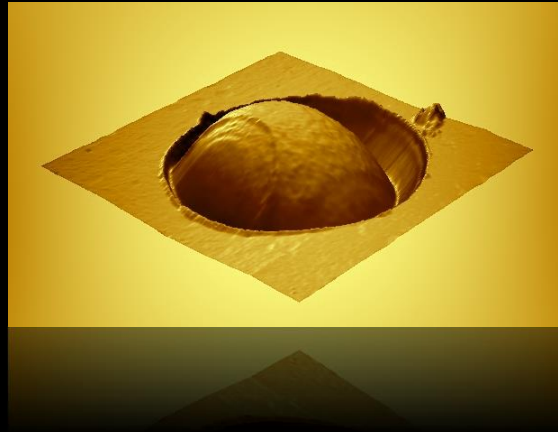


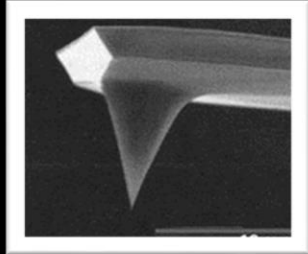
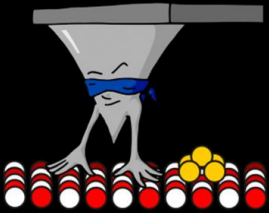
ATOMIC FORCE MICROSCOPY ON MICROBIAL SURFACES



Audrey BEAUSSART & Sofiane EL-KIRAT-CHATEL

LIEC, UMR 7360, NANCY
LCPME, UMR 7564, NANCY

Atomic Force Microscopy (AFM)



« This microscope works by touching objects with a sharp tip instead of looking at them »

The Nobel Prize in physics 1986

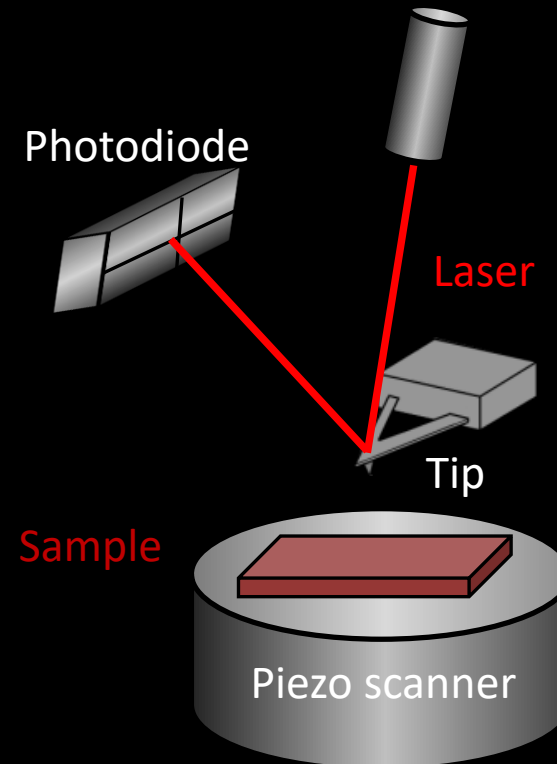
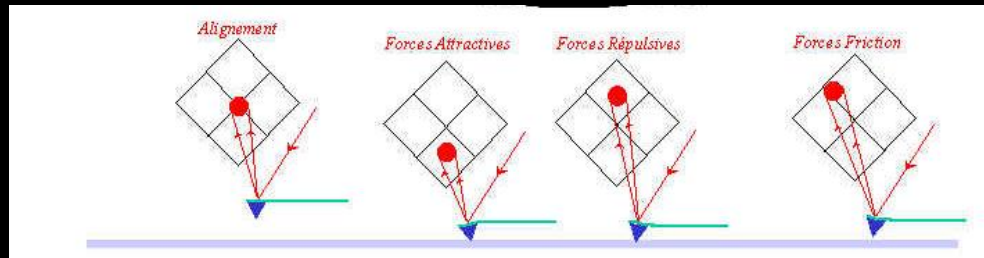


Gerd Binnig

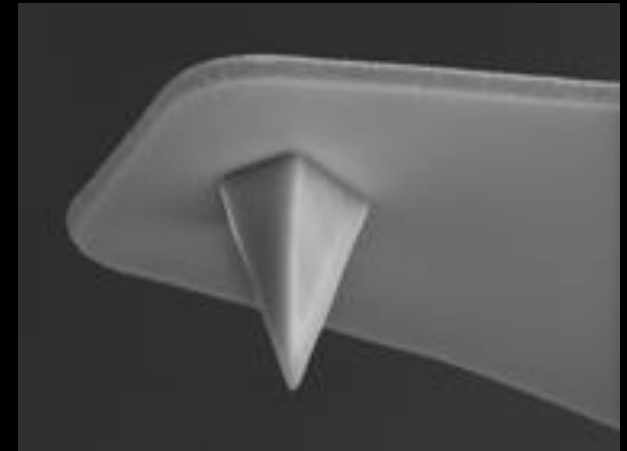
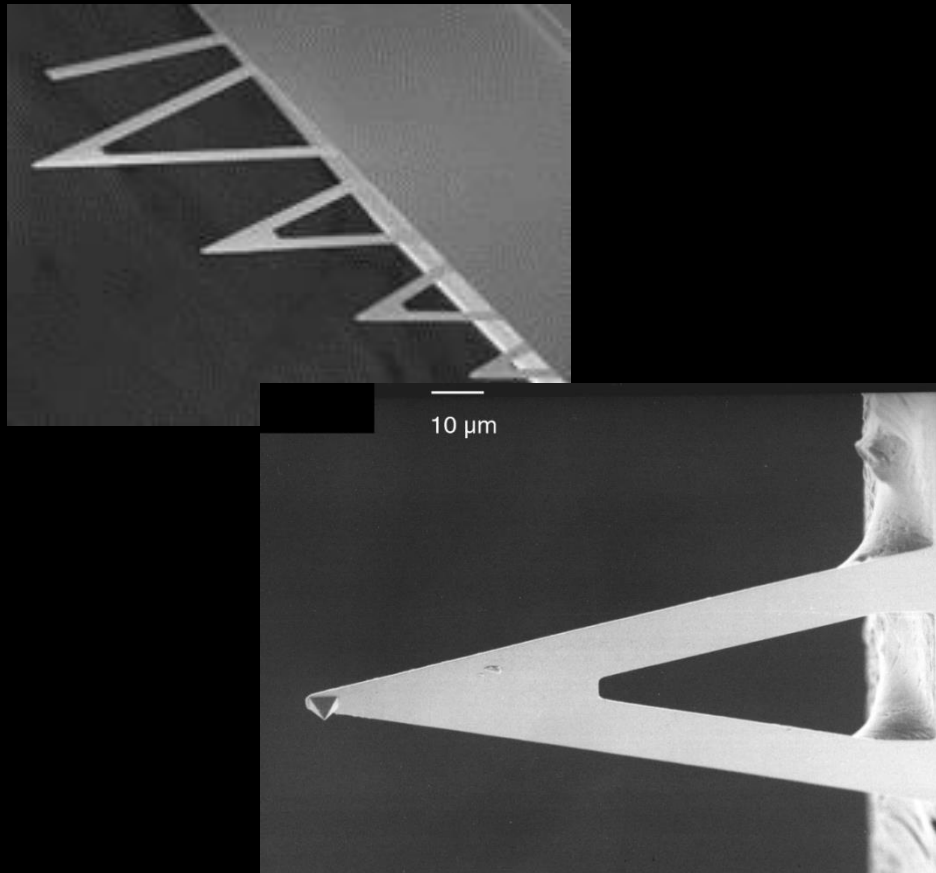


Heinrich Rohrer

Atomic Force Microscopy (AFM)

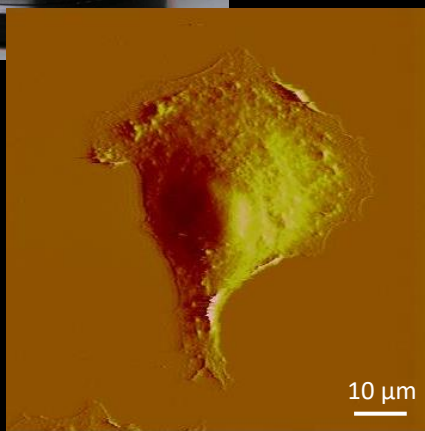
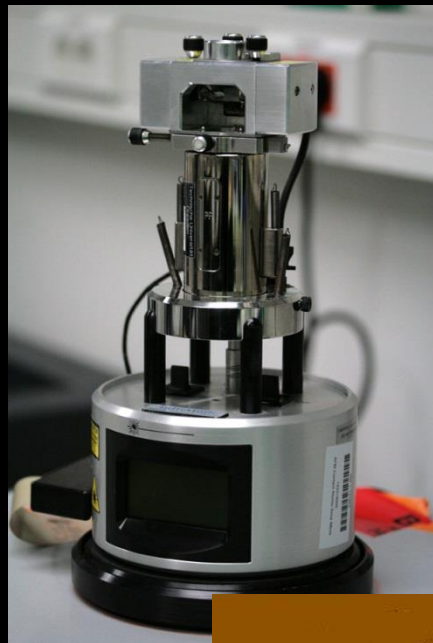


Atomic Force Microscopy (AFM)

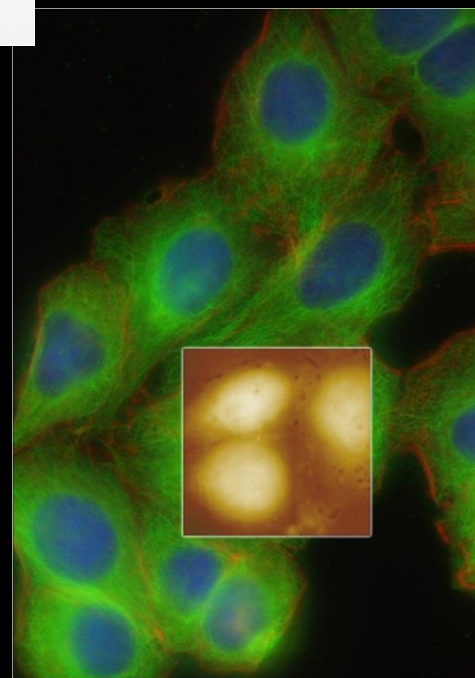
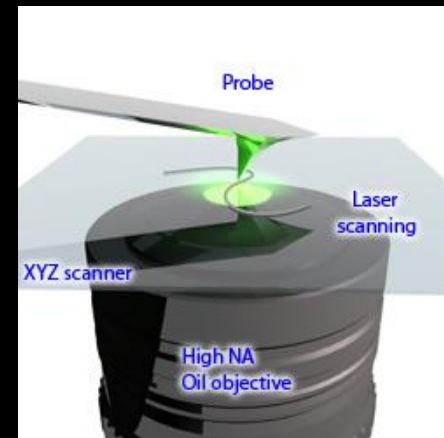


Geometry:	Rotated (Symmetric)
Tip Height (h):	2.5 - 8.0 μm
Front Angle (FA):	$15 \pm 2.5^\circ$
Back Angle (BA):	$25 \pm 2.5^\circ$
Side Angle (SA):	$17.5 \pm 2.5^\circ$
Tip Radius (Nom):	10 nm
Tip Radius (Max):	40 nm
Tip SetBack (TSB)(Nom):	4 μm
Tip Set Back (TSB)(RNG):	0 - 7 μm

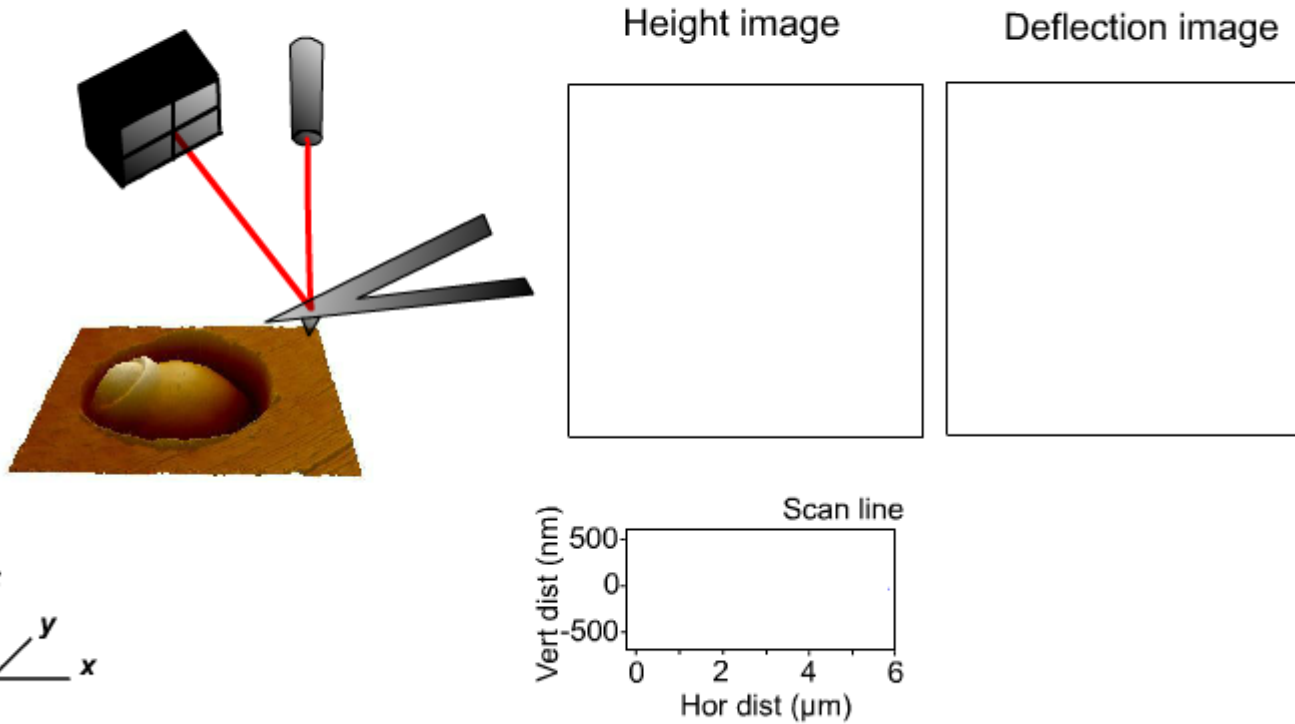
Classical AFM



AFM mounted on an optical system

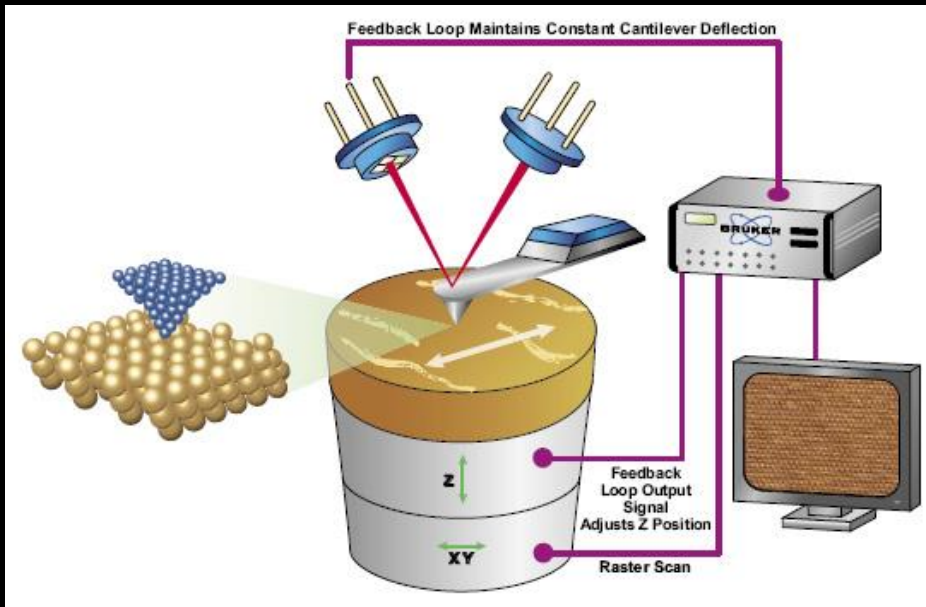


Imaging mode : principle

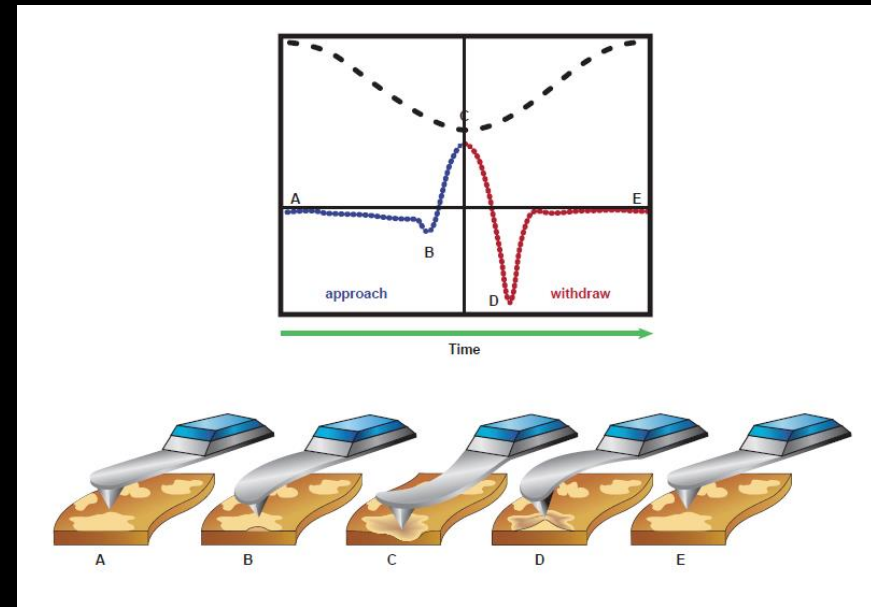


Imaging mode : contact vs peak force tapping

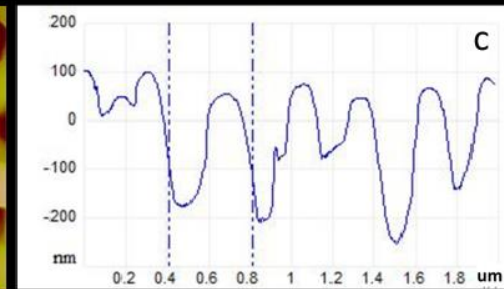
Contact



Peak force tapping



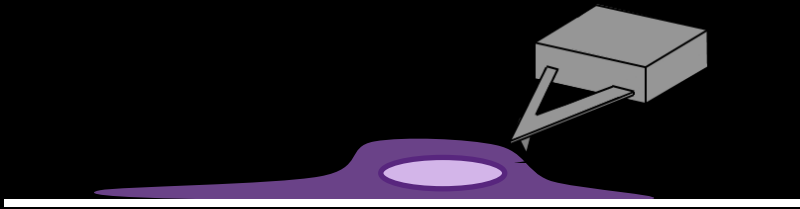
Color code



Immobilization techniques

Mammalian cells

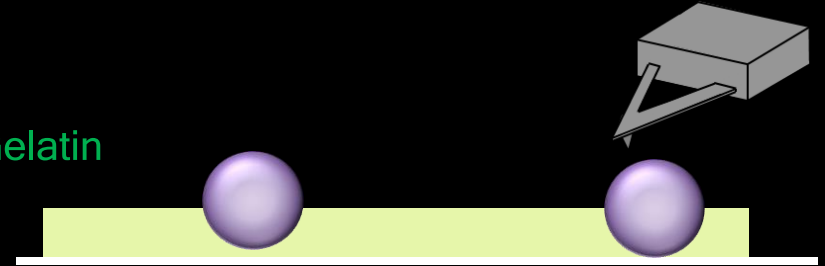
(tend to naturally spread on the surface)



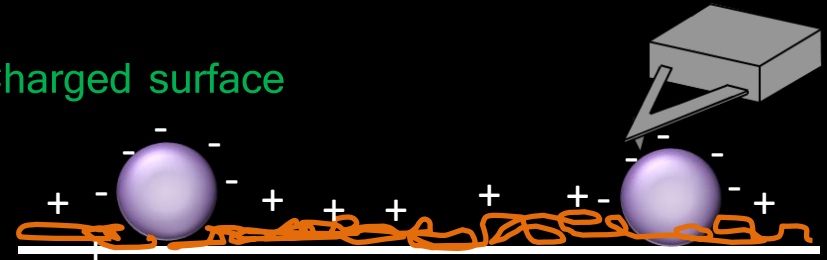
Micro-organisms

(round, difficult to deform)

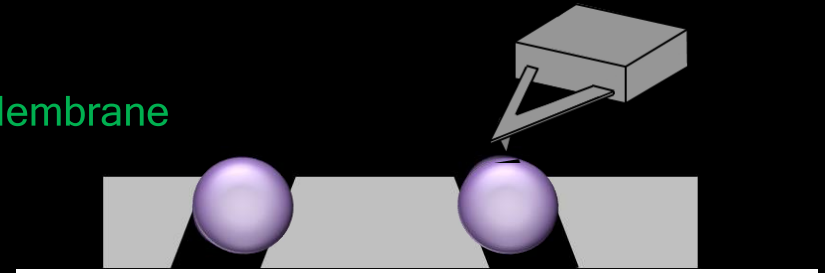
Gelatin



Charged surface

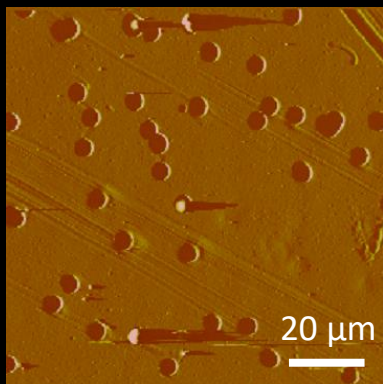
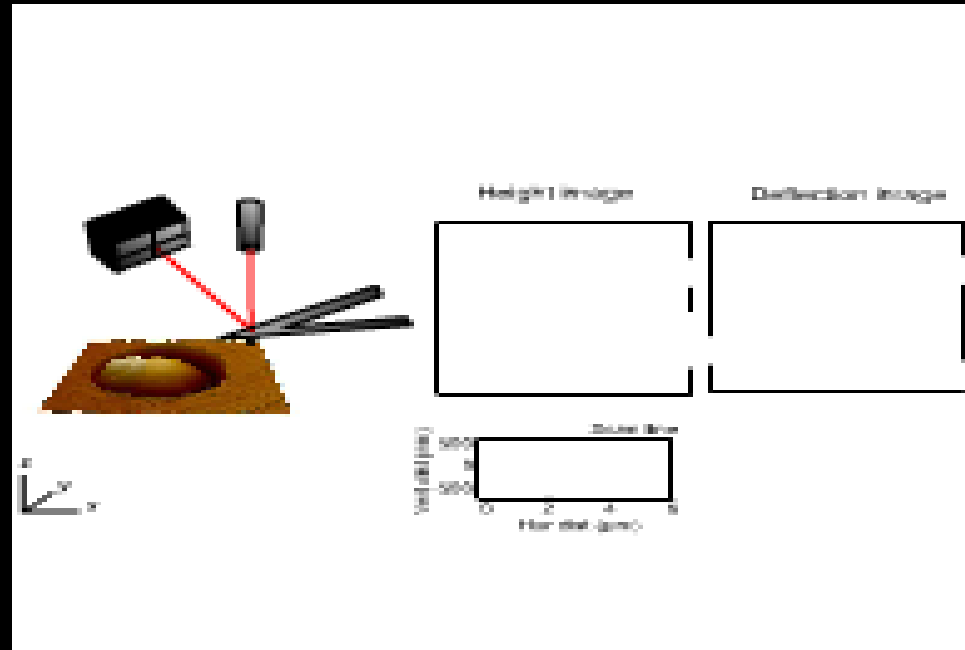
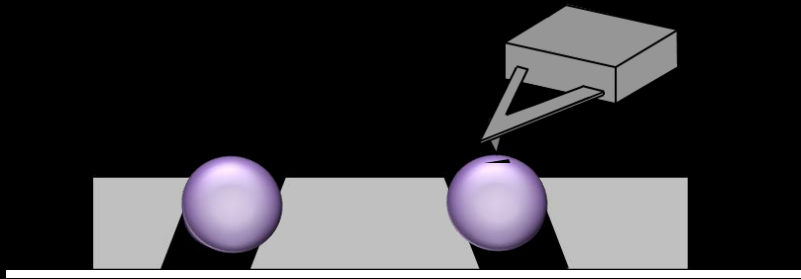


Membrane



AFM imaging of microbes

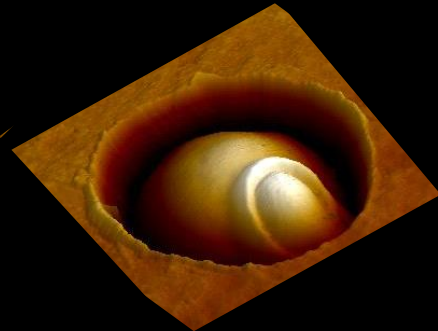
- *Mechanically trapped in membrane*
- *Imaged under small force (100 pN)*
- *In physiological conditions (liquid)*



Imaging mode : examples

Yeast

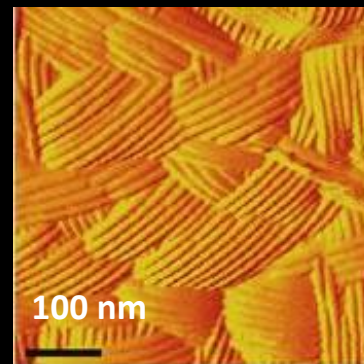
Candida albicans



Fungus

Aspergillus fumigatus

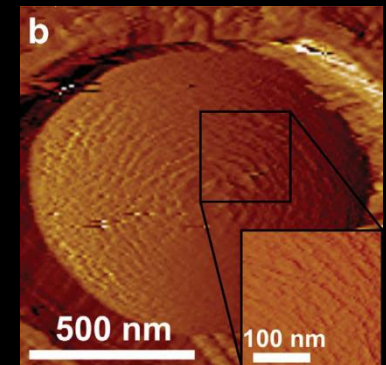
Hydrophobic rodlets



Bacteria

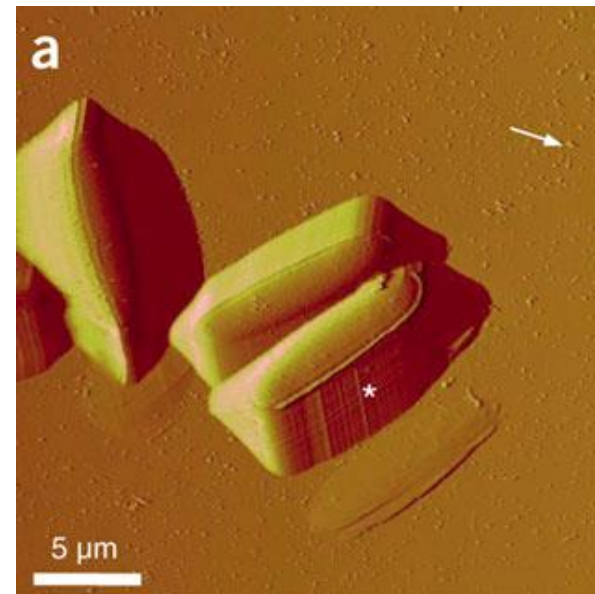
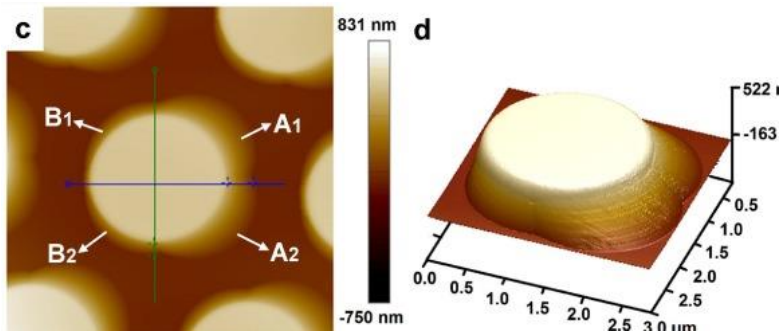
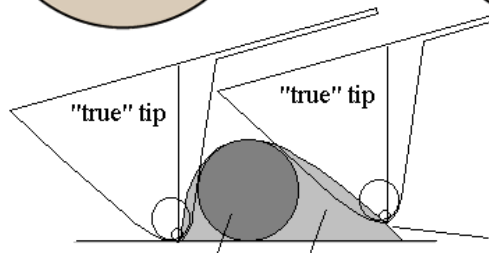
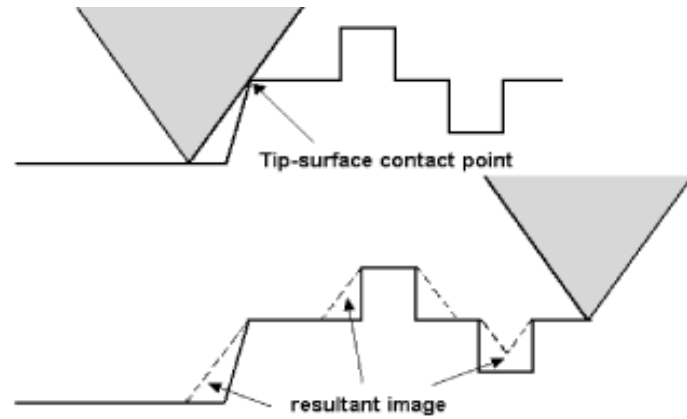
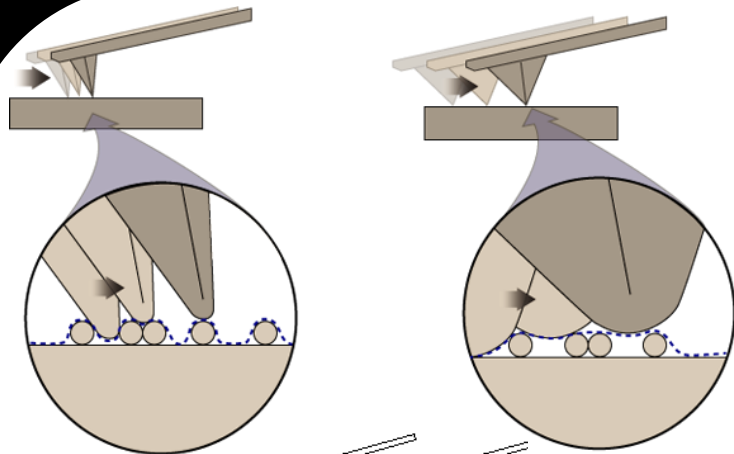
Lactococcus lactis

peptidoglycan



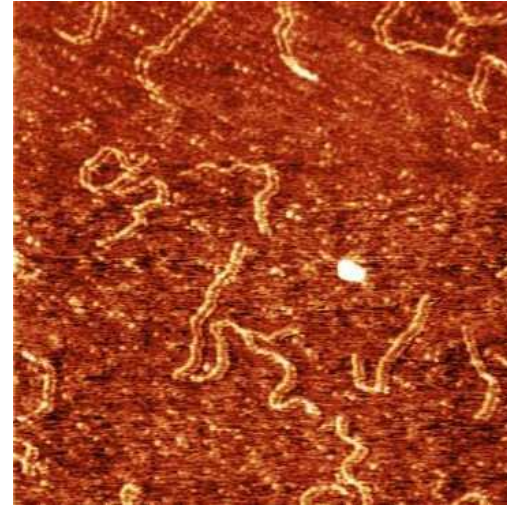
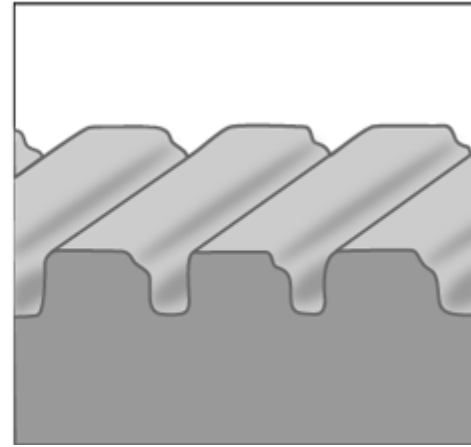
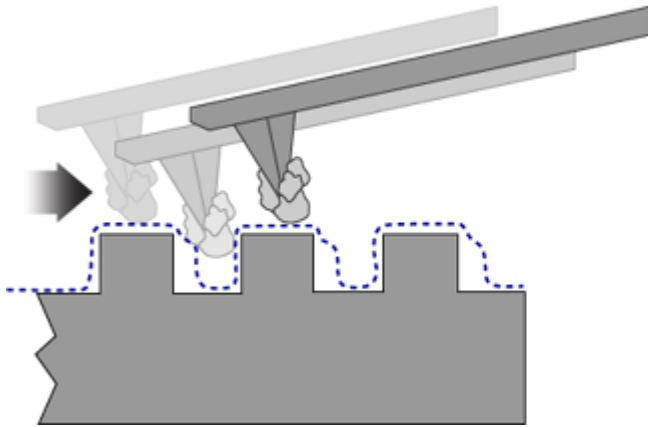
Imaging artefacts

- Tip radius of curvature

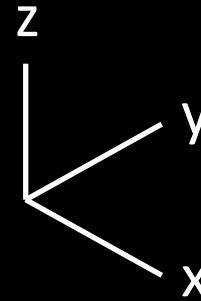
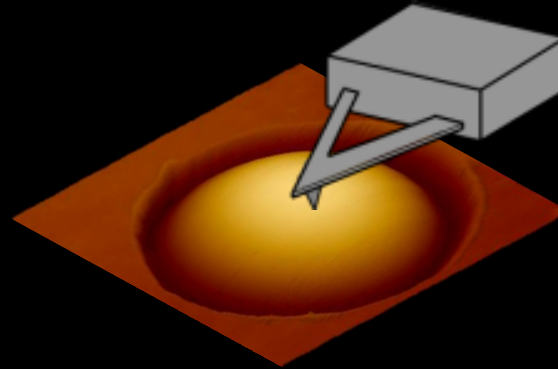


Imaging artefacts

- Tip contamination



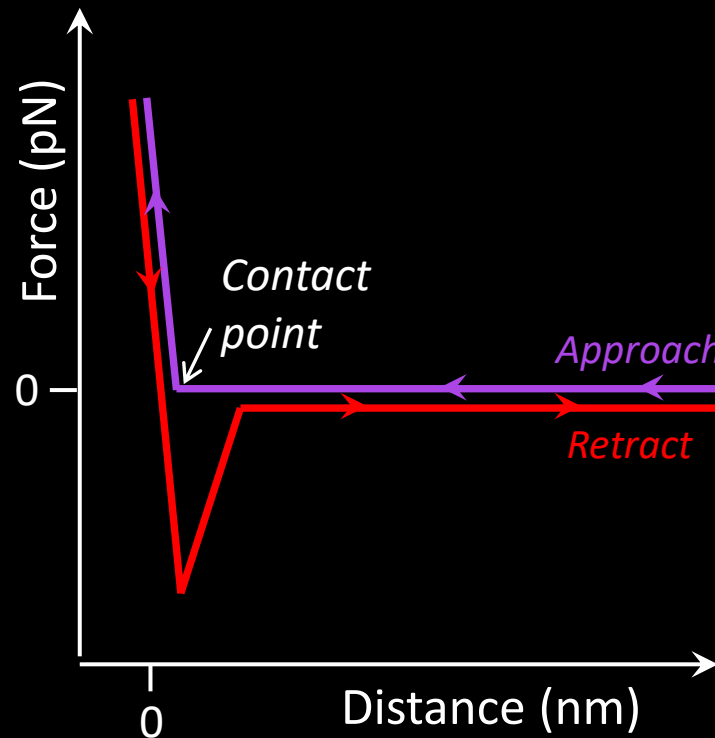
Force Spectroscopy



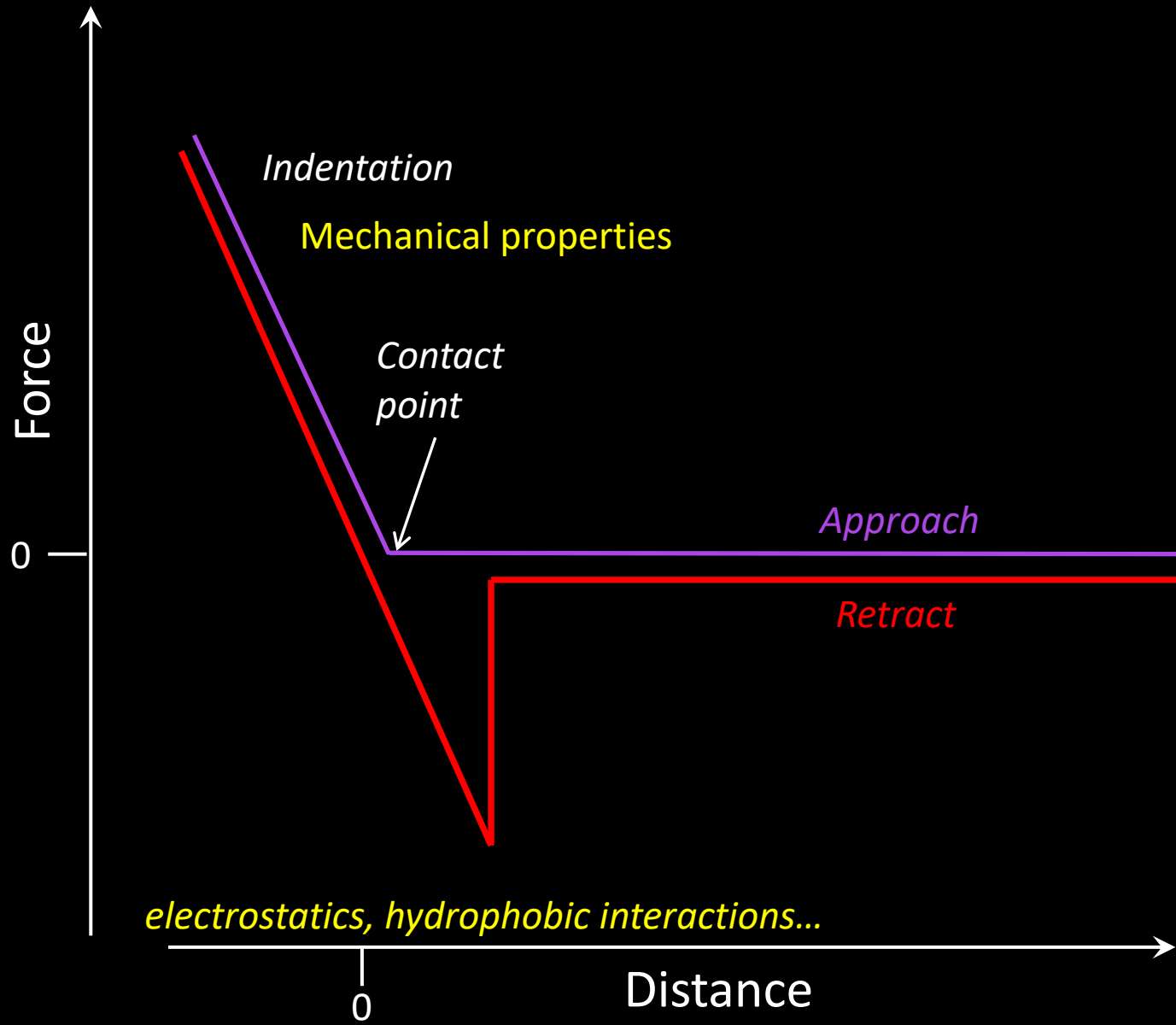
Force-distance curves

- Deflection sensitivity (nm/V)
- Cantilever spring constant (N/m)

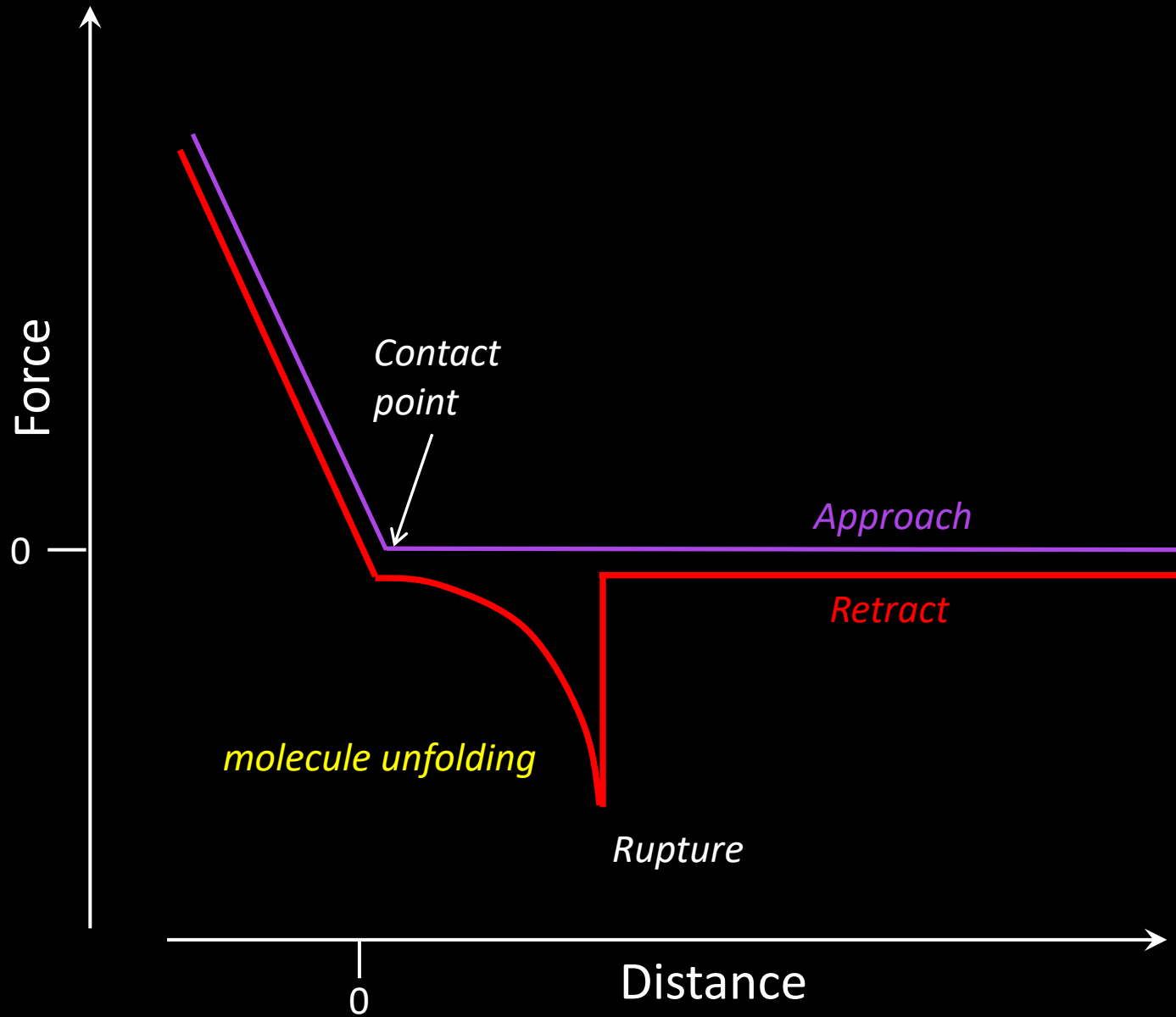
Hooke's law : $F = -k \times d$



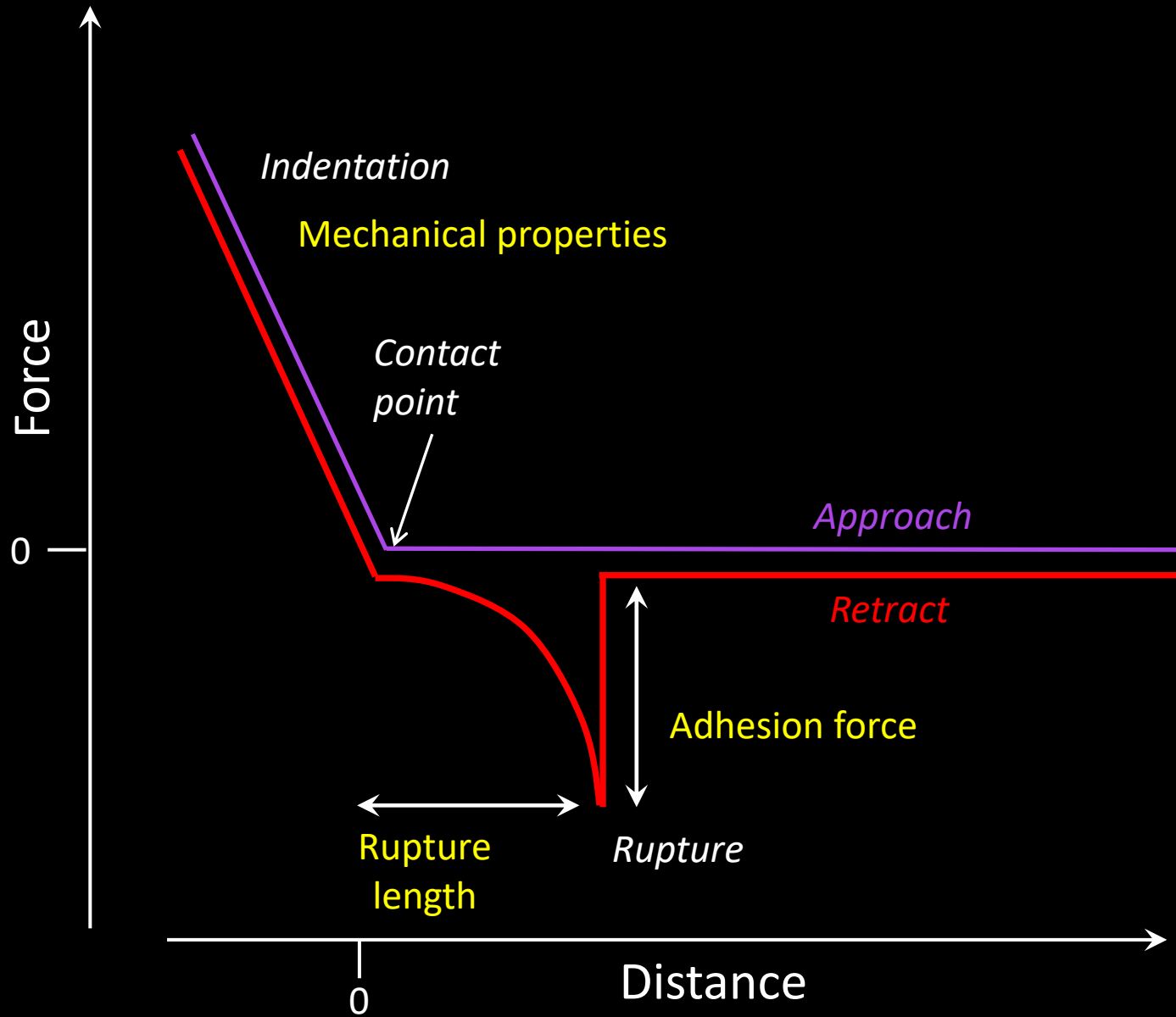
Force-distance curves



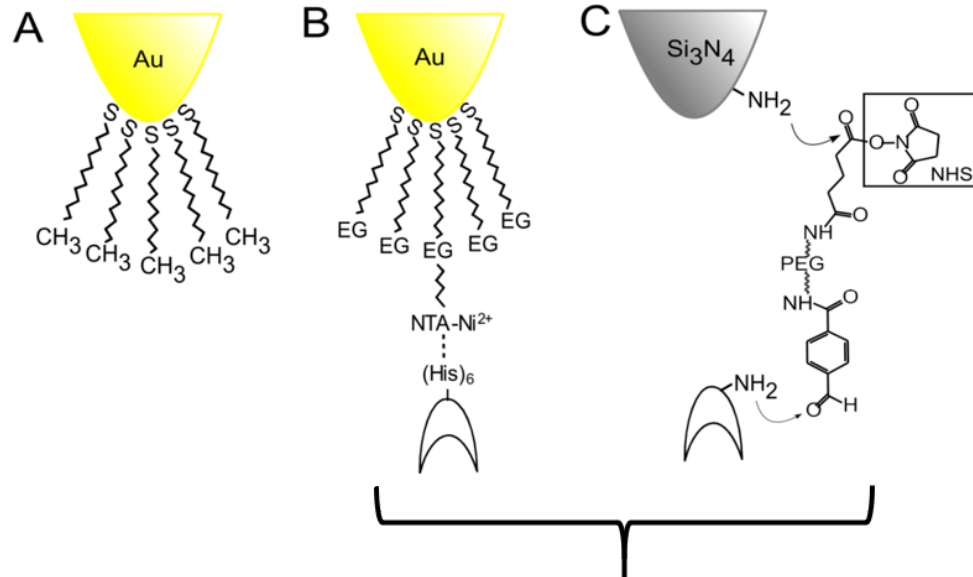
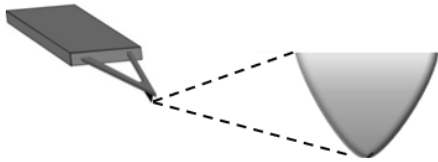
Force-distance curves



Force-distance curves



Tip functionalisation



Nanomechanical
properties

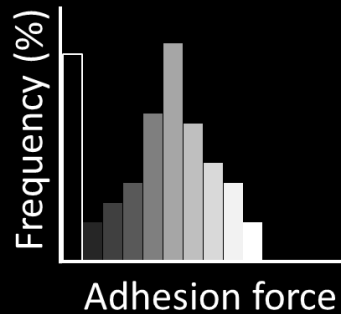
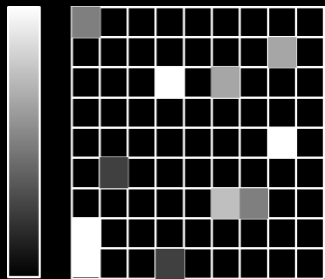
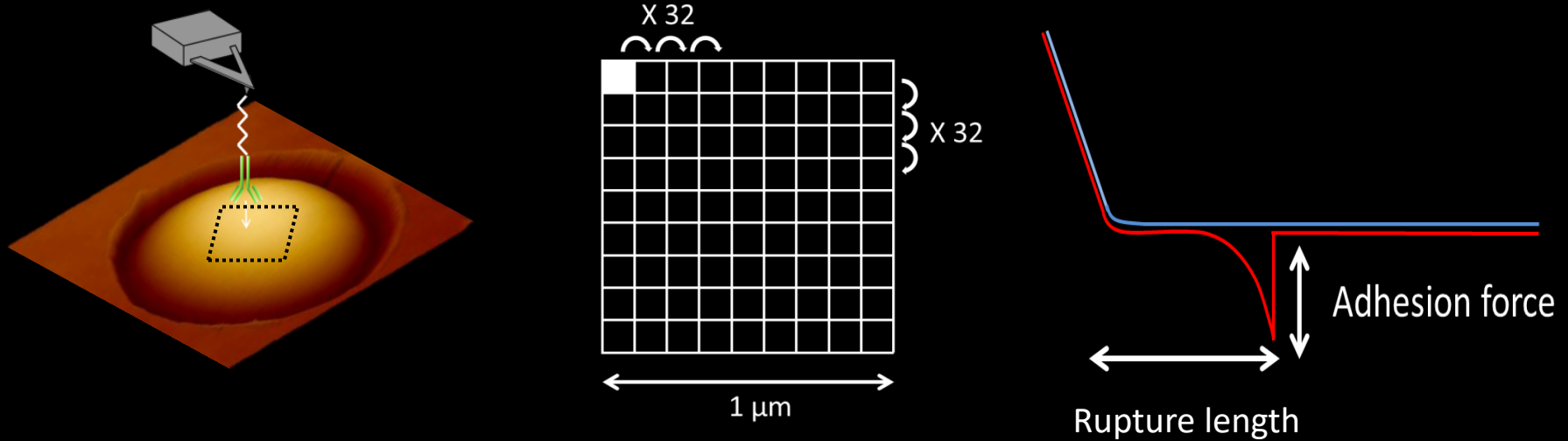
Chemical Force
Microscopy

Single Molecule
Force Spectroscopy

Indentation
(courbe approche)

Extension / interaction
(courbe retrait)

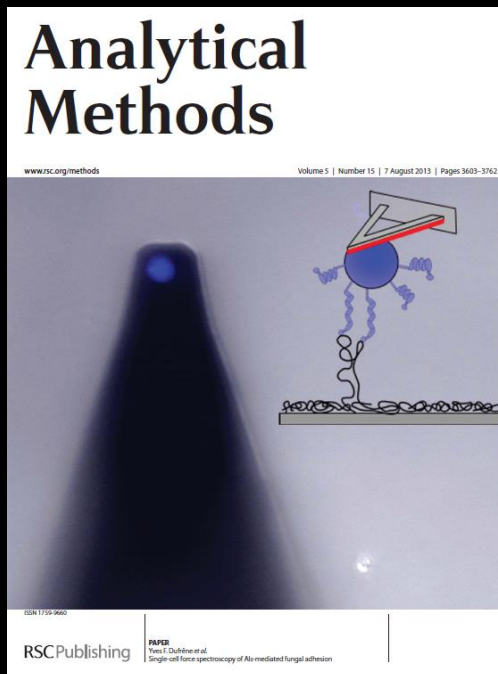
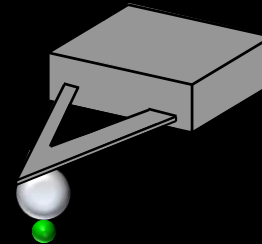
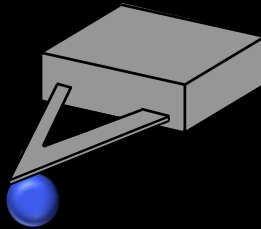
Mapping of individual molecules



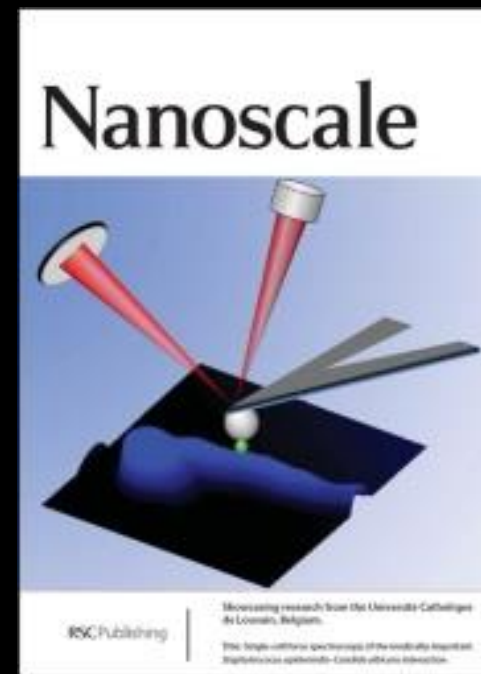
Mapping of individual molecules

- Distribution
- Mechanical properties
- Dynamics

Single Cell Force Spectroscopy



D. Alsteens, A. Beaussart *et al.*, 2013

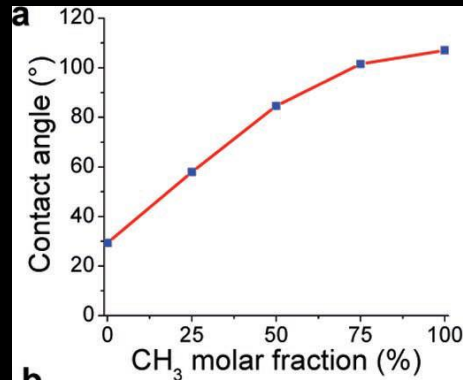
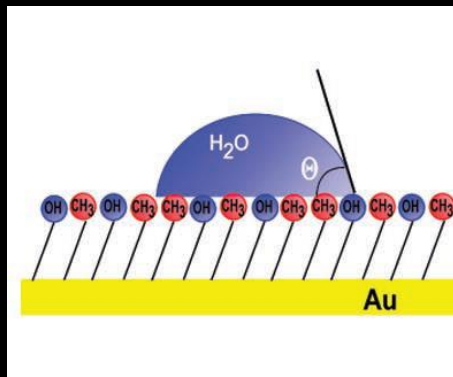
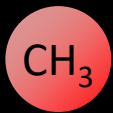


A. Beaussart, P. Herman *et al.*, 2013

Chemical force microscopy

- Macroscopic measurements

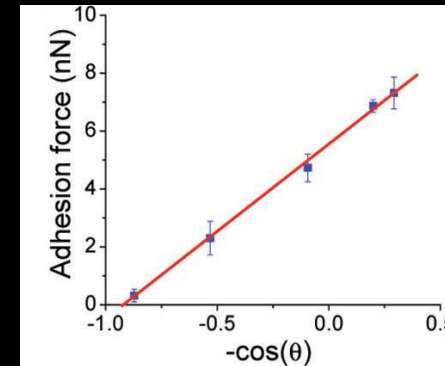
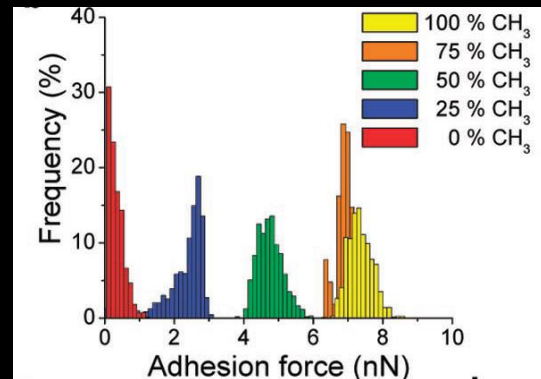
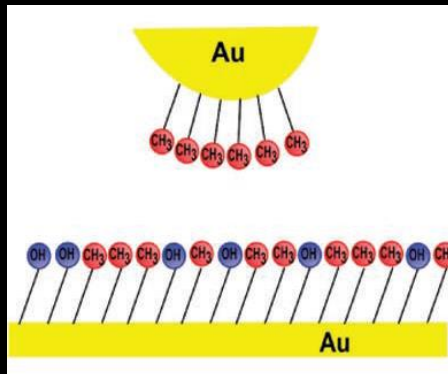
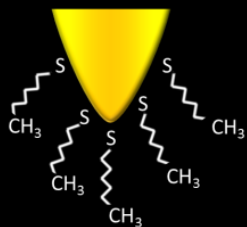
Water contact angle



Nanoscale measurements

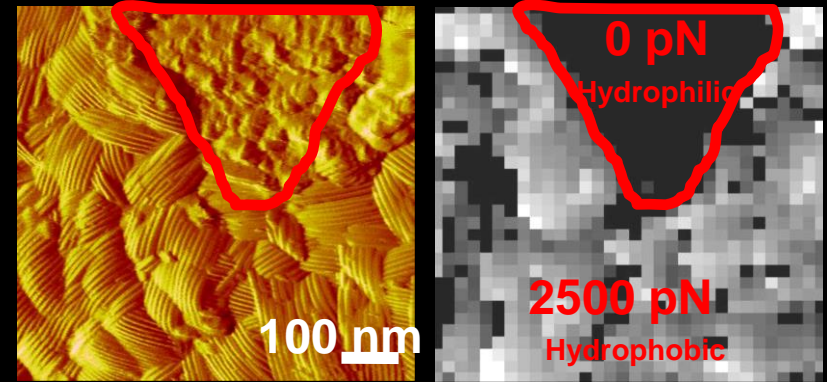
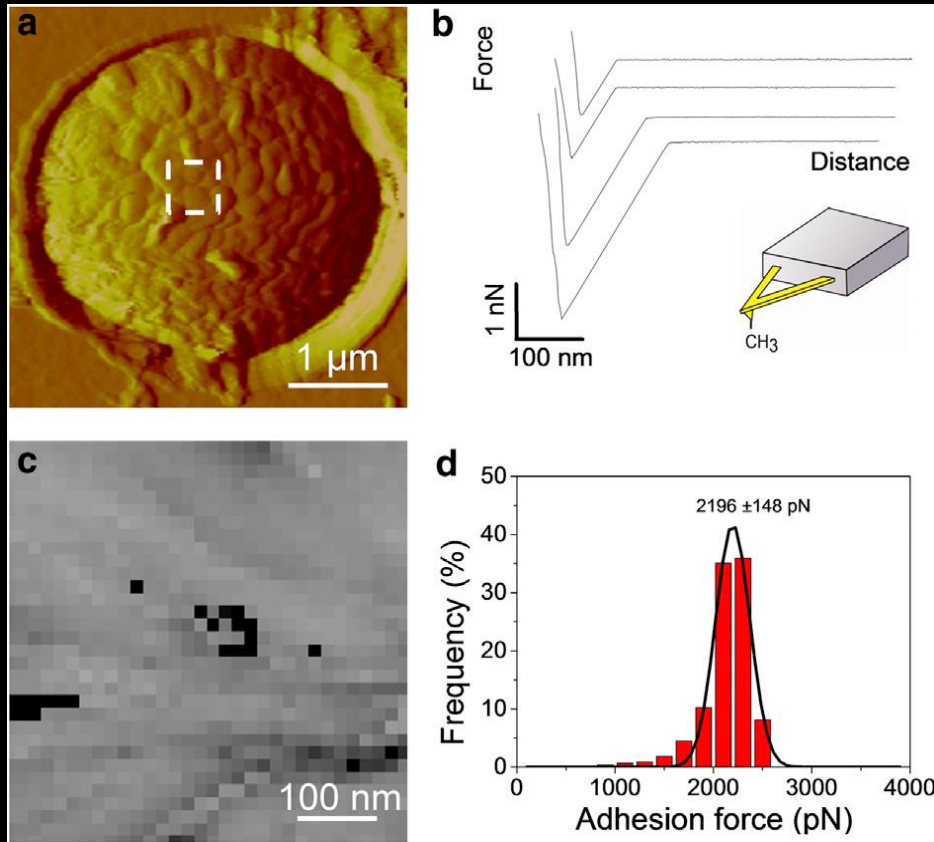
- Nanoscale measurements

Chemical force microscopy



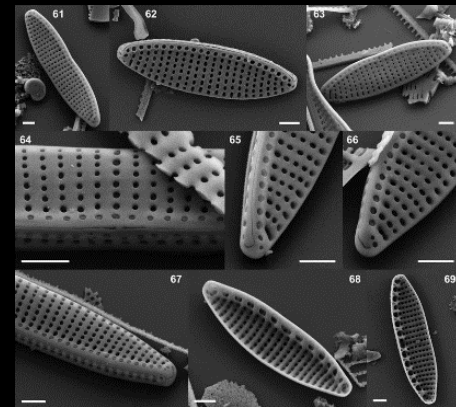
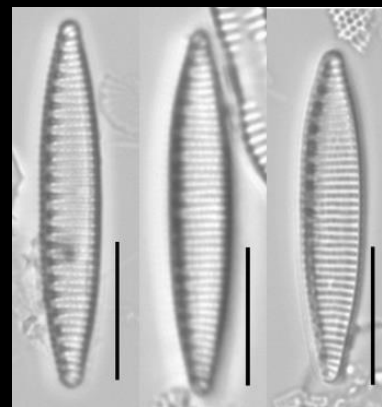
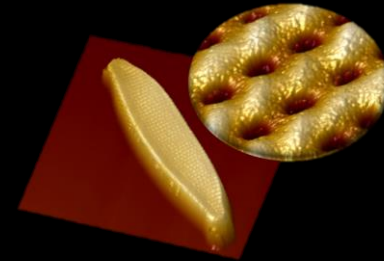
Macroscopic measurements

Chemical force microscopy of *A. fumigatus*



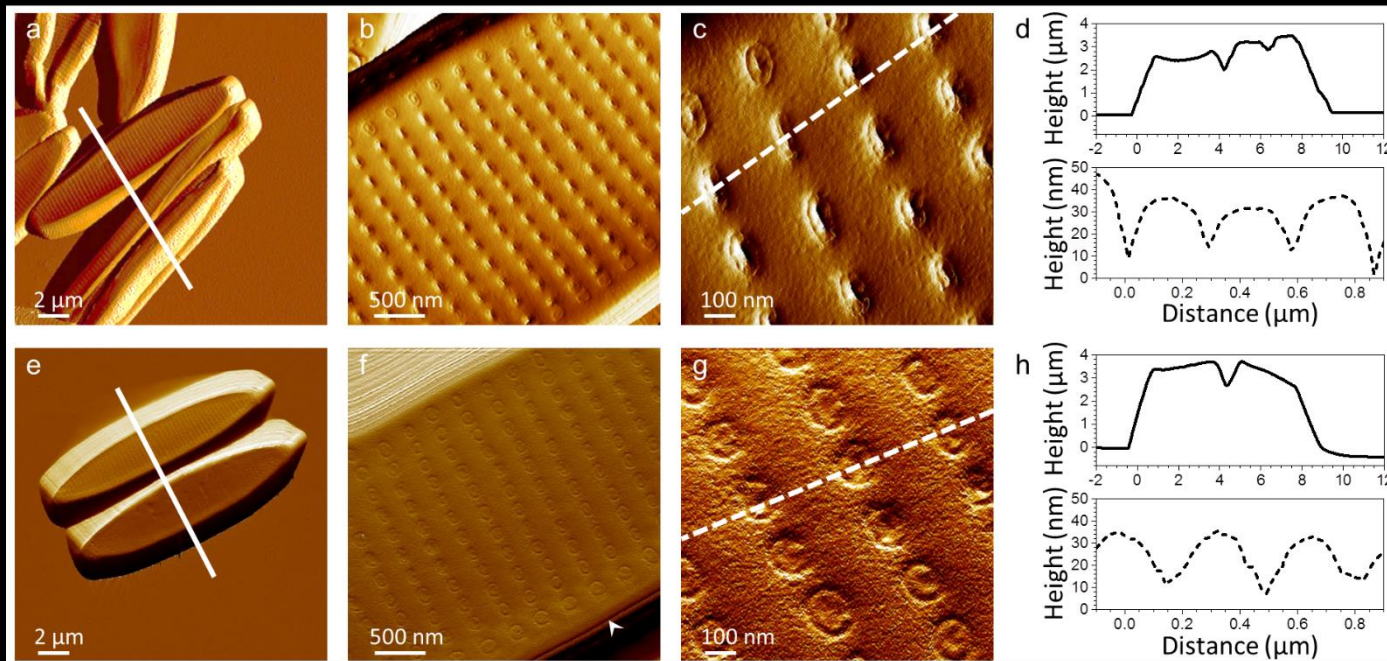
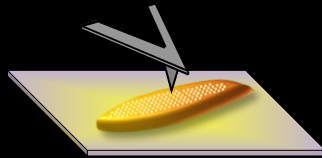
Nitzschia palea

- Photosynthetic microalgae
- Fresh water
- Motile
- Silica frustule with pores
- Adhere to surfaces and form mixed biofilms with other species
- Production of extracellular polymeric substances: EPS
- Bioindicator of water quality

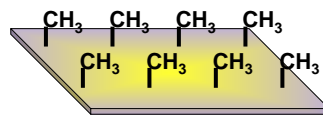


What are the adhesive mechanisms of *N. palea* towards surfaces of different chemistry?

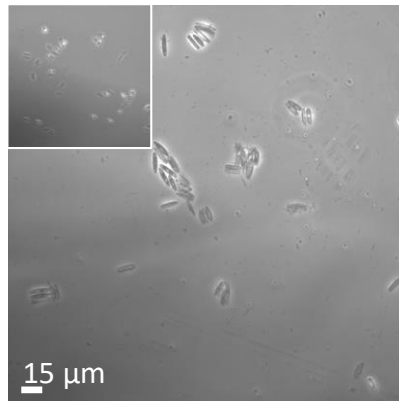
Chemical force microscopy of *Nitzschia palea*



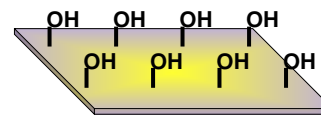
Adhesion of *Nitzschia palea*



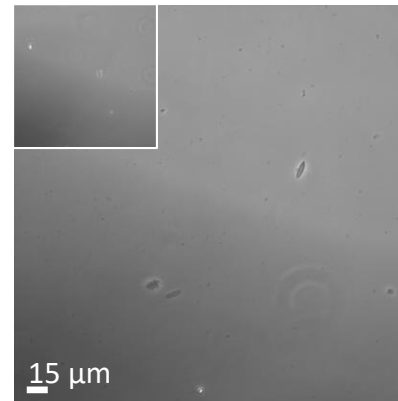
Hydrophobic



~720 cells/mm²



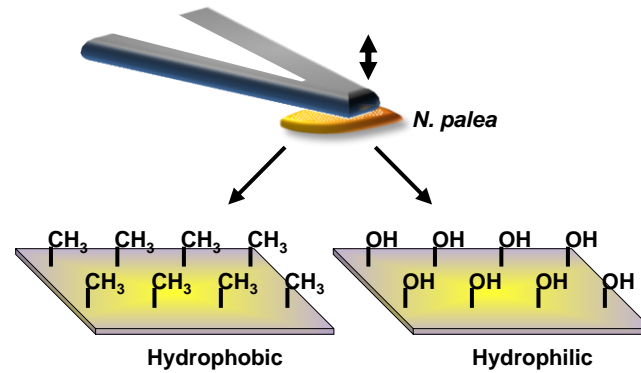
Hydrophilic



~24 cells/mm²

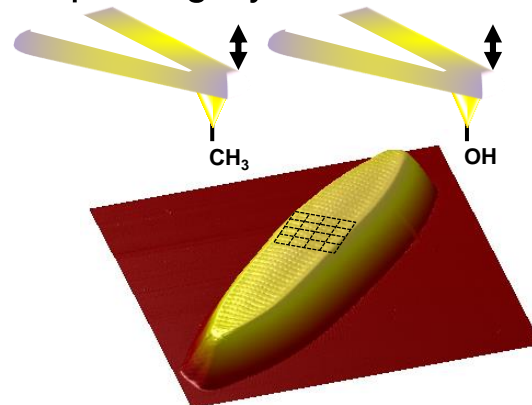
Adhesion of *Nitzschia palea*

> Single cell force spectroscopy

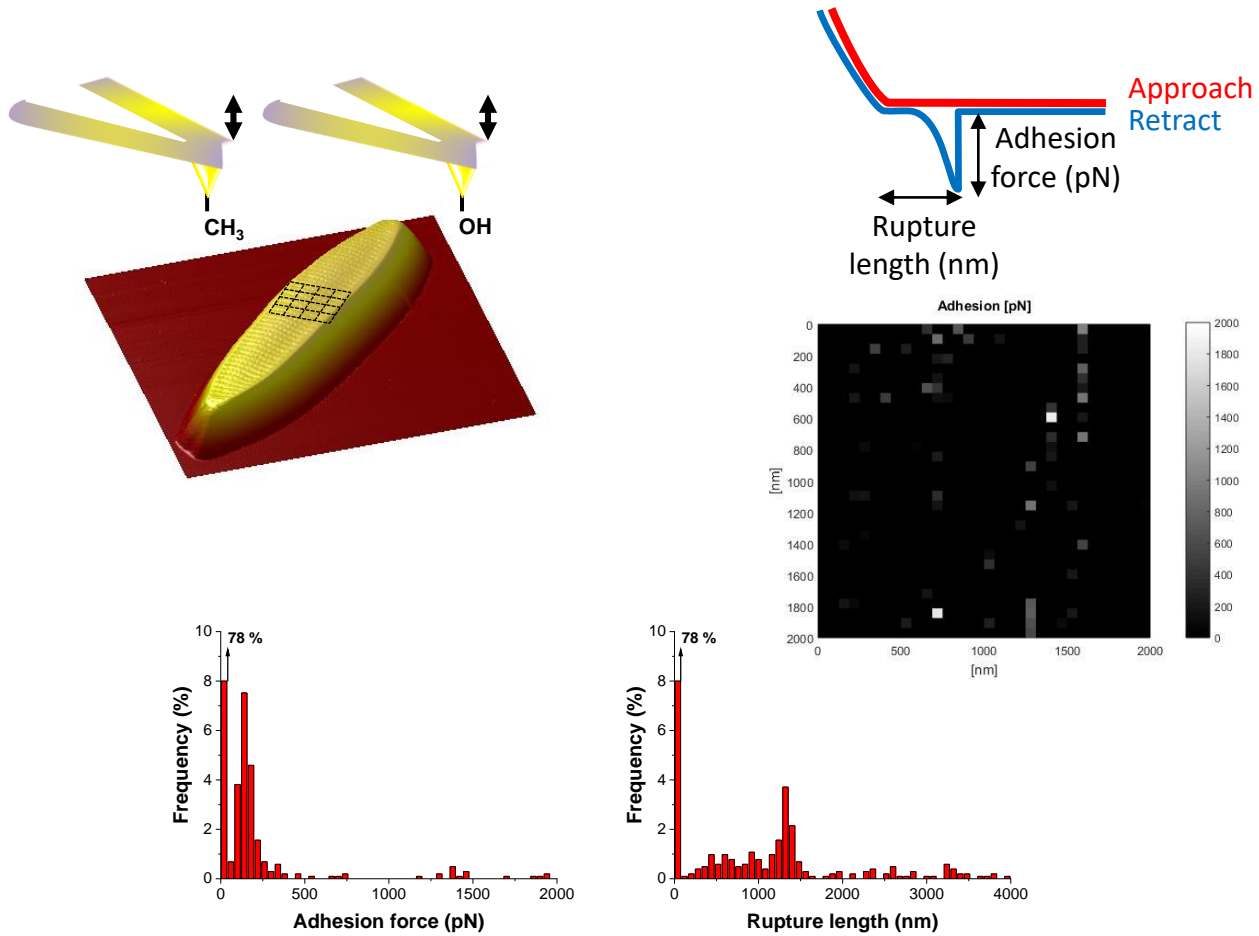


Other
workshop

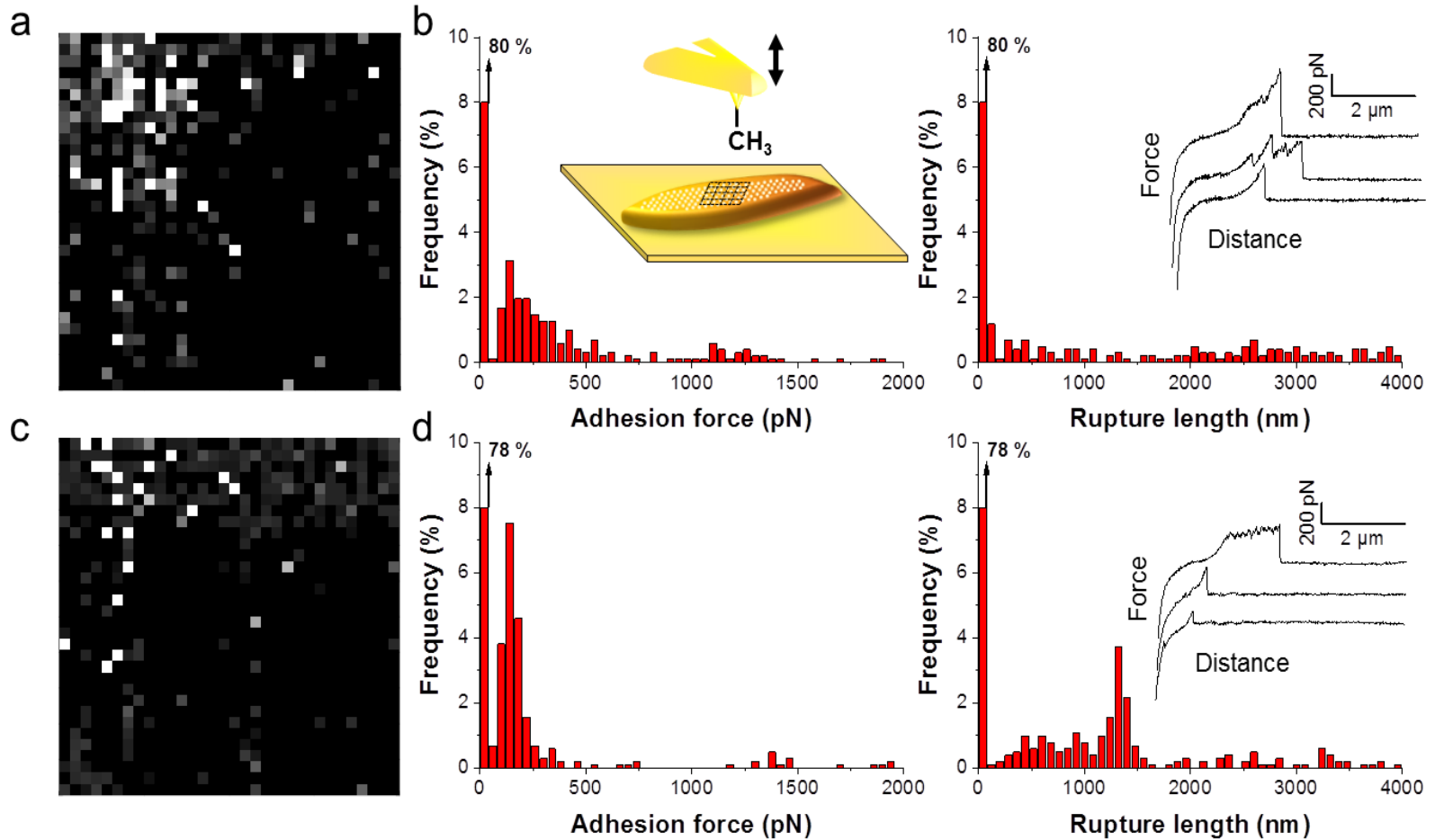
> Single molecule probing by chemical force spectroscopy



CFM of *Nitzschia palea*

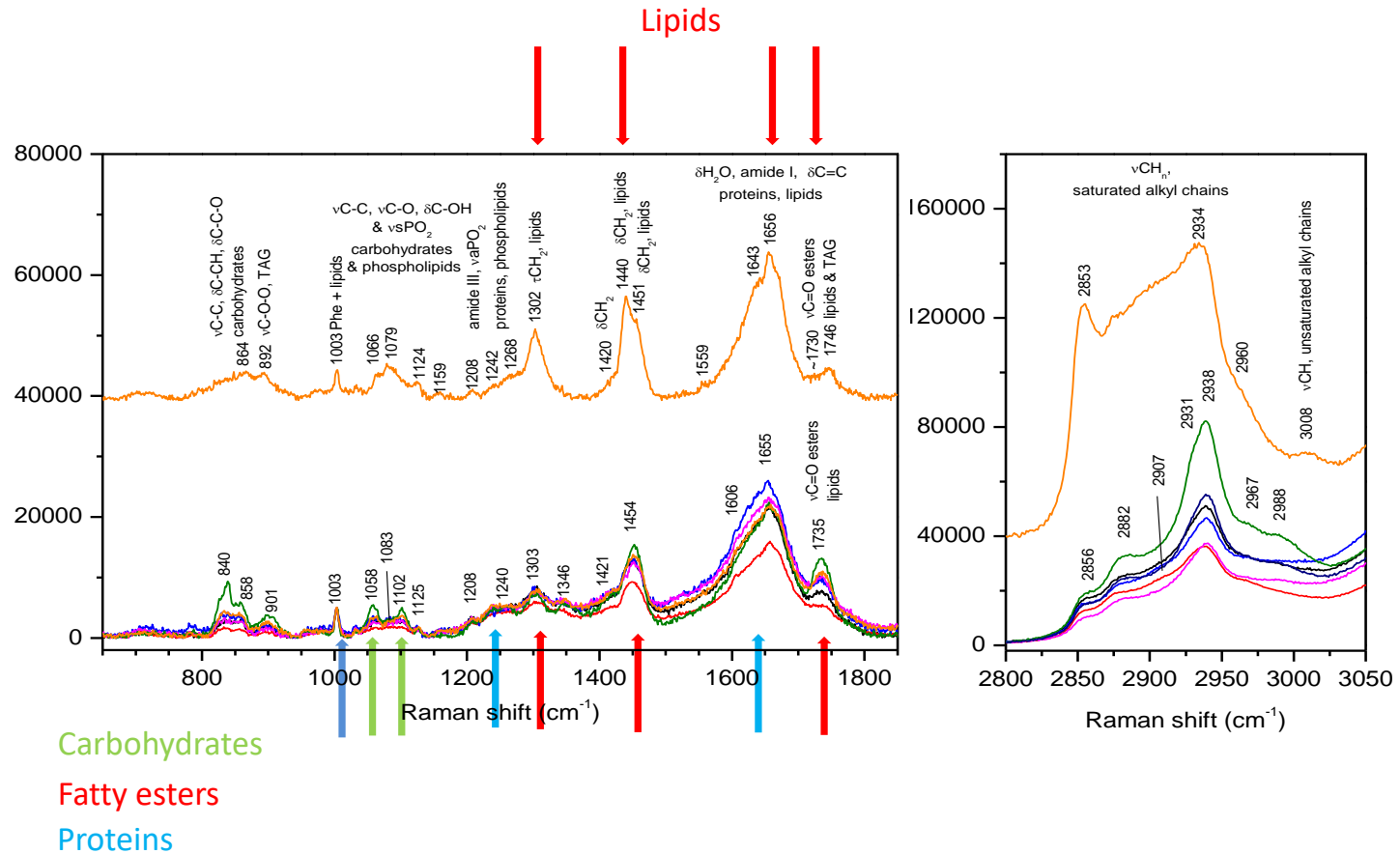


CFM of *Nitzschia palea*

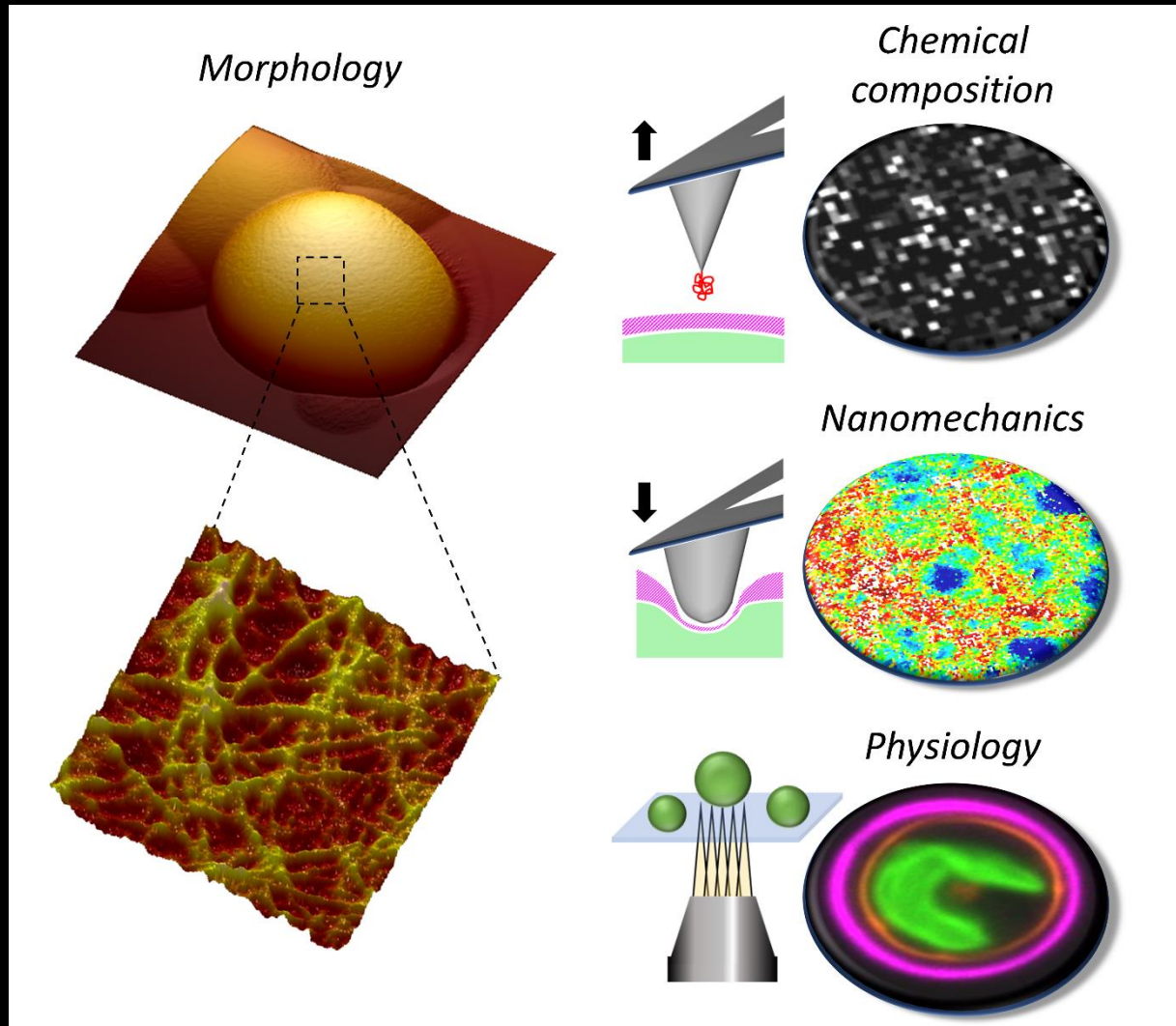


Raman of *Nitzschia palea*

(Glyco)-lipids are the major EPS of *N. palea*



Applications of AFM to green microalgae: *Chlorella vulgaris*



Applications of AFM to green microalgae: *Chlorella vulgaris*

