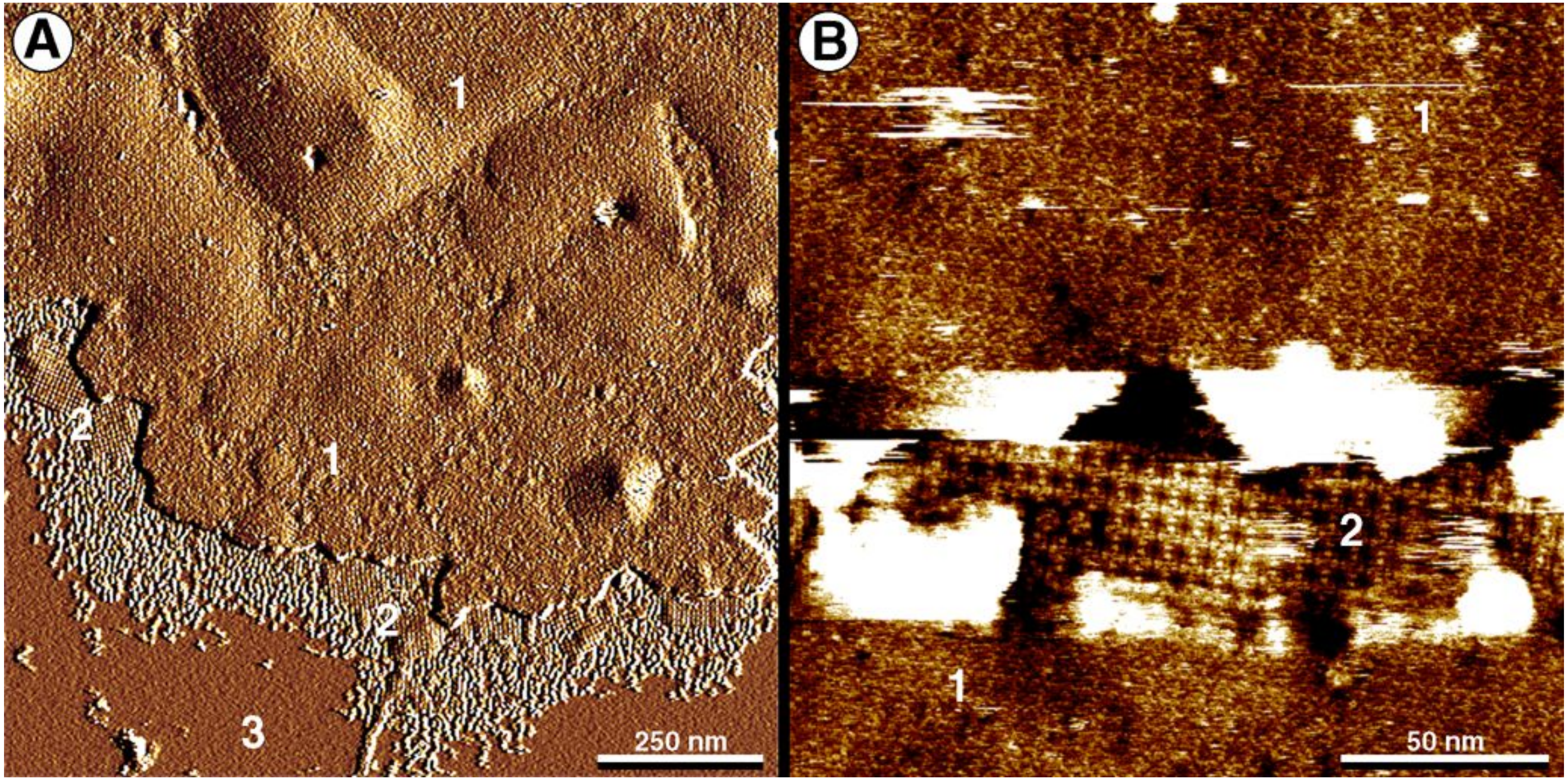


Shiels *et al.* (2000), FASEB, 14, 2207-2212.

Lens fiber cells: Architecture in the eye

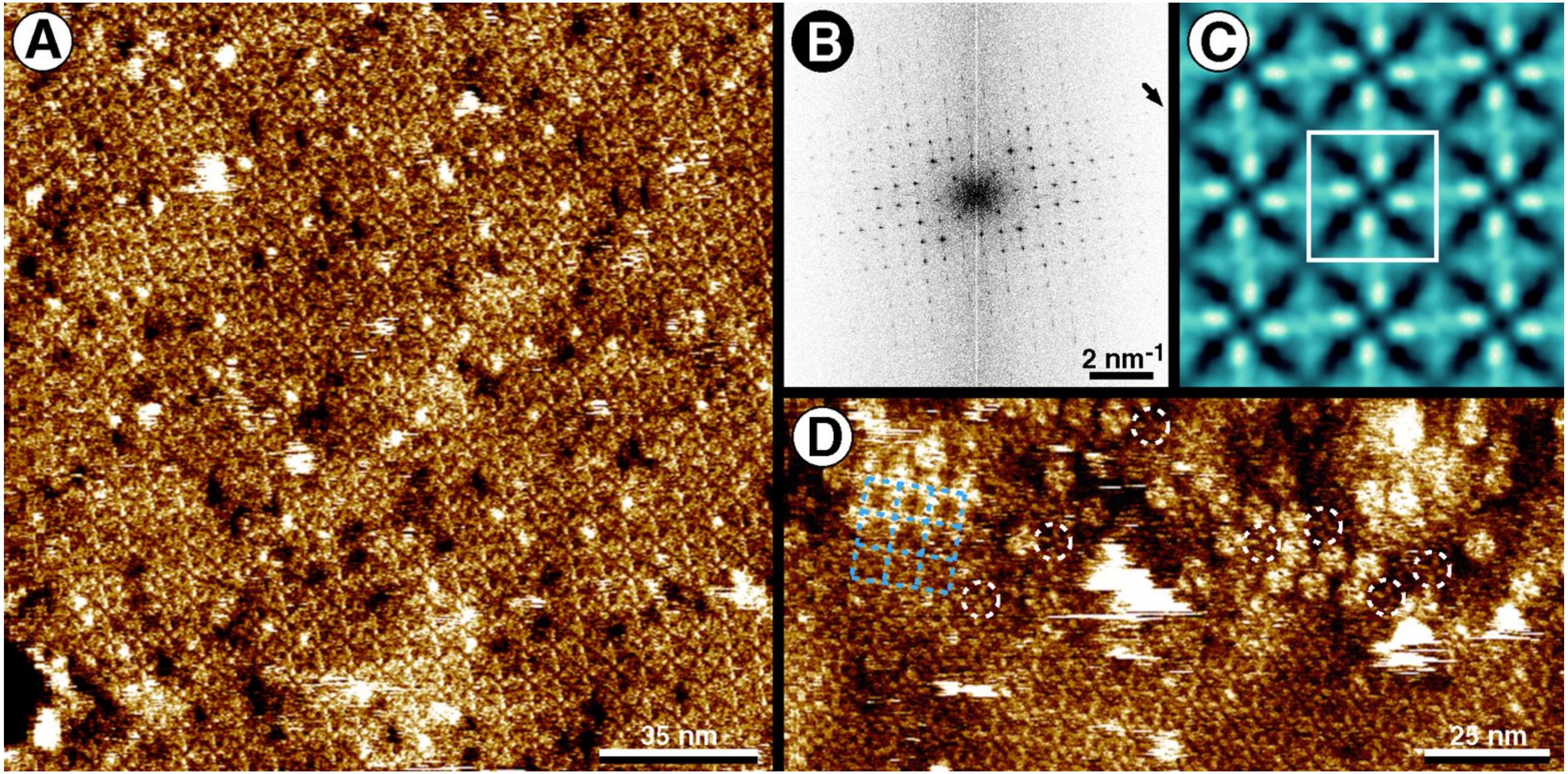


AFM of double-layered 2D crystals of AQP0



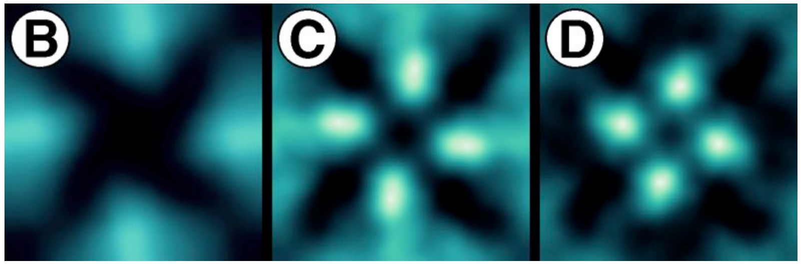
Fotiadis *et al.* (2000), J. Mol. Biol.

The cytoplasmic surface of AQP0



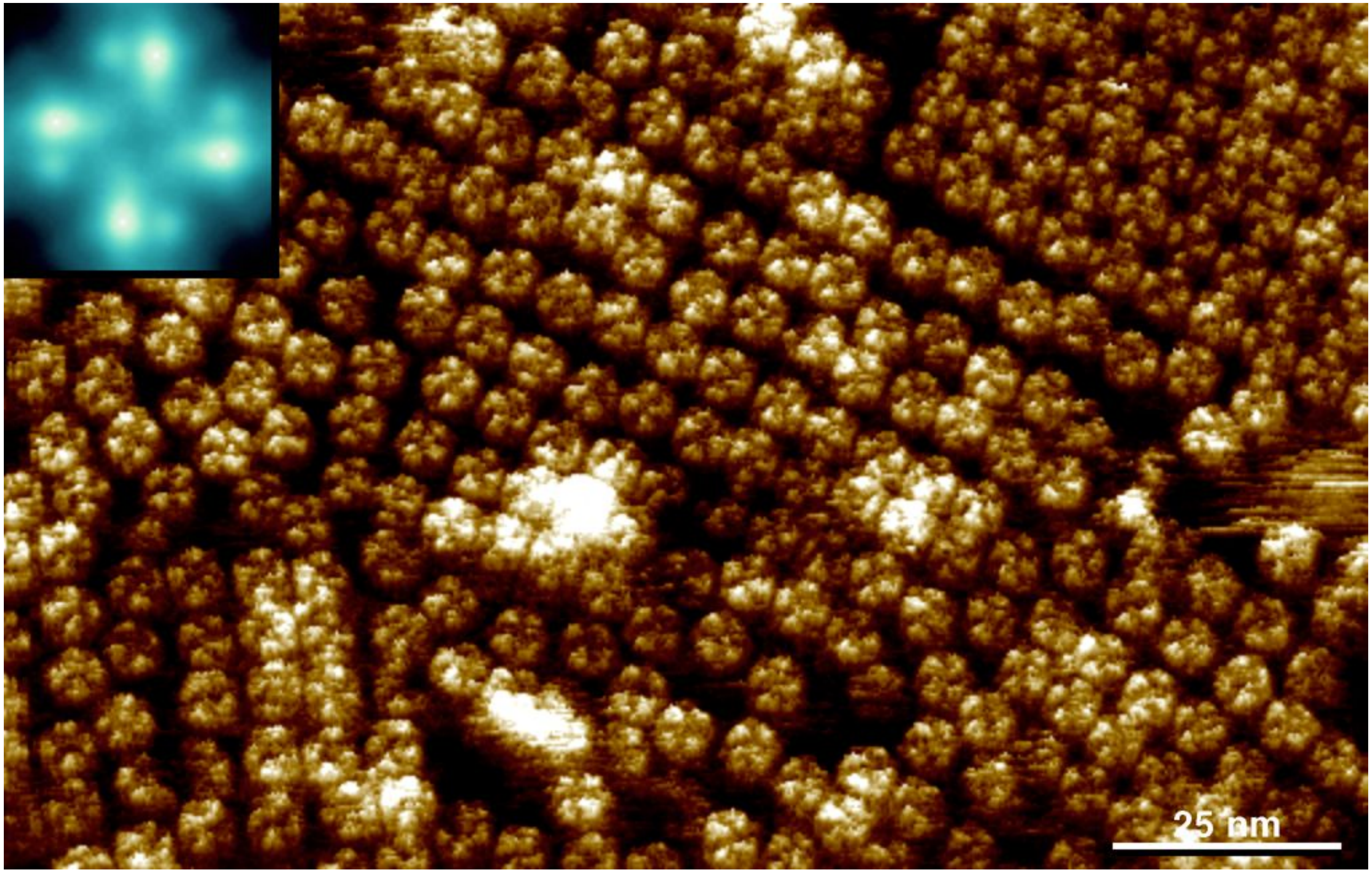
Arrow: 6.1 Å

C-terminus: ~7 kDa



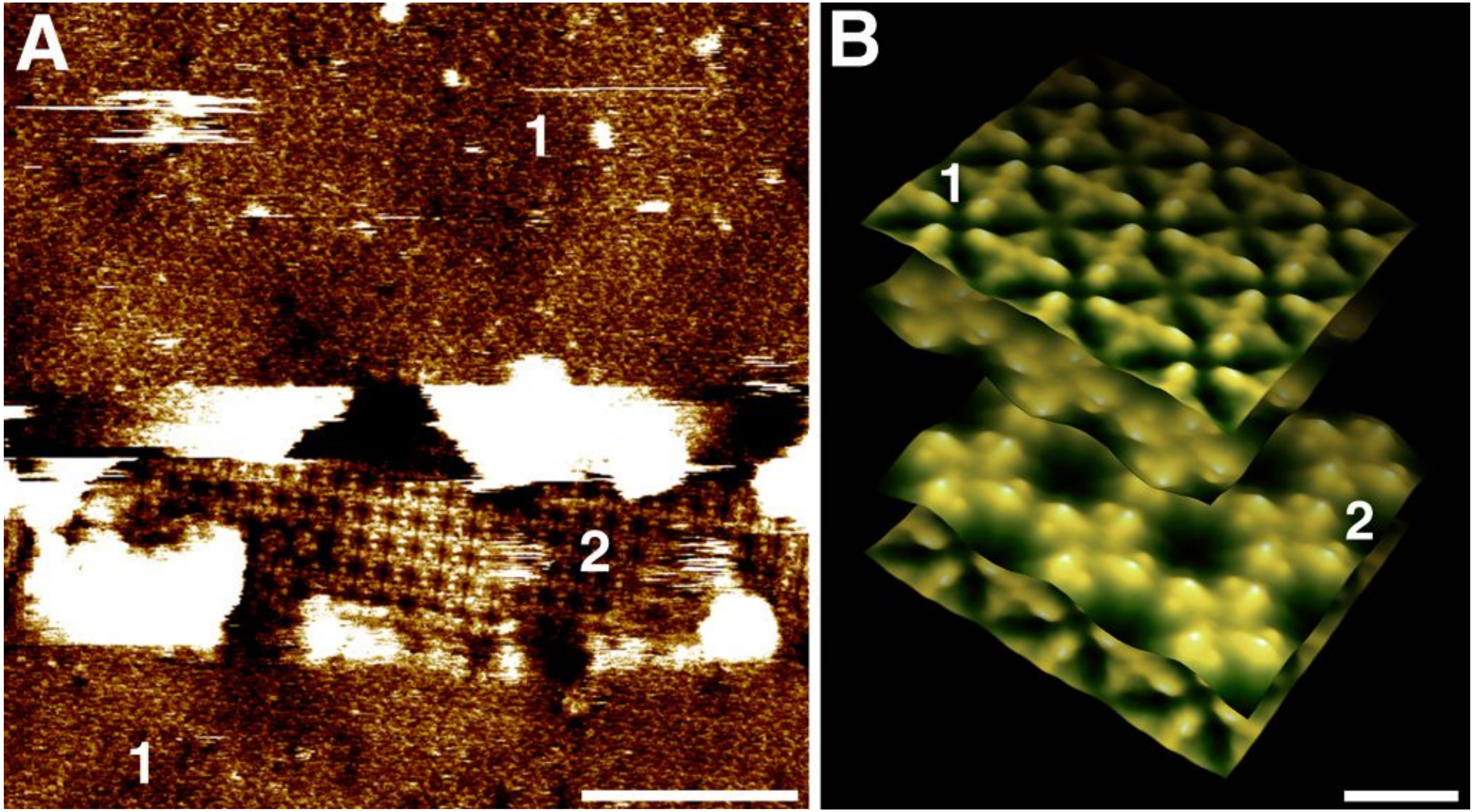
Fotiadis *et al.* (2000), J. Mol. Biol.

The extracellular surface of AQP0



Fotiadis *et al.* (2000), J. Mol. Biol.

Structure and interaction mechanisms of stacked 2D AQP0 crystals



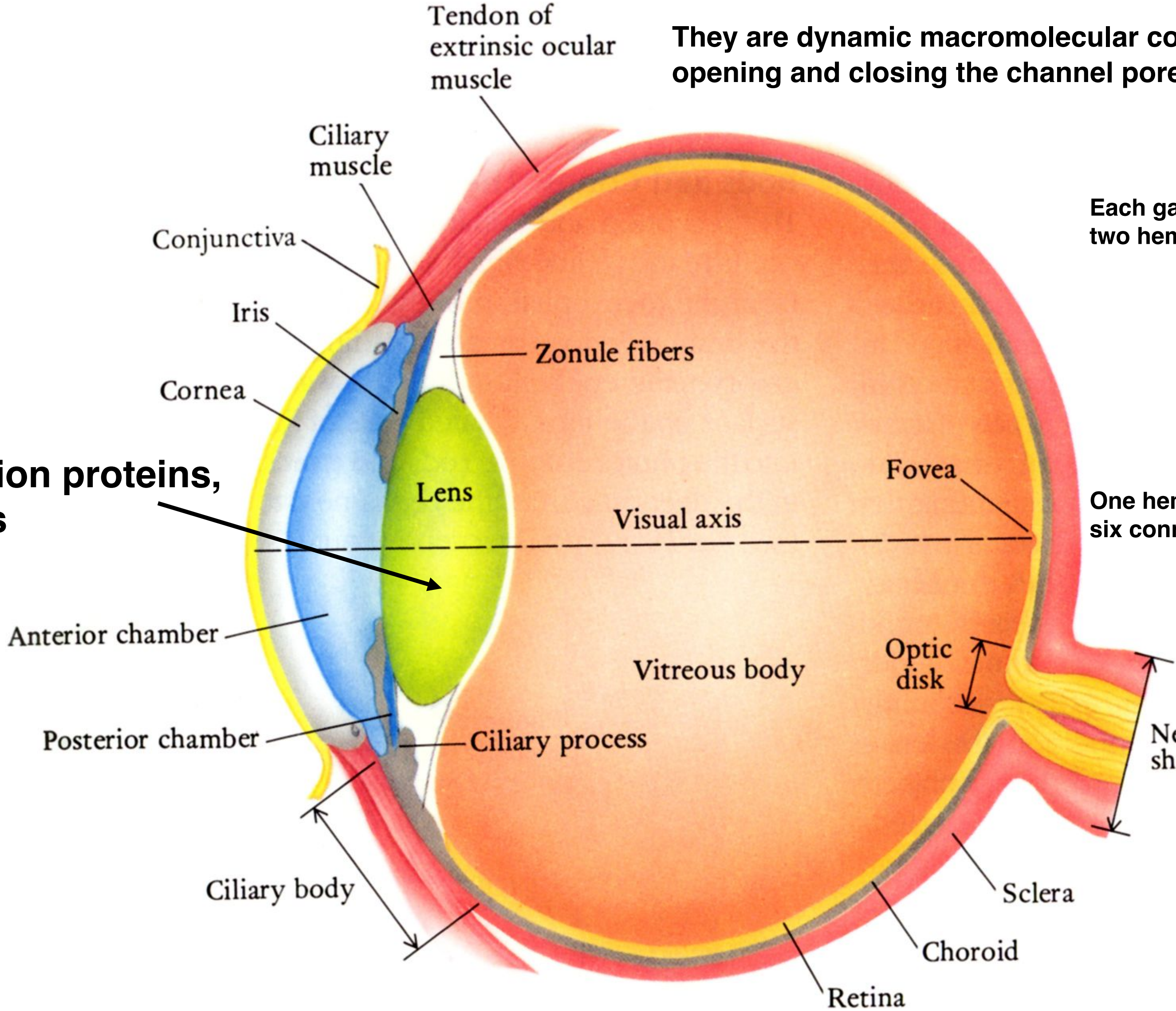
Fotiadis *et al.* (2000), J. Mol. Biol.

Horizontal section of the vertebrate eye

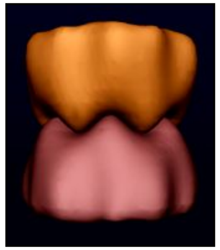
Gap junction channels regulate cell-cell communication by passing metabolites, ions, and signaling molecules.

They are dynamic macromolecular complexes capable of opening and closing the channel pore in response to stimuli.

Gap junction proteins, connexins

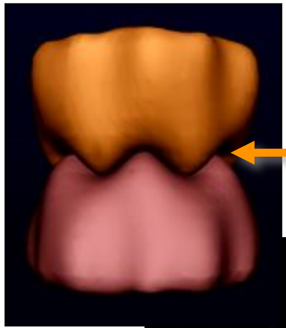


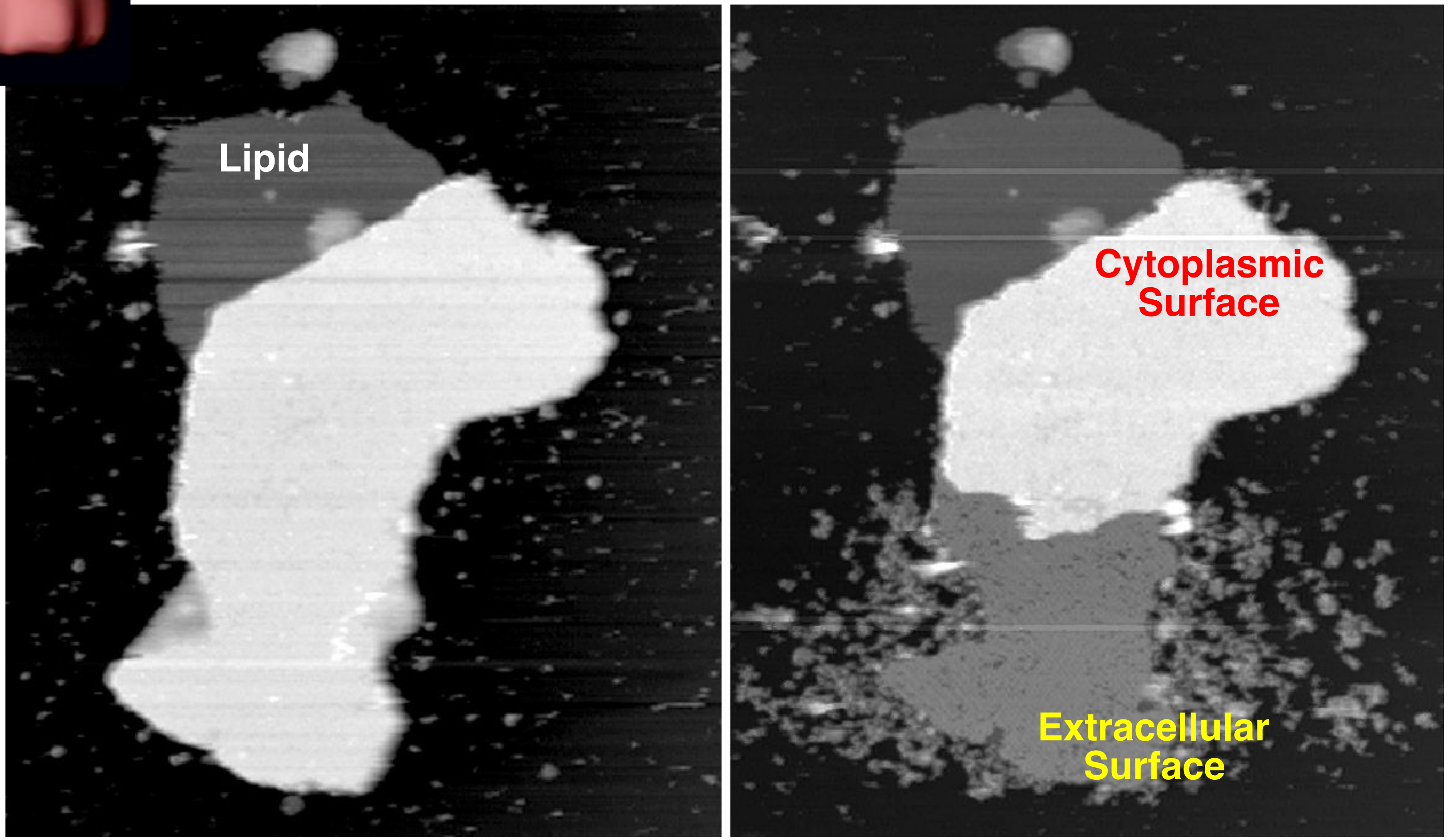
Each gap junction is composed of two hemichannels (connexons).



One hemichannel is composed of six connexin molecules.

Force dissection of a gap junction plaque

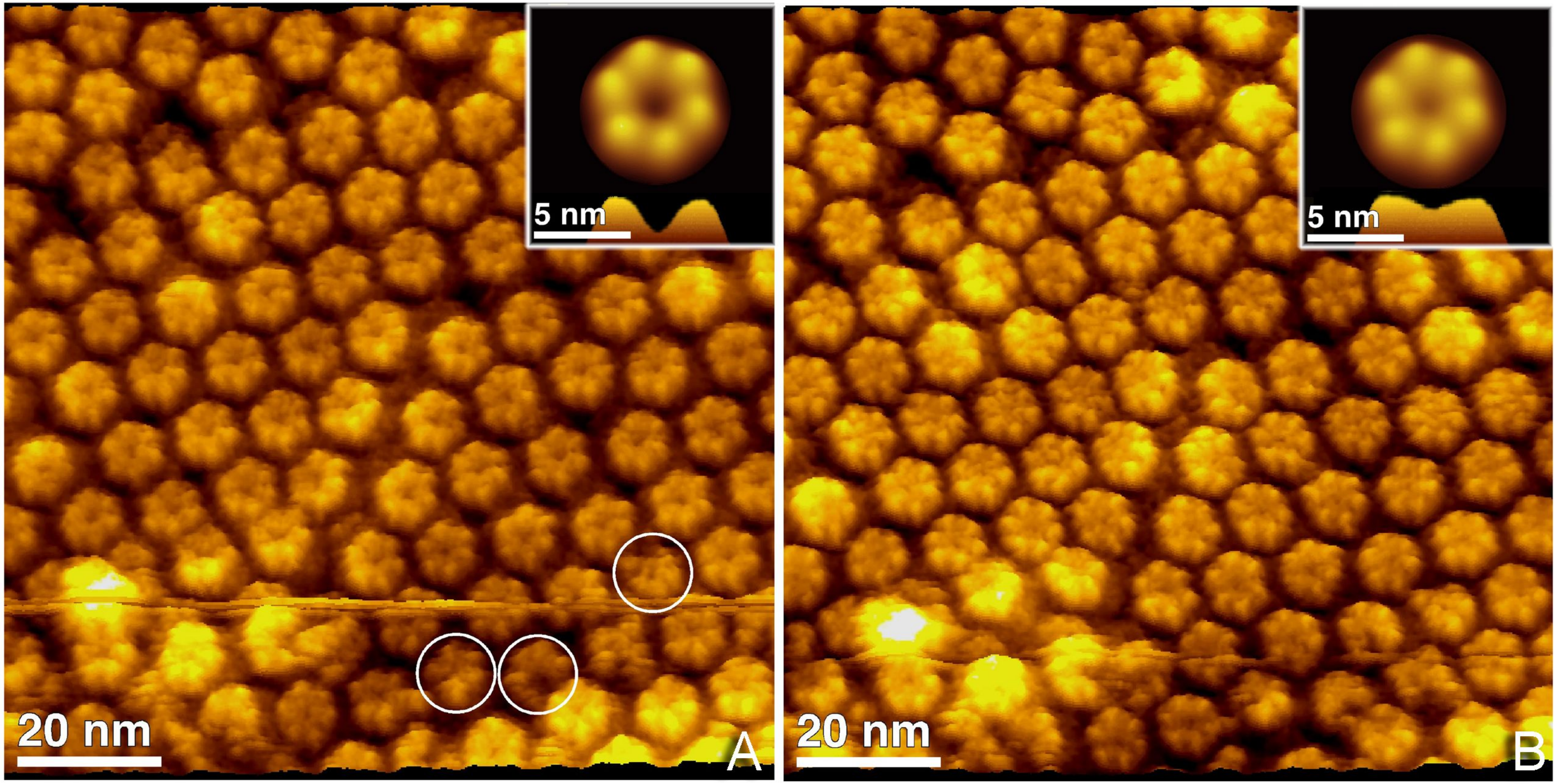




Isolated Cx26 gap junction plaque from HeLa cells (cervical cancer cells) as imaged by AFM

Yu *et al.* (2007), J. Biol. Chem.

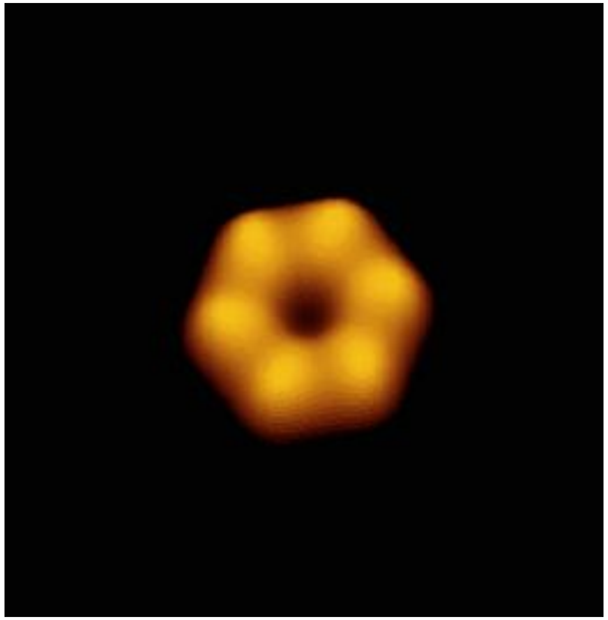
Conformational changes in surface structures of isolated connexin 26 gap junctions



- Ca^{2+} + 0.5 mM Ca^{2+}

Müller *et al.* (2002), EMBO J.

Comparison of features +/- Ca^{2+} reveals a large change in the extracellular pore of hemichannels.



	- Ca^{2+} size ± SD [nm]	+ Ca^{2+} size ± SD [nm]
Outer diameter of extracellular pore	4.9 ± 0.3	4.8 ± 0.3
Inner diameter of extracellular pore	1.3 ± 0.3	0.5 ± 0.3
Gap junction height	17.4 ± 0.7	18 ± 0.9

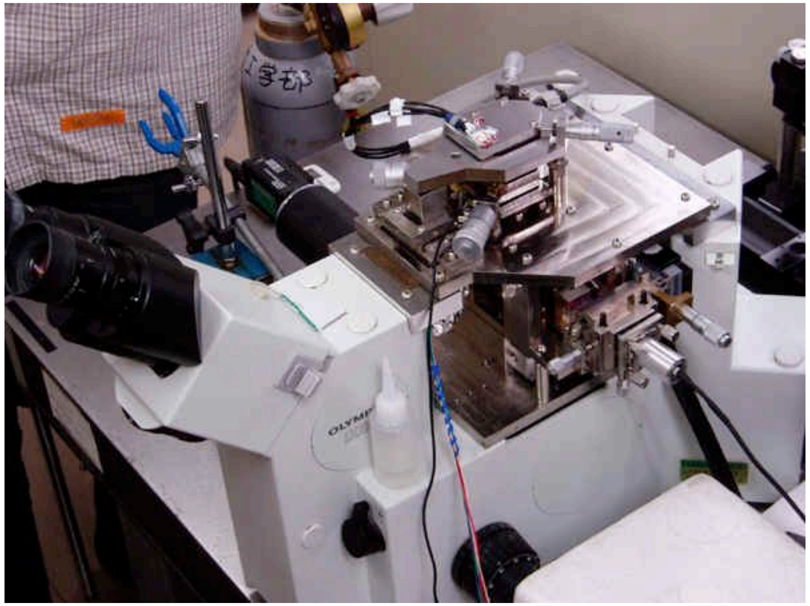
Problem:

Acquisition of one topograph with a commercial AFM takes >1.5 min.

→ Biological processes occur on a much faster time scale.

Solution:

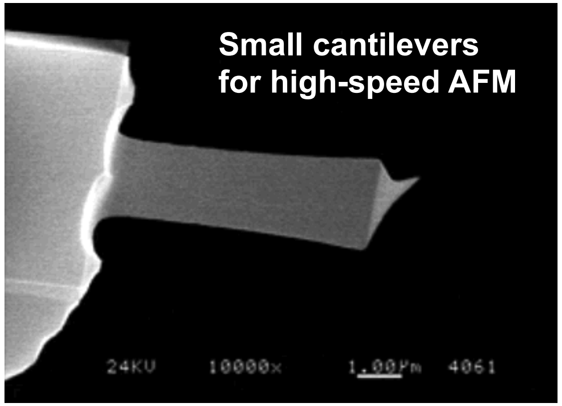
Development of high-speed AFM



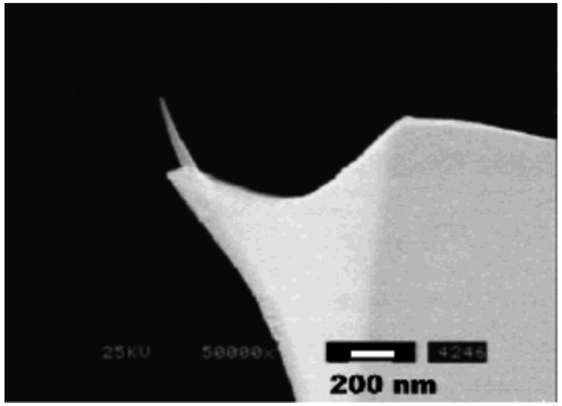
Toshio Ando's group at Kanazawa University, Japan

Ando *et al*. (2001), PNAS

State of the art high-speed AFM

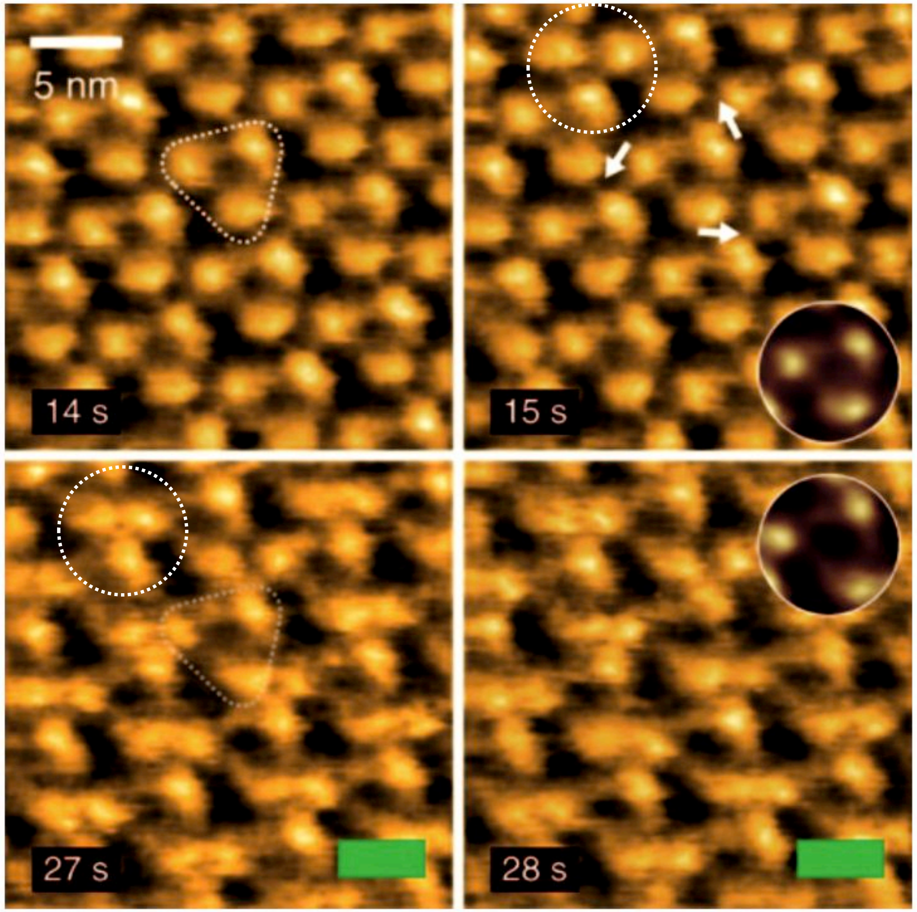


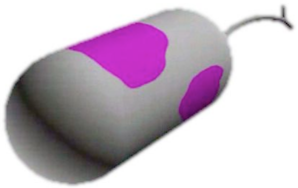
l= 7 μm, w= 2 μm, d= 90 nm
k= 0.2 N/m
Resonance frequency= 1.2 MHz (in water)



After plasma etching: tip radius ~5 nm

Reversible light-induced dynamic structural changes of the bacteriorhodopsin mutant D96N*





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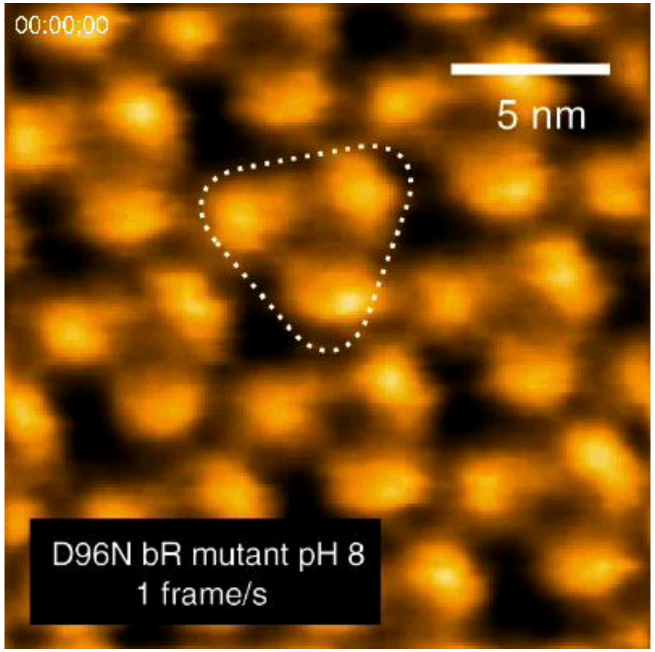

* Bacteriorhodopsin mutant D96N has a photocycle of ~10 s.
wt-bacteriorhodopsin photocycle: ~10 ms (one H^+ is pumped out of the cell)

Shibata *et al.* (2011), Angew. Chem. Int. Ed.

Thank you for your attention !

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27th January 2015

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