# Measurement technic Moravia

## Sales and servise of laboratory instruements

X-ray diffraction, elemental analysis

Surface analysis

Measurement of light and radiance

Systems for deposition and etchning

Spectral methods

Others

# Content

- Introduction
- History of AFM
- Technology background
- Contact mode
- Tapping mode

# Location


Our territory: Czech republic and Slovakia

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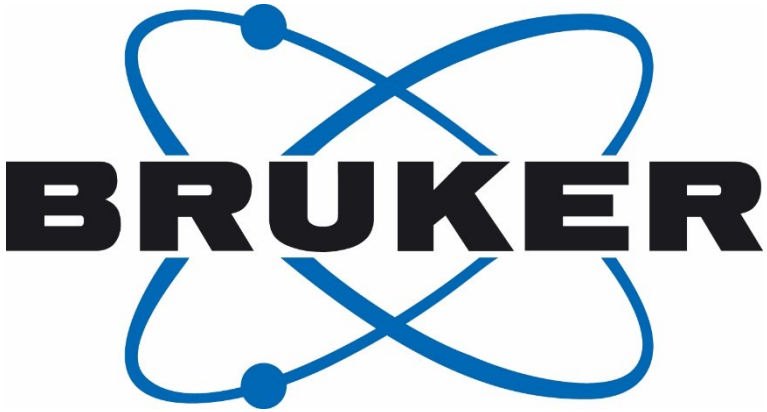
+420 513 034 409

ID: 293 16 715, VAT: CZ293 16 715

# Our partners











# Content

**There's Plenty of Room at the Bottom....**

**Tam dole je spousta místa**

LOGIN / REGISTER

## WELCOME ... At The Bottom

Welcome to the gallery of images created using the SPM method. SPM – Scanning Probe Microscopy – gives us a glimpse into the world of molecules, atoms, nanoparticles and the smallest tiny objects. Here you can see fascinating images from different fields of science (and art!) that show the beauty and complexity of the nanoworld.

You can also give a like to images based on what you like or has some scientific value. It's a gallery for anyone interested in exploring and sharing. We welcome every nice picture and every user. We wish you a pleasant browsing and discovering experience!

### How does it work...

- Create simply an account and upload your AFM images
- Collect likes
- The image with the most likes will be selected for our year 2024 calendar
- You can send image to category ART, if you think that it is beautiful or to the category SCIENCE, if it has some scientific value. Or you can send it to the both categories, because science can be beautiful too, can't it?
- This year, in October, we will stop the Contest and we will make Calendar from the best images we will get. Do you want one? Let us know!

# History AFM

1981 – Scanning tunneling microscopy

1986 – Nobel price STM (Binning, Rohrer)

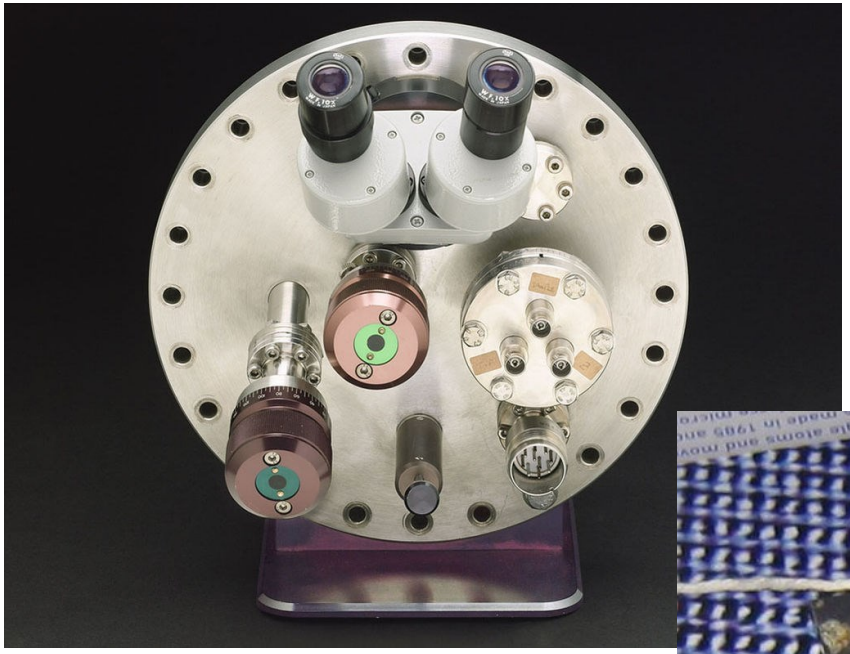
1986 – AFM

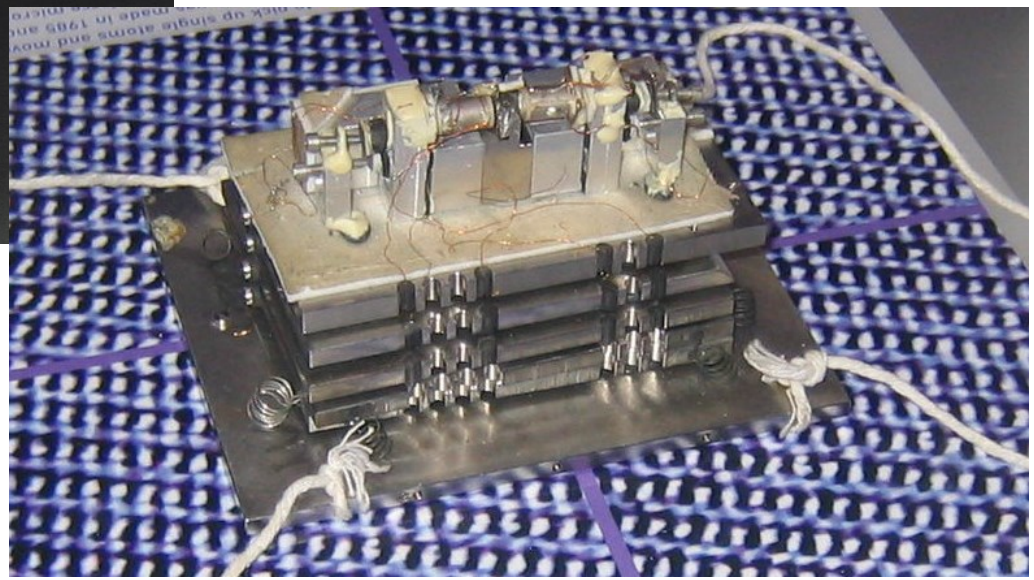
1989 – First commercial AFM

1992 – Tapping mode, measuring in fluids

2010 – PeakForce tapping

# First STM a AFM

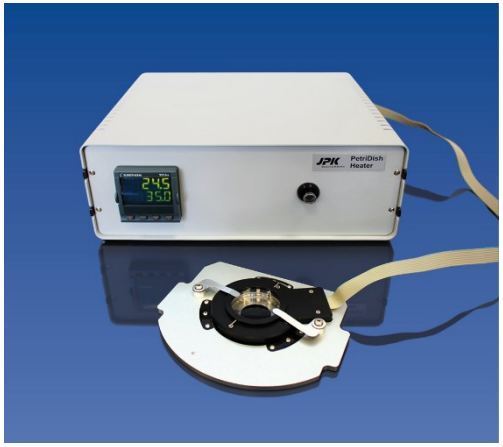


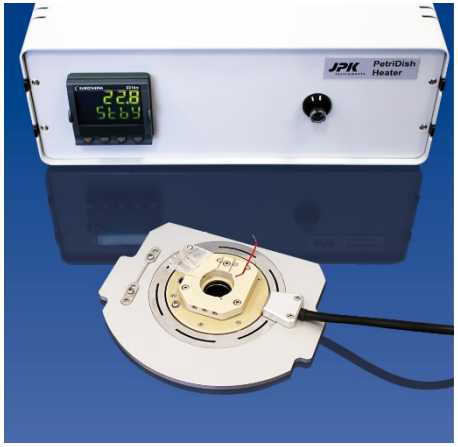


# Nowadays AFM

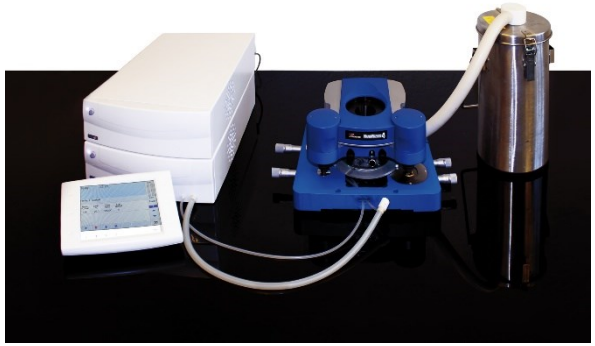

# Nowadays AFM

# Accesories


JPK
PetriDish
Heater
24.5
35.0

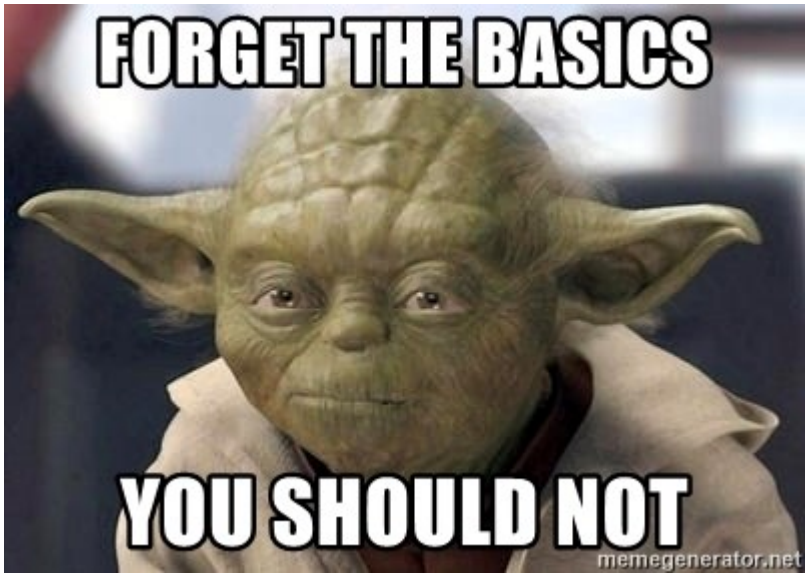

JPK
PetriDish
Heater
22.8
Set 37


JPK
BioCell
23.0
37.0

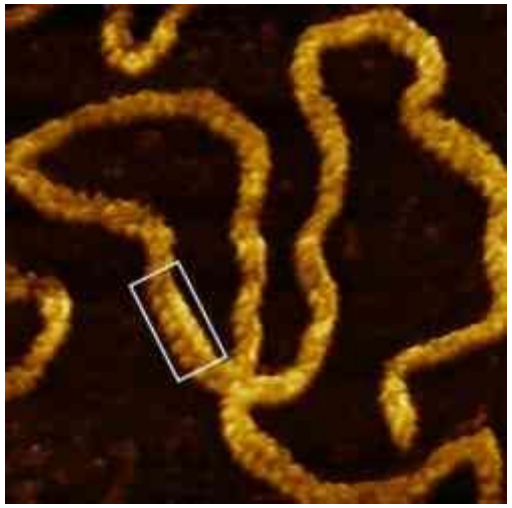


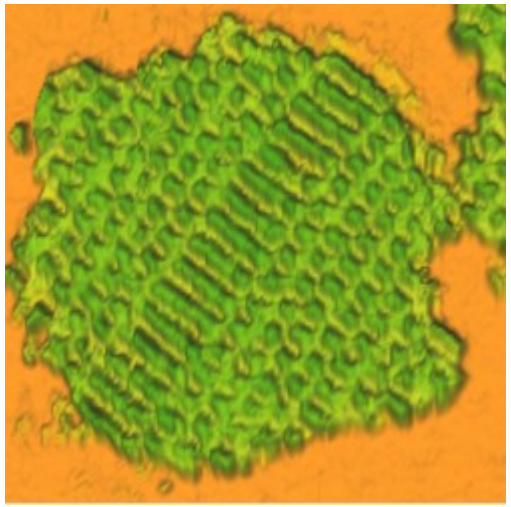
# Basics

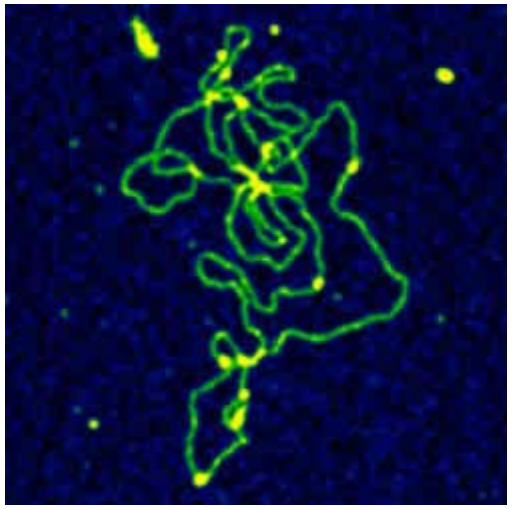
- What do we measure?
- How big is our scale?
- How is it possible?

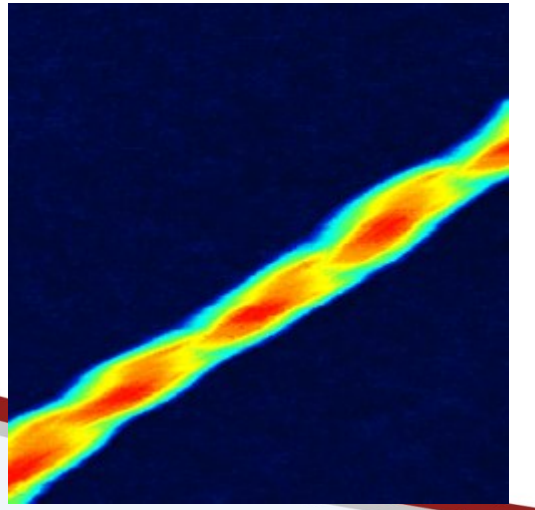


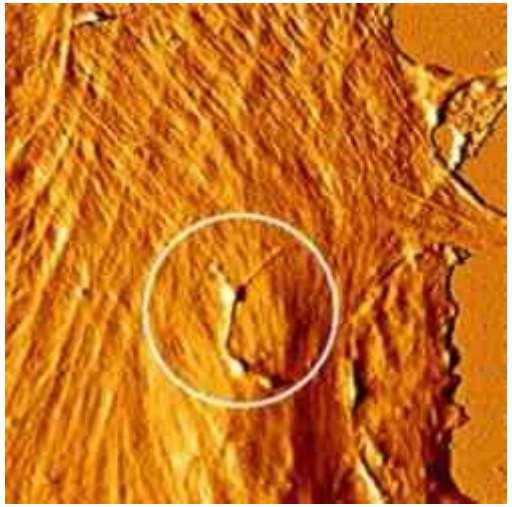
# What do we measure?



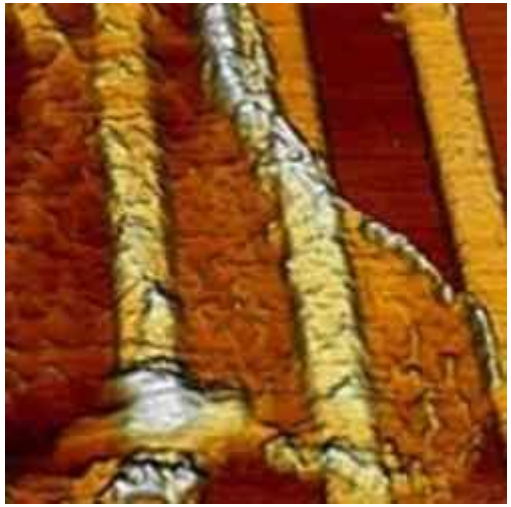
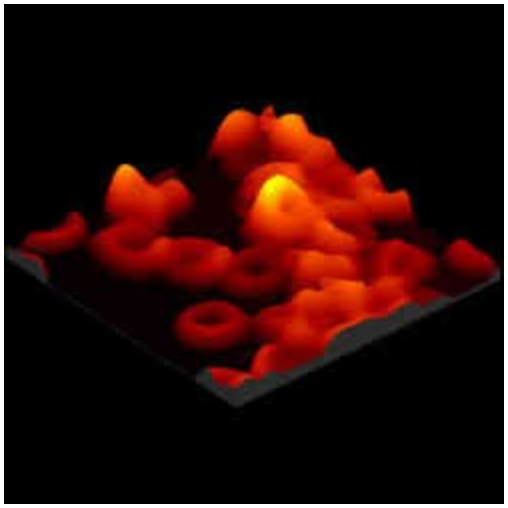
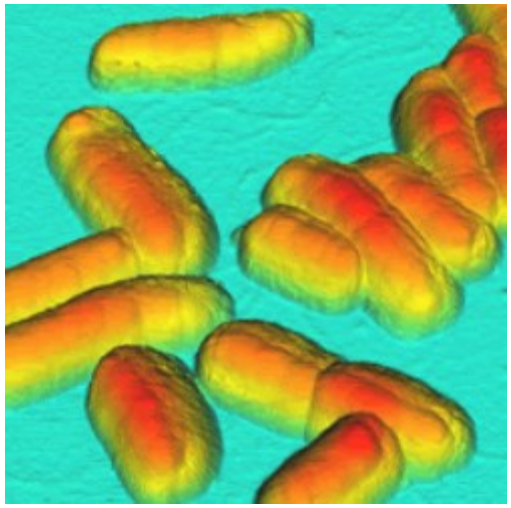
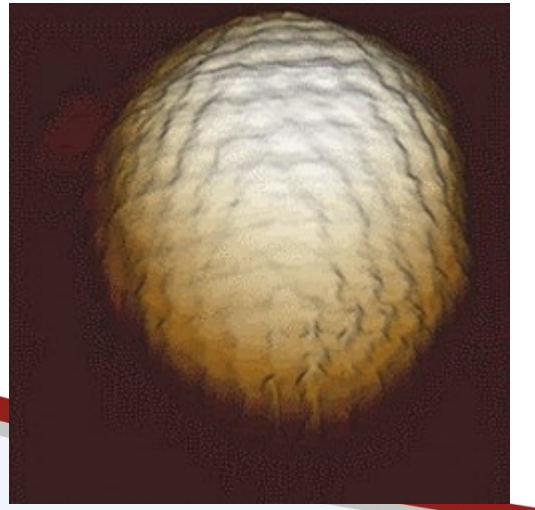
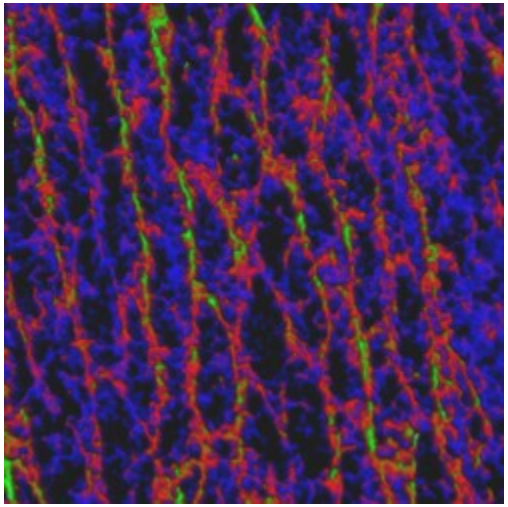
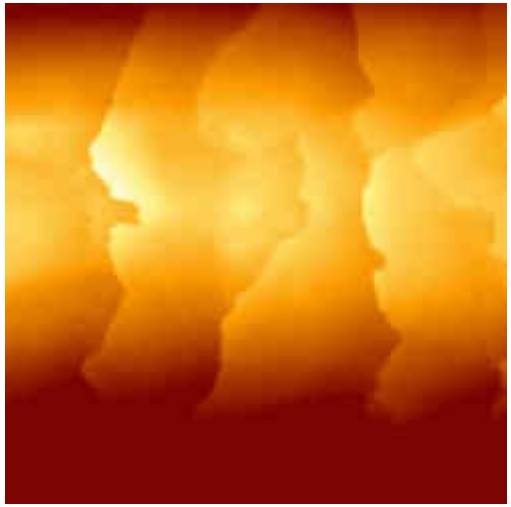




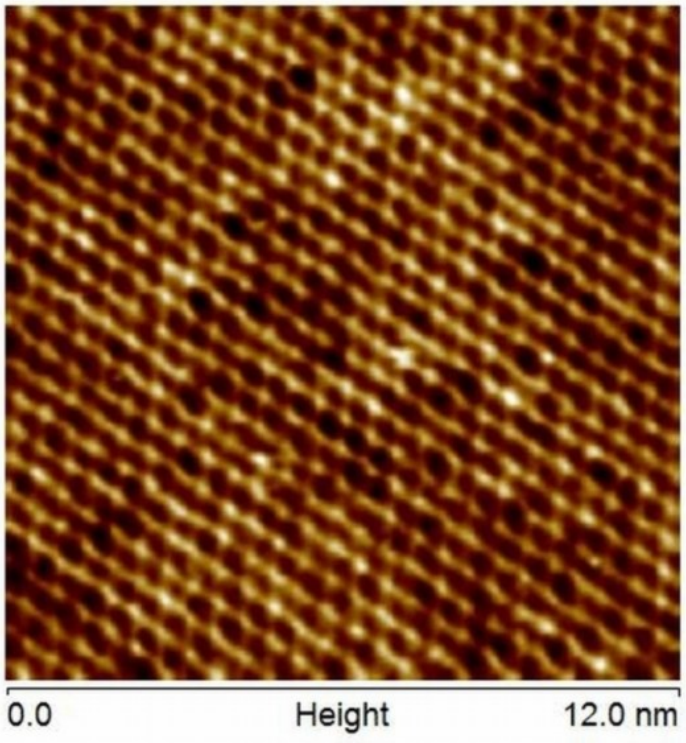




# What do we measure?

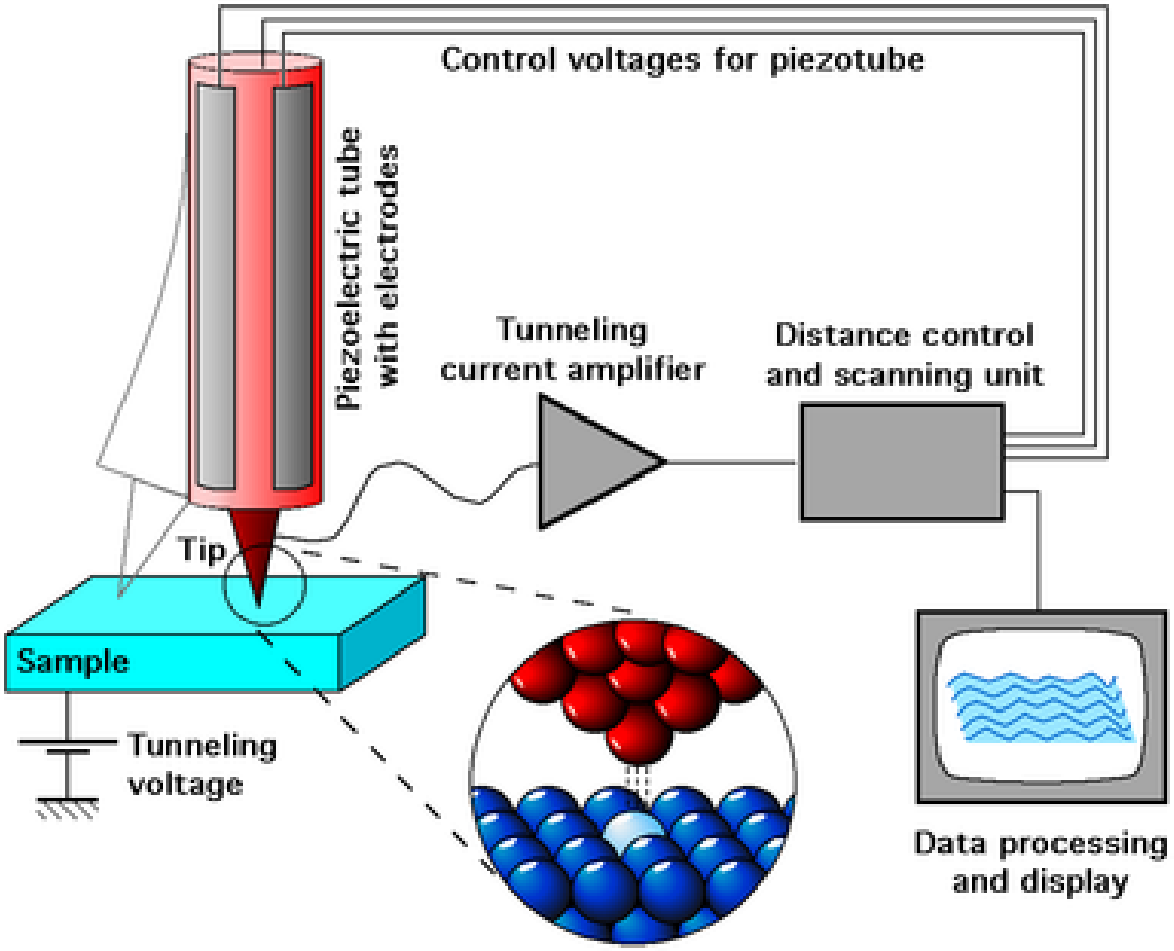







# How big is our scale

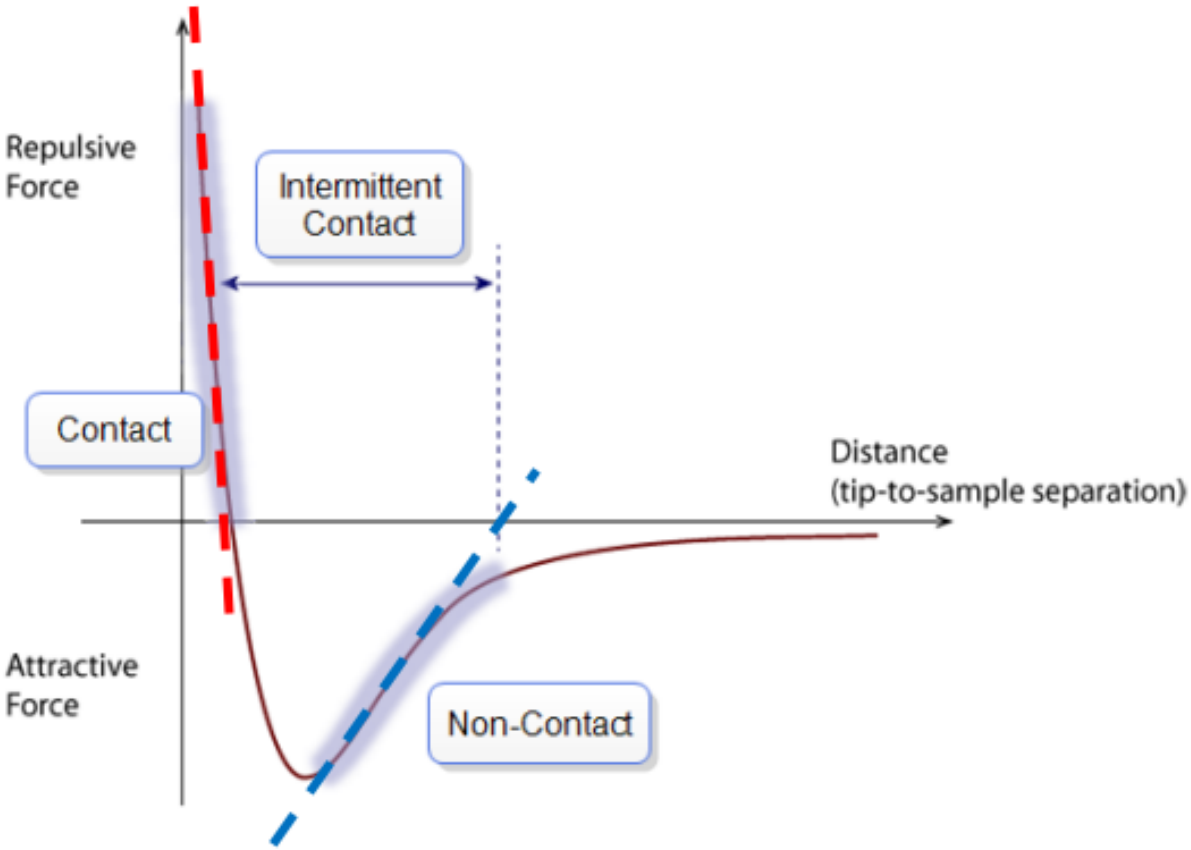


# How is it possible?

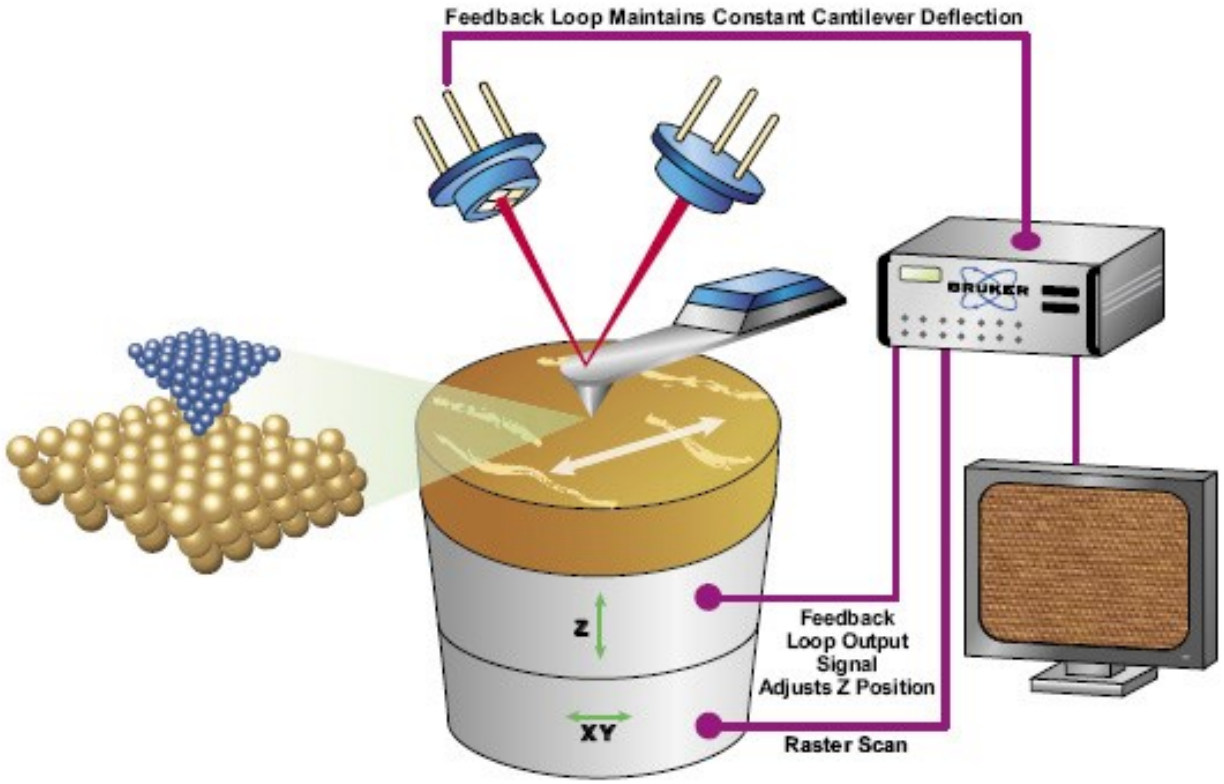
# STM


Control voltages for piezotube
Piezoelectric tube with electrodes
Tunneling current amplifier
Distance control and scanning unit
Tip
Sample
Tunneling voltage
Data processing and display

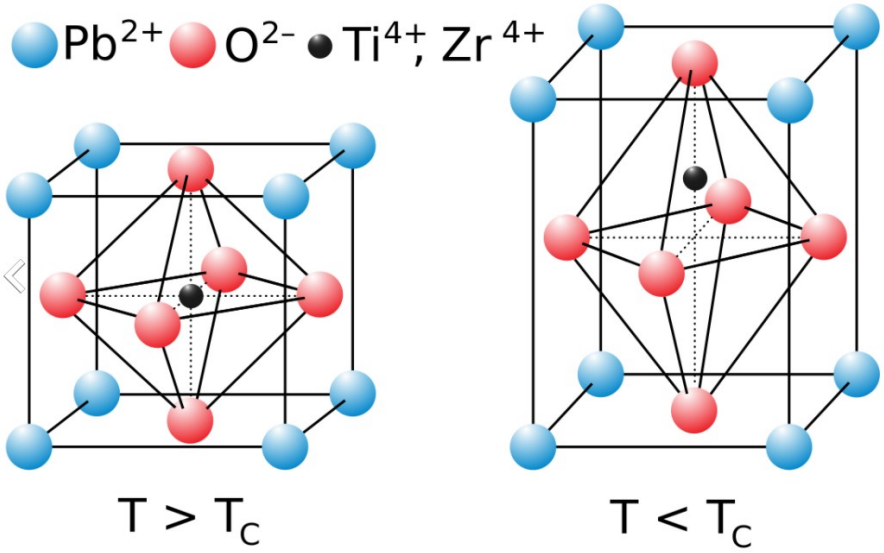
# Attractive and repulsive forces

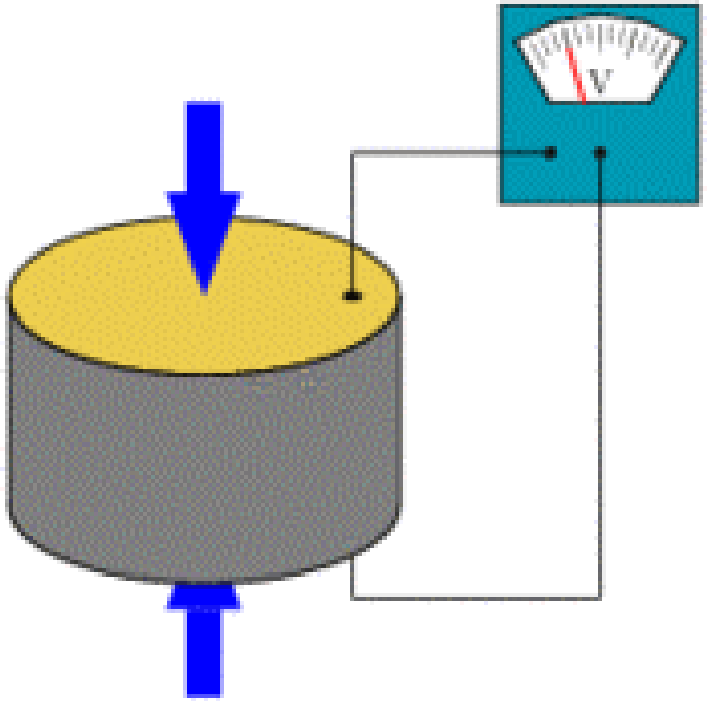


# AFM

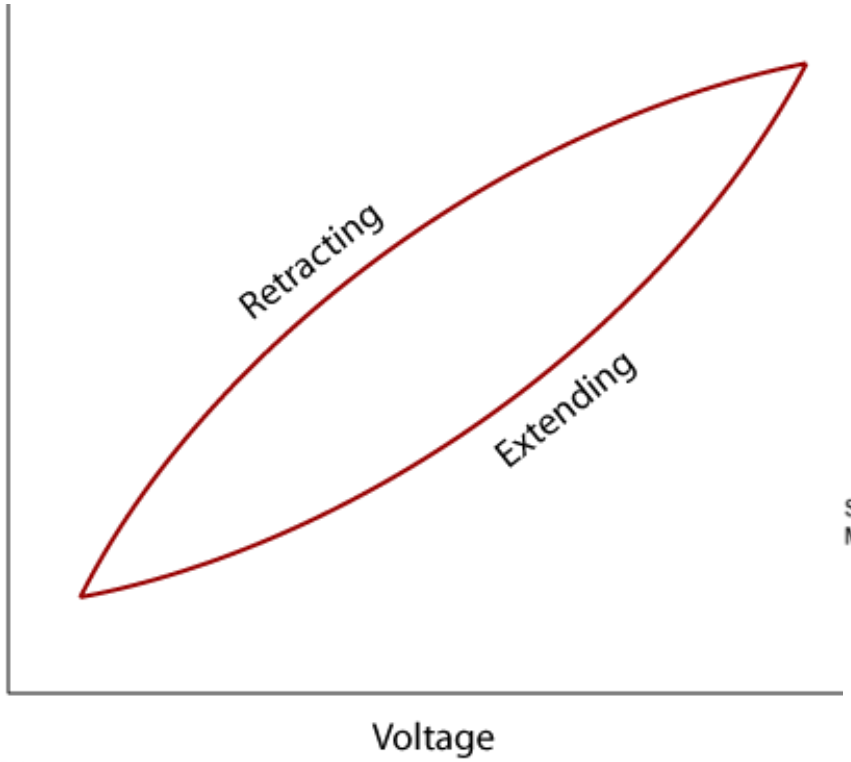

Feedback Loop Maintains Constant Cantilever Deflection
BRUKER
Z
XY
Feedback
Loop Output
Signal
Adjusts Z Position
Raster Scan

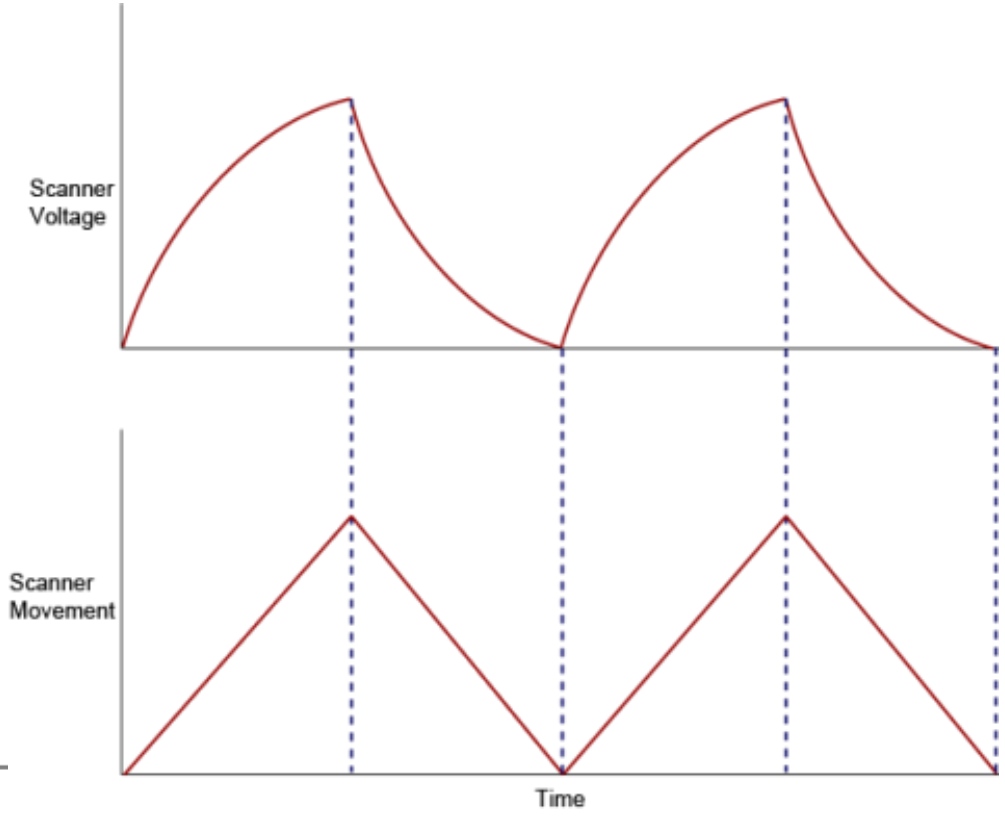
# Piezo elements



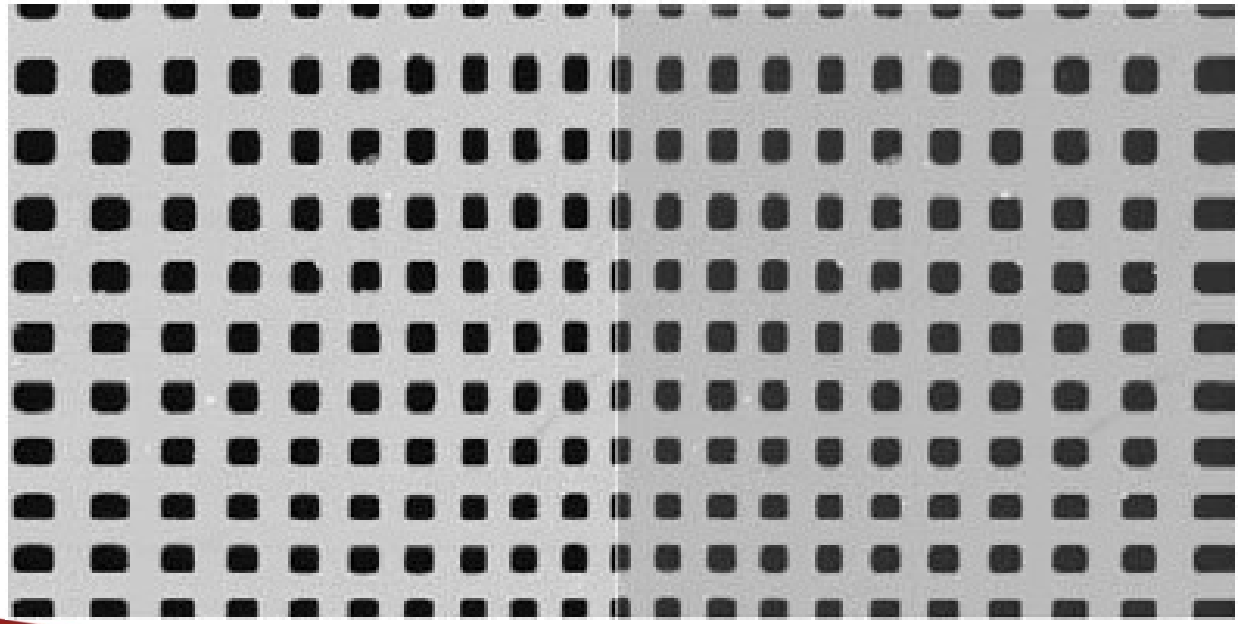
# Open loop x closed loop



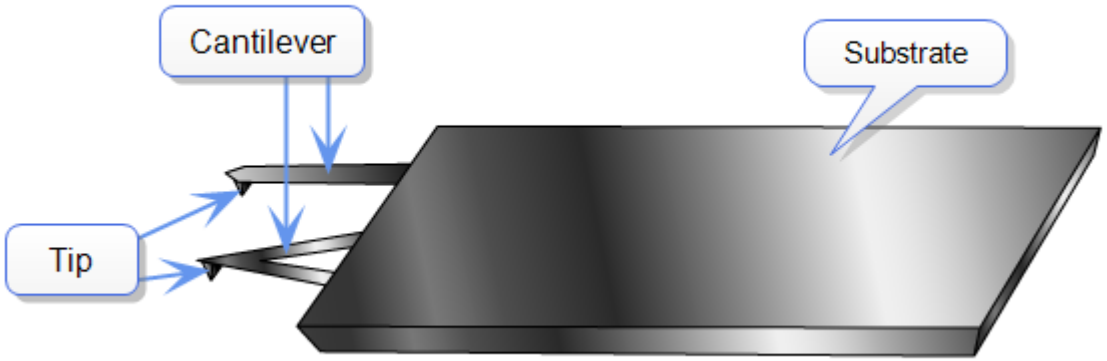
# Open loop x closed loop

- **Closed loop – Sensor detect the actual position and correct the nonlinearity**
- **Open loop - Calibration routine determines the nonlinearity movement of piezo**

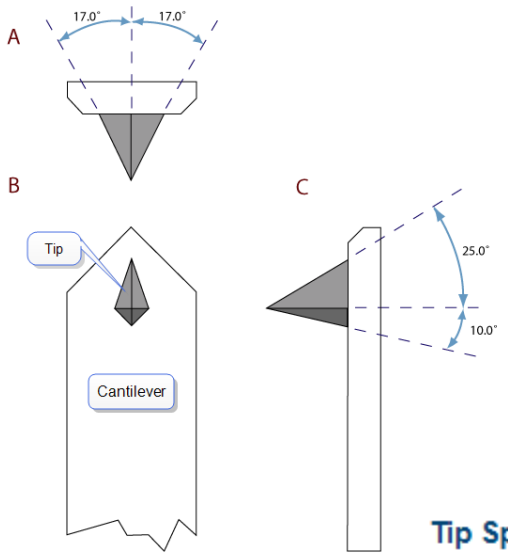


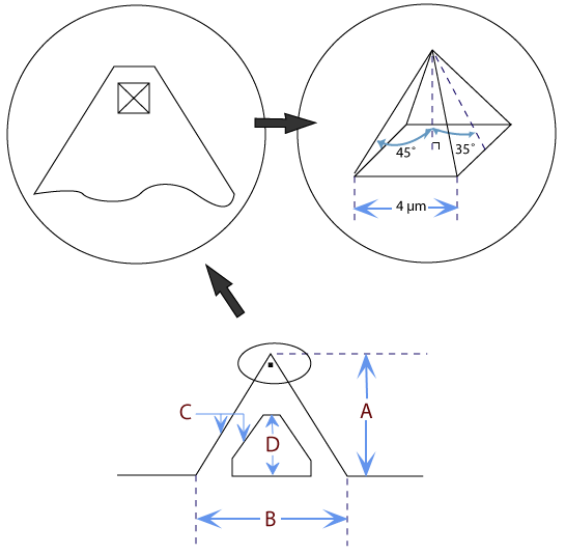
# Probes

- Si or $Si_3N_4$
- 2 main geometries
- Surface treatment, doping …

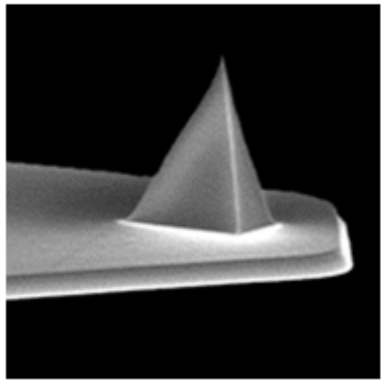


# Probes



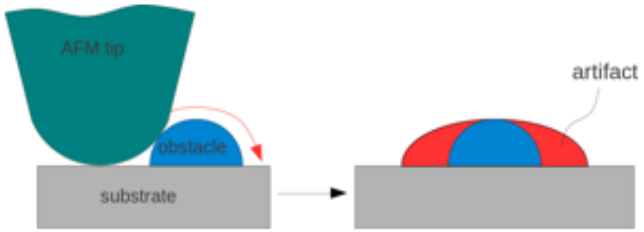




## Tip Specification

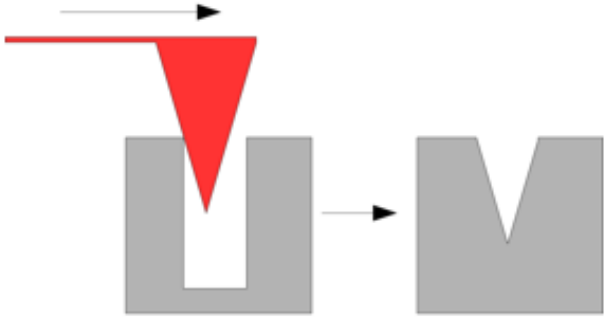


| | |
|---|---|
| Geometry: | Rotated (Symmetric) |
| Tip Height (h): | 2.5 - 8.0µm |
| Front Angle (FA): | 15 ± 2.5° |
| Back Angle (BA): | 25 ± 2.5 ° |
| Side Angle (SA): | 22.5 ± 2.5 ° |
| Tip Radius (Nom): | 2 nm |
| Tip Radius (Max): | 12 nm |
| Tip SetBack (TSB)(Nom): | 4 µm |
| Tip Set Back (TSB)(RNG): | 0 - 7 µm |

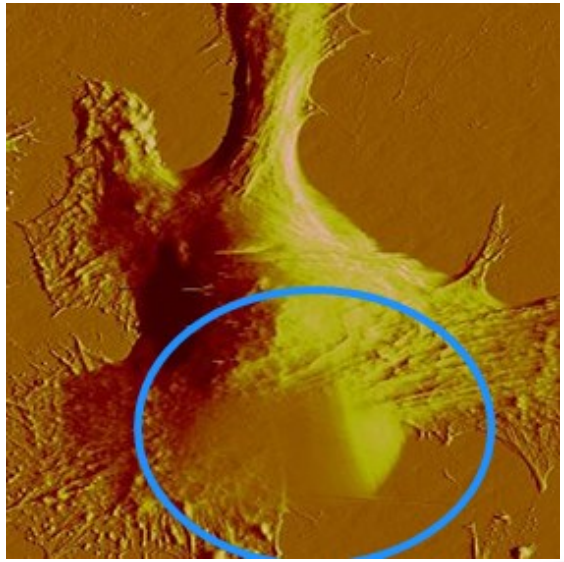
# Probes



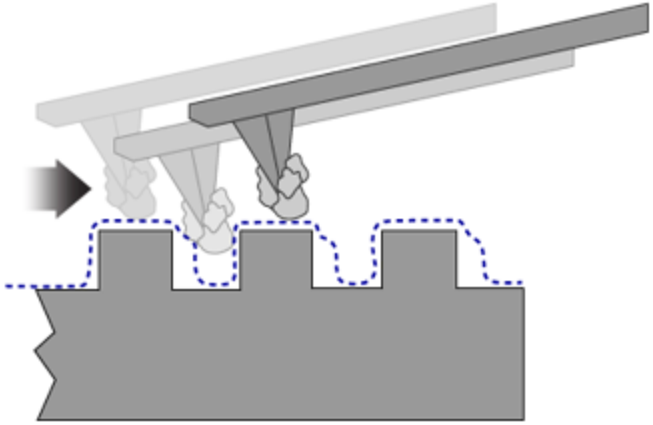

Tip radius

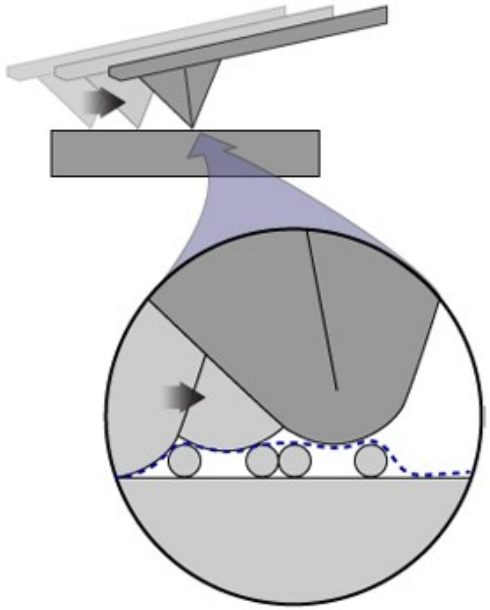


Tip half angle



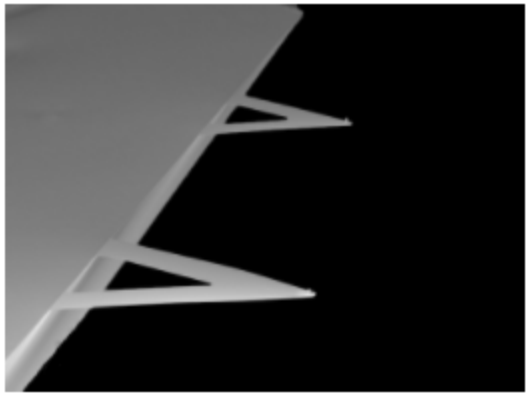
# Probes





# Probes

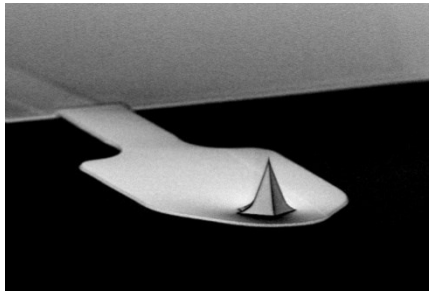
## Cantilever Specification

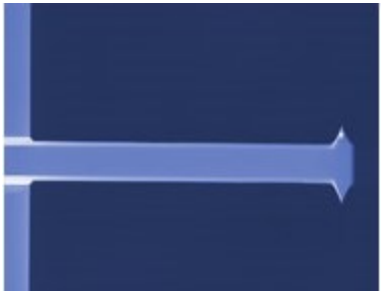


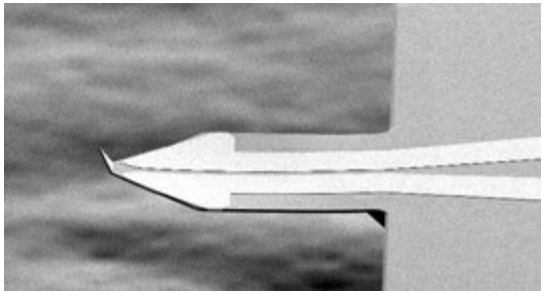
| | |
|---|---|
| **Material:** | Silicon Nitride |
| **Geometry:** | Triangular |
| **Cantilevers Number:** | 4 |
| **Cantilever Thickness (Nom):** | 0.6 µm |
| **Cantilever Thickness (RNG):** | 0.55 - 0.65 µm |
| **Back Side Coating:** | Reflective Gold |
| **Top Layer Back:** | 45 ± 5 nm of Ti/Au |

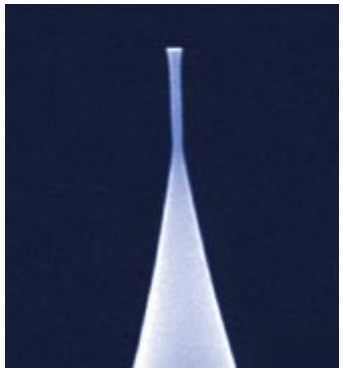
| Shape | Resonant Freq. kHz | | | Spring Const. N/m | | | Length µm | | | Width µm | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nom. | Min. | Max. | Nom. | Min. | Max. | Nom. | Min. | Max. | Nom. | Min. | Max. |
| A Triangular | 65 | 50 | 80 | 0.35 | 0.175 | 0.7 | 120 | 115 | 125 | 25 | 20 | 30 |
| B Triangular | 23 | 16 | 28 | 0.12 | 0.06 | 0.24 | 205 | 200 | 210 | 40 | 35 | 45 |
| C Triangular | 56 | 40 | 75 | 0.24 | 0.12 | 0.48 | 120 | 115 | 125 | 20 | 15 | 25 |
| D Triangular | 18 | 12 | 24 | 0.06 | 0.03 | 0.12 | 205 | 200 | 210 | 25 | 20 | 30 |

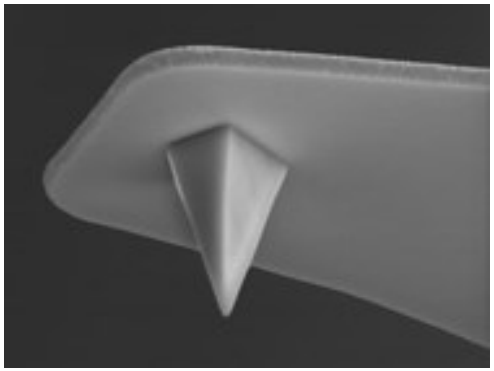
# Probes

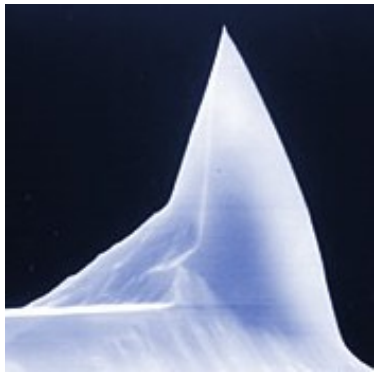








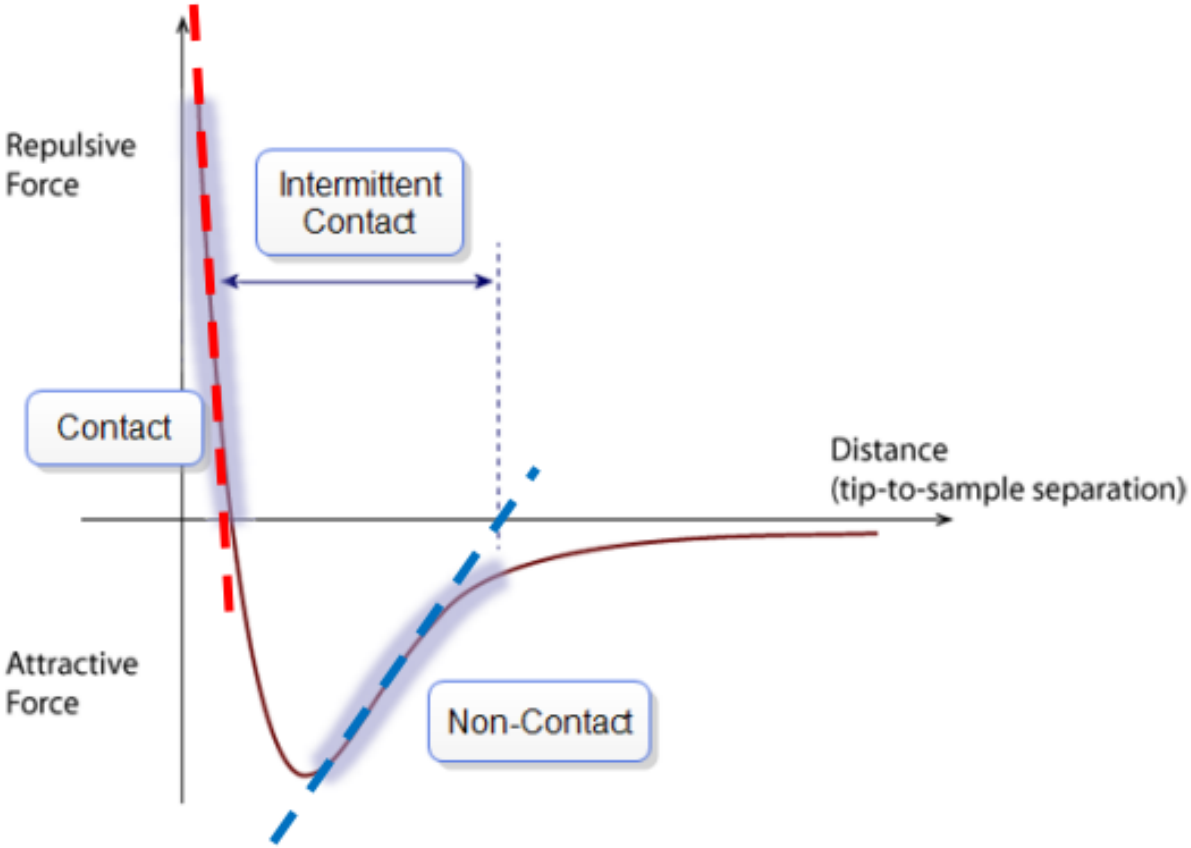




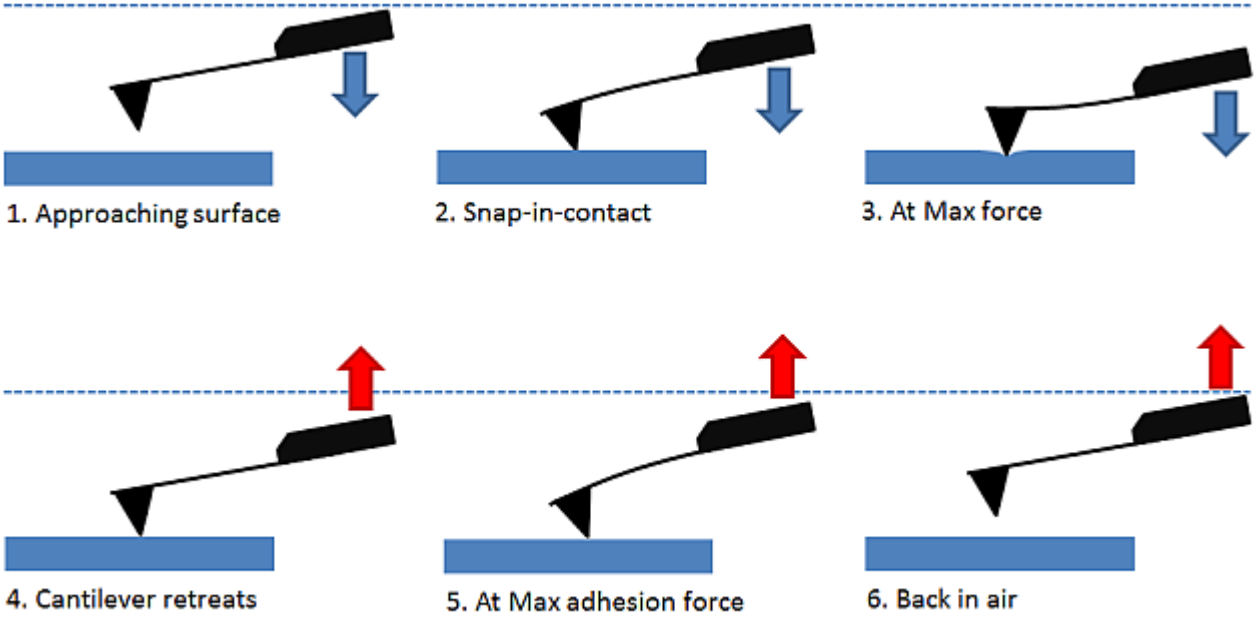
# Selection of probes

- Working mode?
- Cantilever stiffness?
- Tip radius?
- Electric, magnetic or other properties?
- Price?

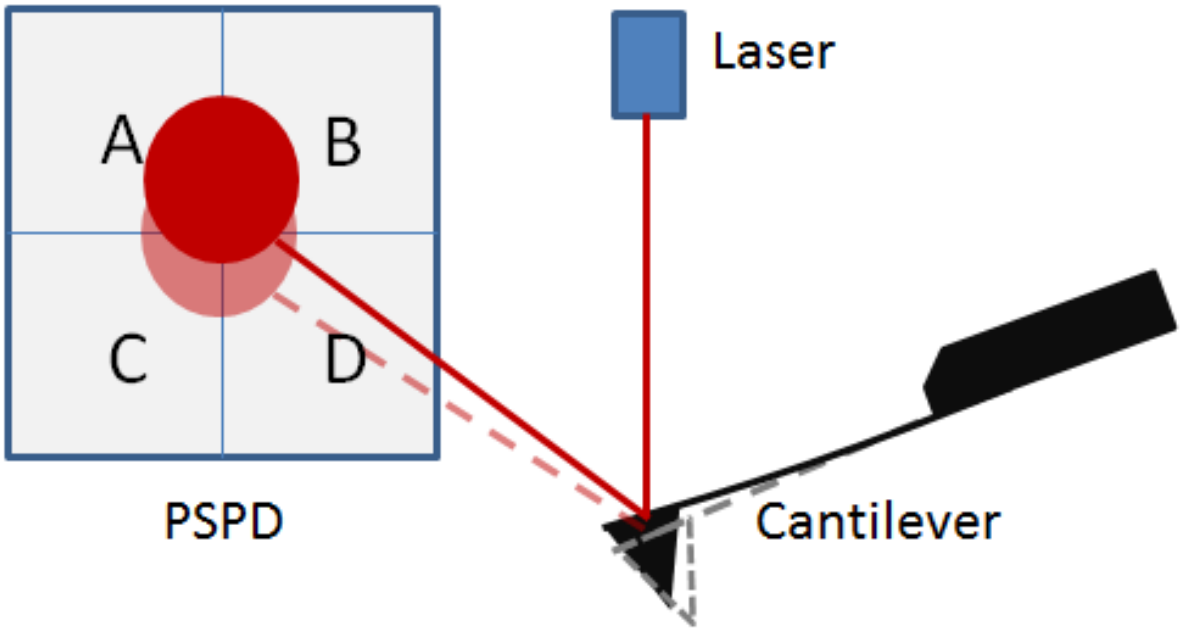
# Engage


Repulsive
Force
Intermittent
Contact
Contact
Distance
(tip-to-sample separation)
Attractive
Force
Non-Contact

# Engage

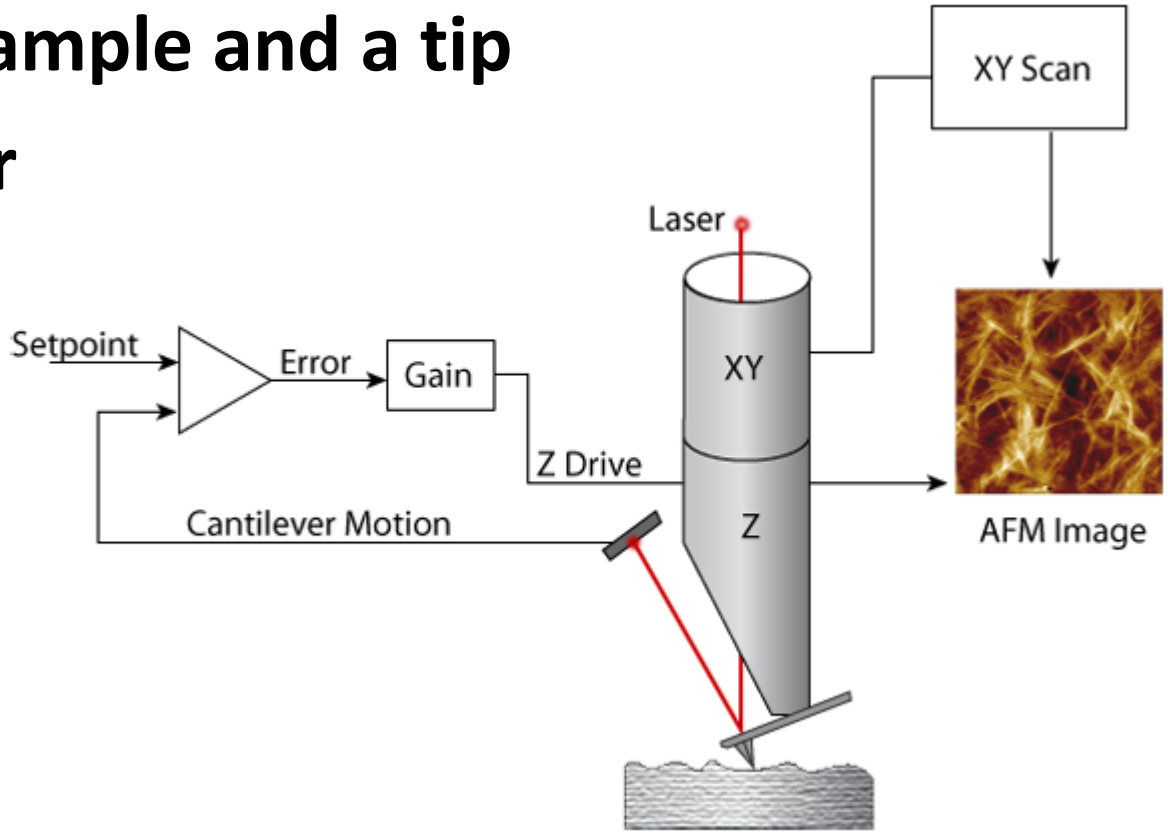

1. Approaching surface
2. Snap-in-contact
3. At Max force
4. Cantilever retreats
5. At Max adhesion force
6. Back in air

# Engage



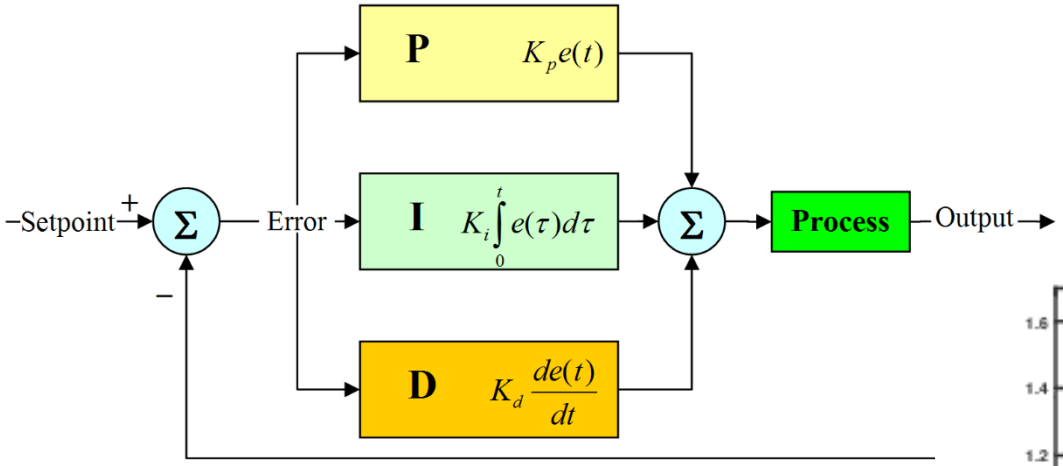
# Feedback

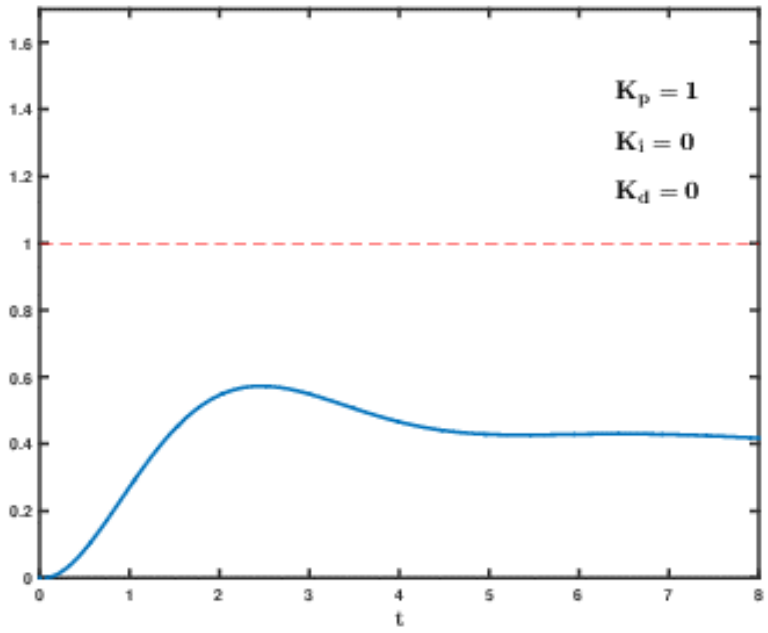
- **Goal is to keep the same distance between a sample and a tip**
- **PID regulator**



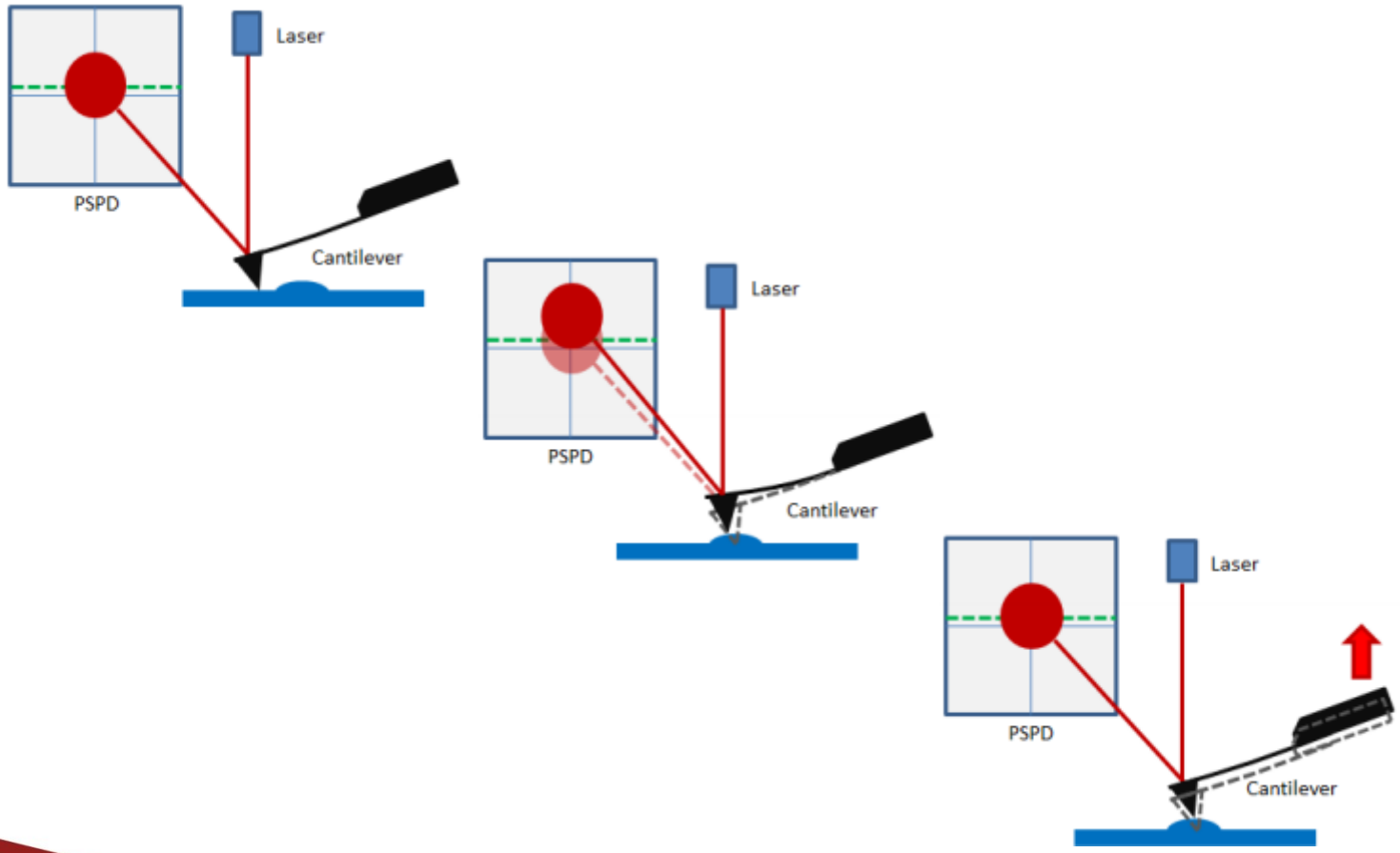
# Feedback

## PID regulátor

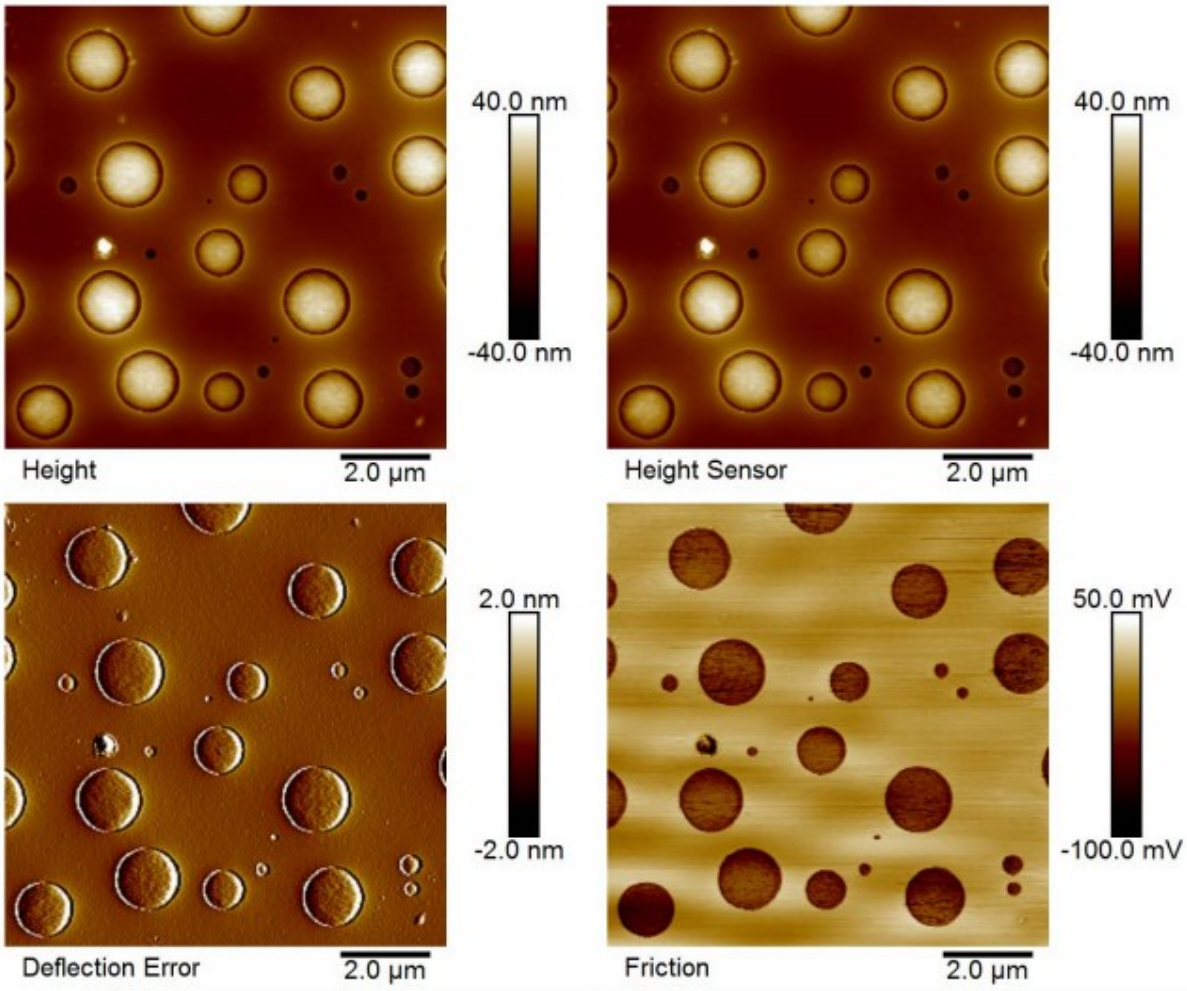


# Contact mode



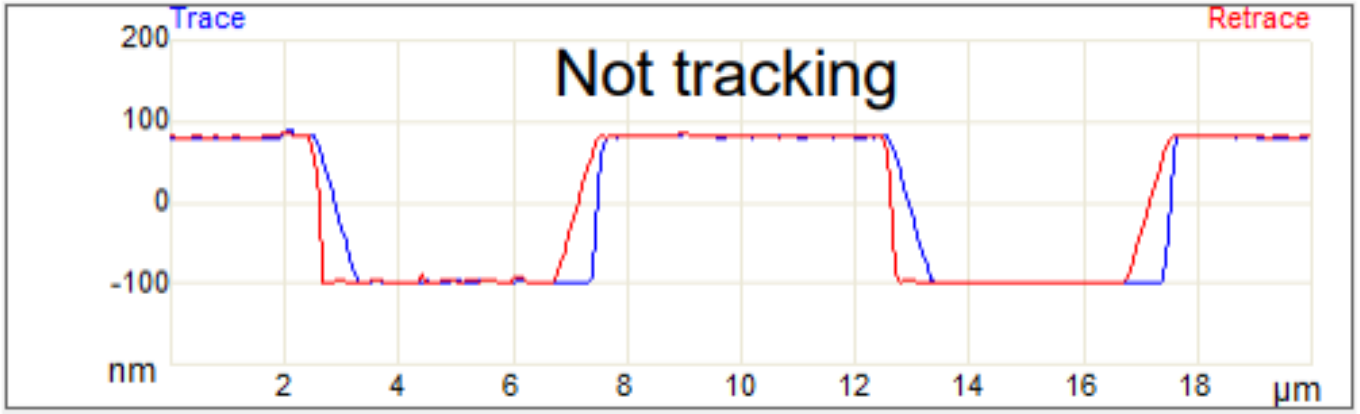
# Signals

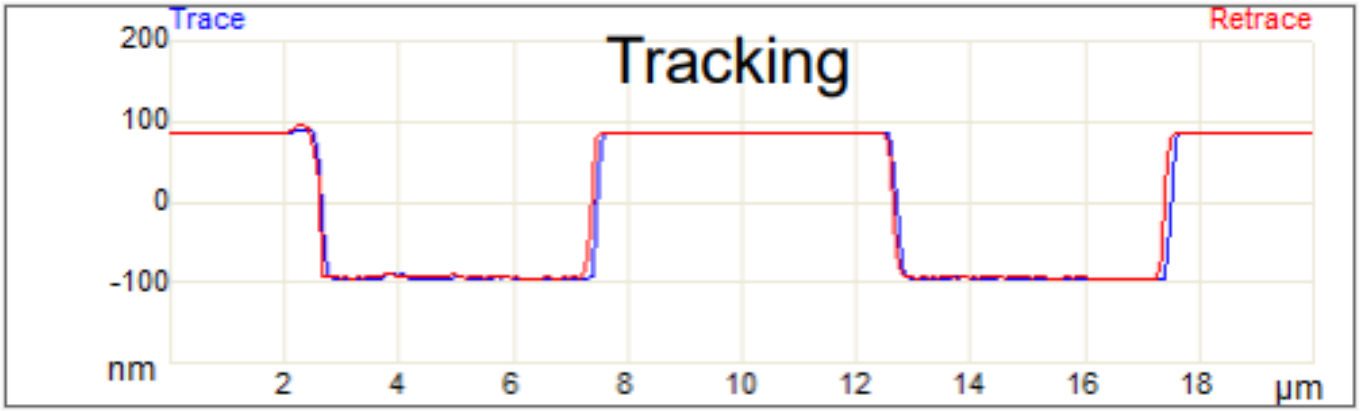

40.0 nm
-40.0 nm
Height
2.0 µm
40.0 nm
-40.0 nm
Height Sensor
2.0 µm
2.0 nm
-2.0 nm
Deflection Error
2.0 µm
50.0 mV
-100.0 mV
Friction
2.0 µm

# Parameters

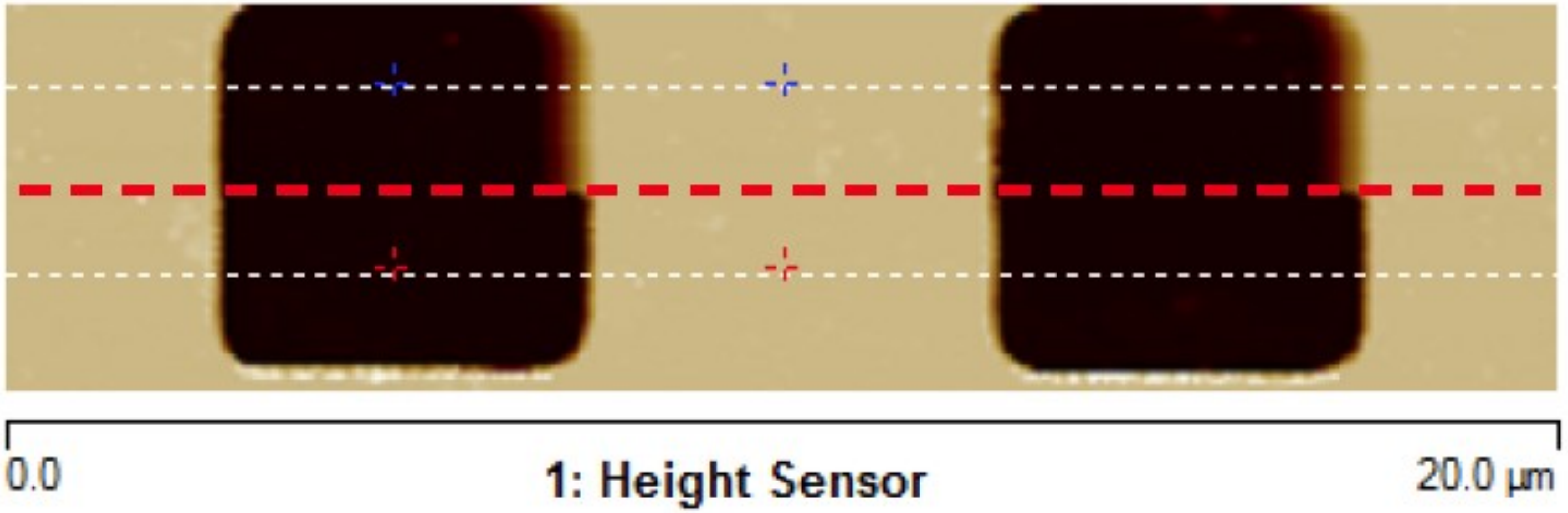
| | |
|---|---|
| ⊟ Scan | |
| ├ Scan Size | 10.0 µm |
| ├ Aspect Ratio | 1.00 |
| ├ X Offset | 0.000 nm |
| ├ Y Offset | 0.000 nm |
| ├ Scan Angle | 90.0 ° |
| ├ Scan Rate | 1.00 Hz |
| └ Samples/Line | 256 |
| ⊟ Feedback | |
| ├ Integral Gain | 20.00 |
| ├ Proportional Gain | 20.00 |
| └ Deflection Setpoint | 1.000 V |
| ⊟ Limits | |
| ├ Z Range | 10.9 µm |
| ├ Reduced Z Delay | 2.00 s |
| └ Z Auto Center Boundary | 0 % |

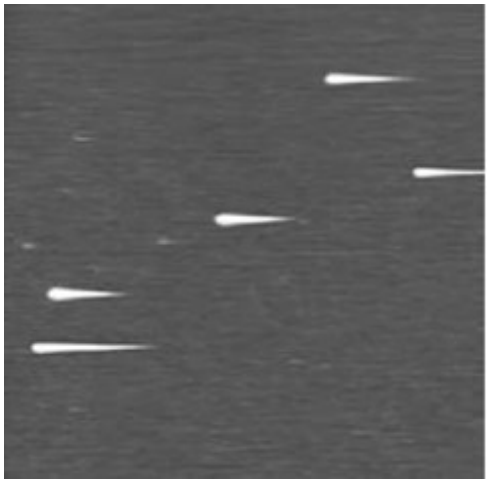
# Setting the parameters



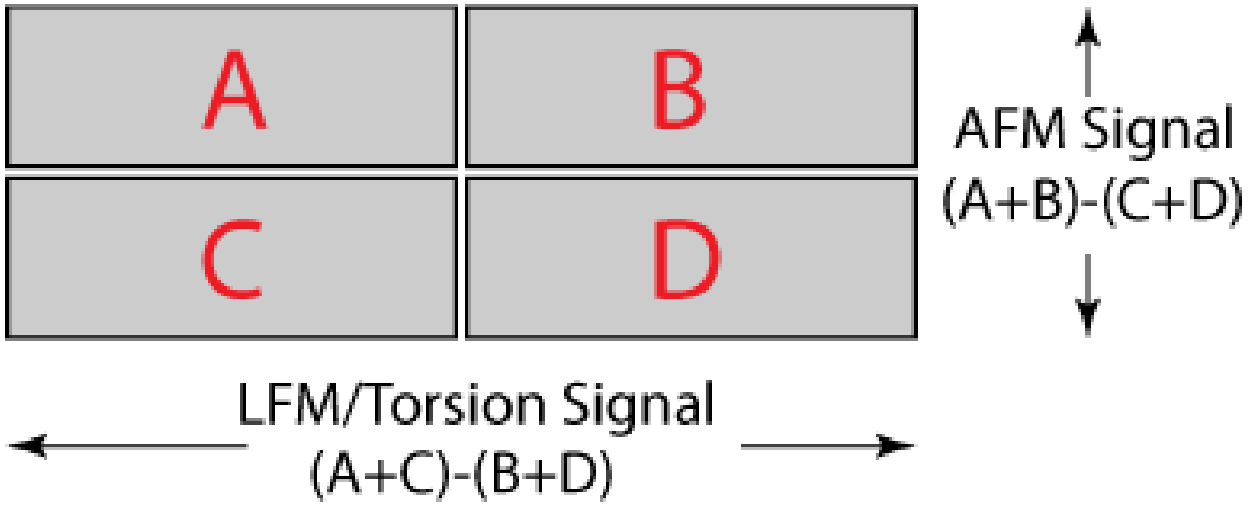



# Setting the parameters

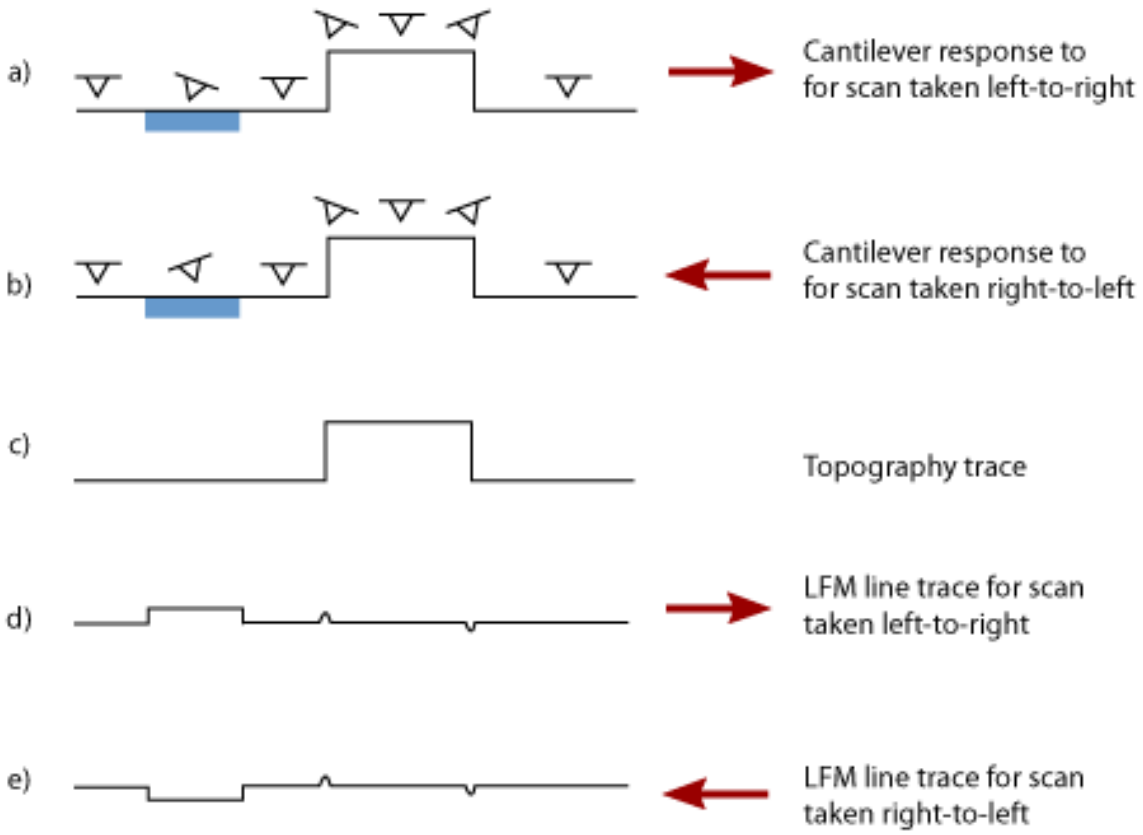


# Microscopy of lateral forces



# Microscopy of lateral forces

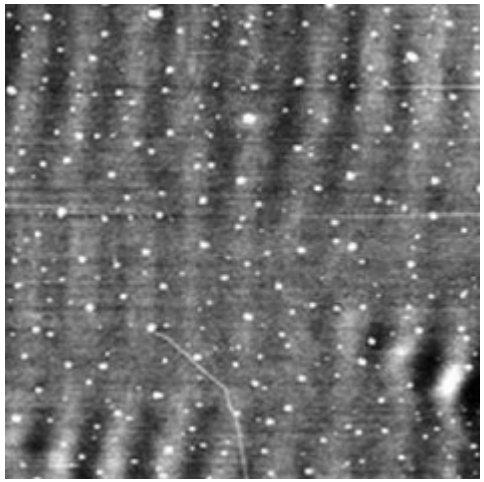


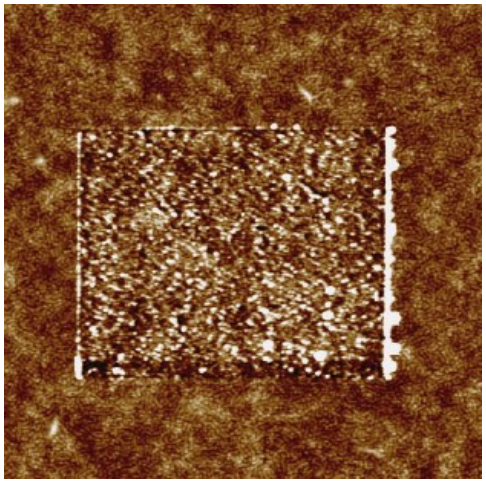
# Benefits of contact

- Easy measurement
- Direct force control
- Microscopy of lateral forces
- High speed
- Tip in contact with sample (good for electric measurement)

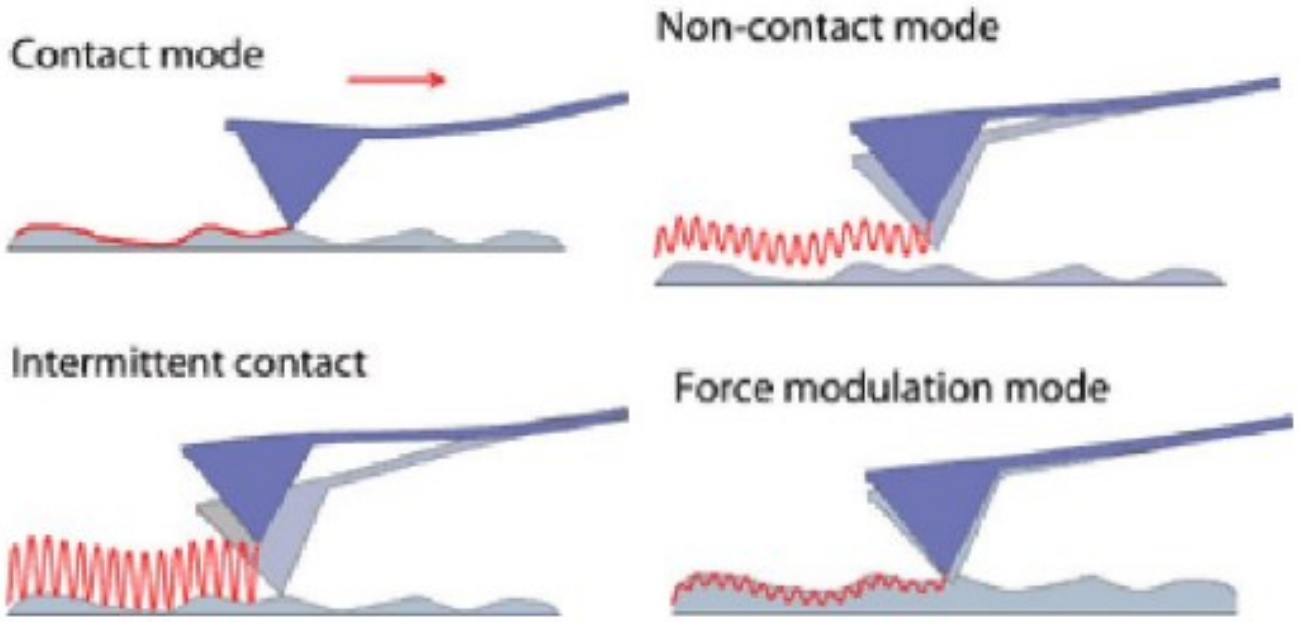
# Disadvantages of contact mode

- Tip in contact with sample
  - Destruction of a sample
  - Higher wear of a tip
- Sensitive for optical interference and drift of laser signal

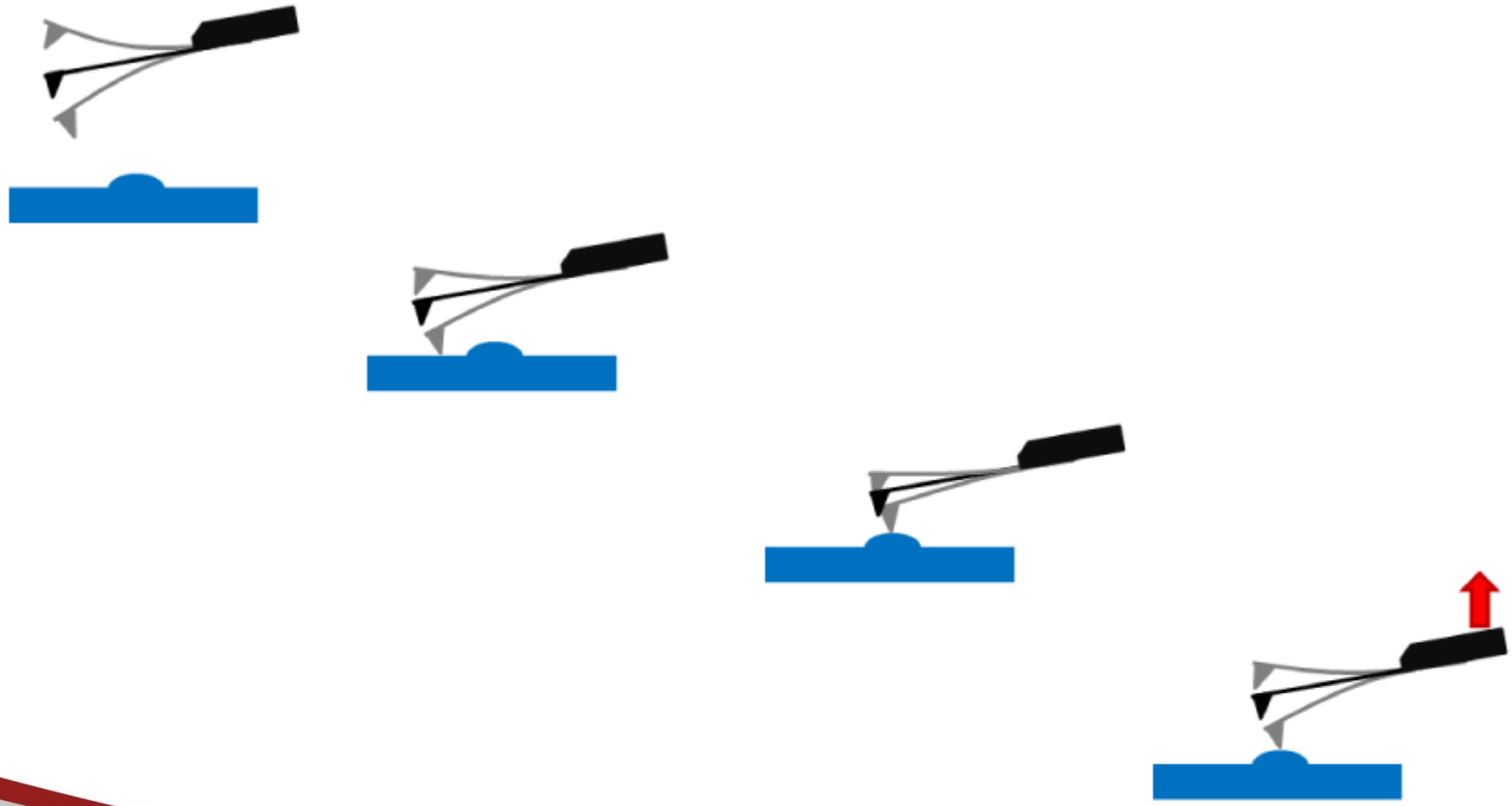




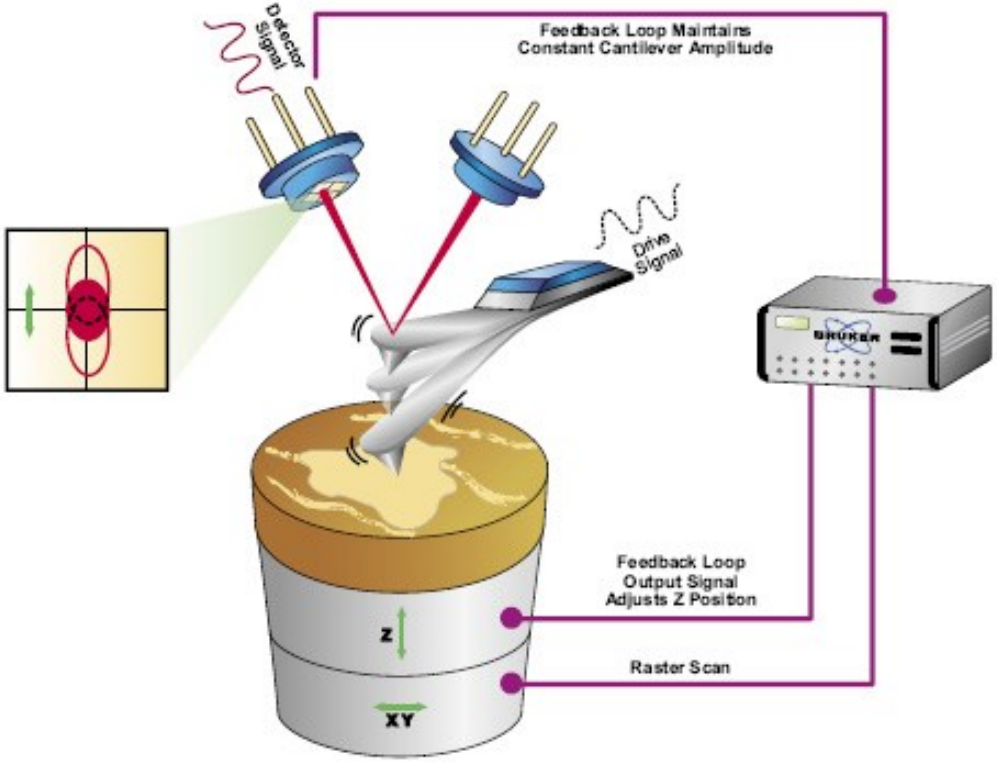
# Types of modes



# Tapping mode

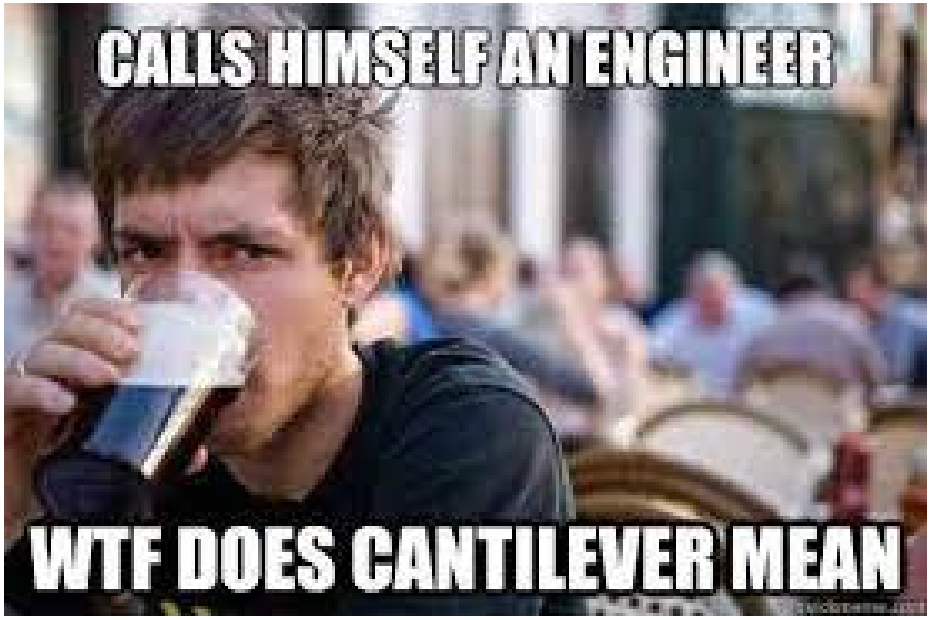


# Tapping mode

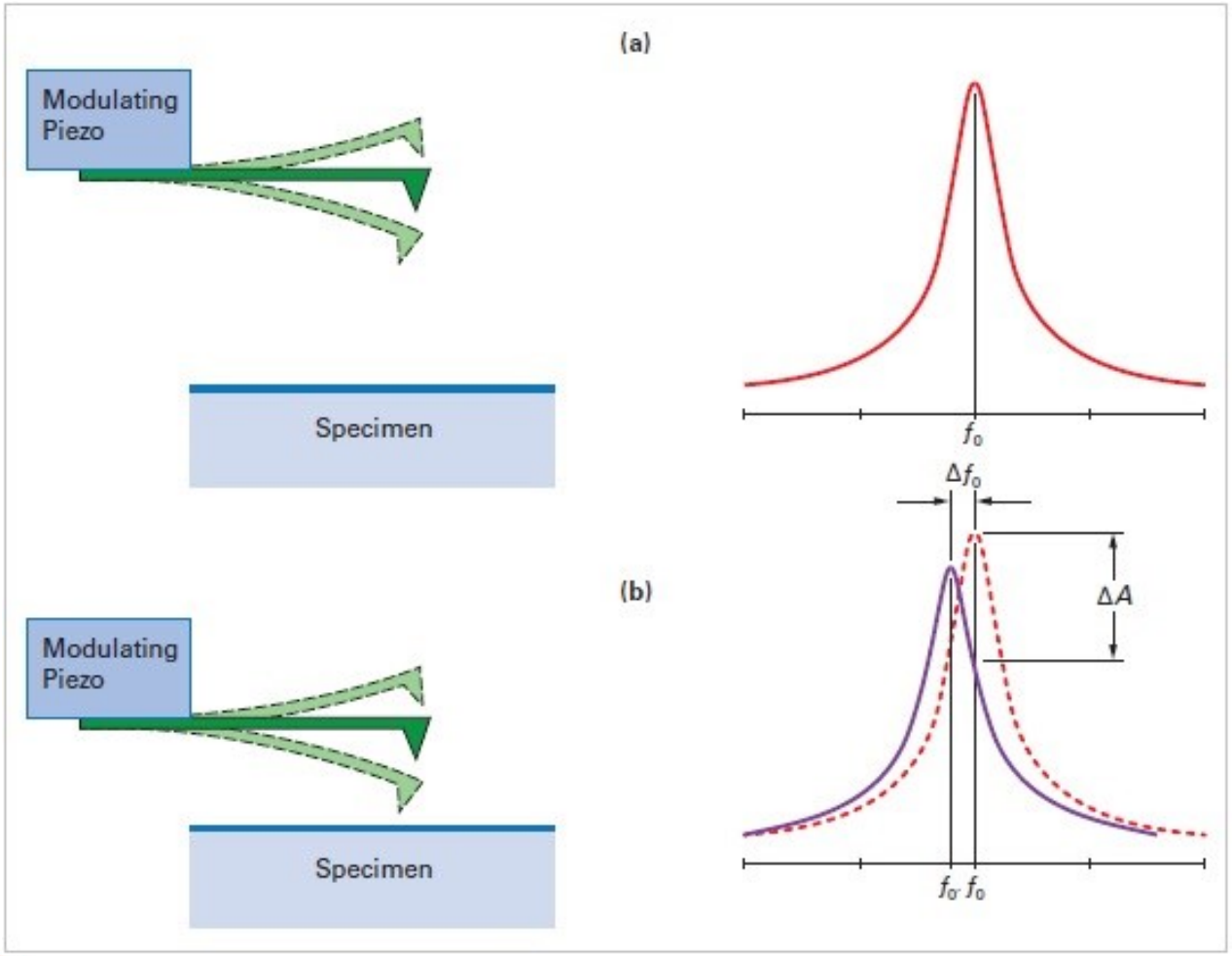


# Oscillation of a cantilever

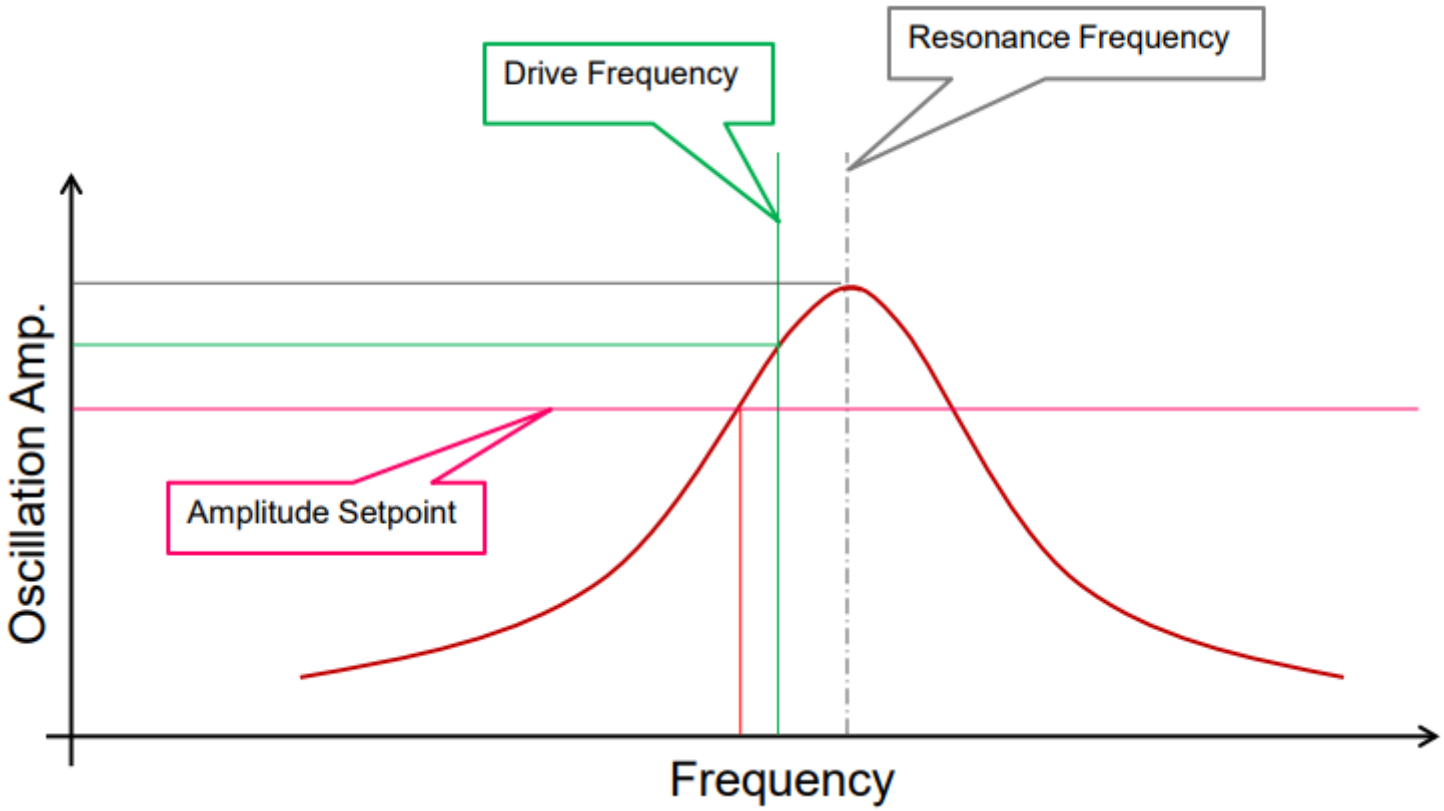
- Piezo element in probe holder or head
- Need to find resonance frequency
- Measuring the difference to amplitude setpoint



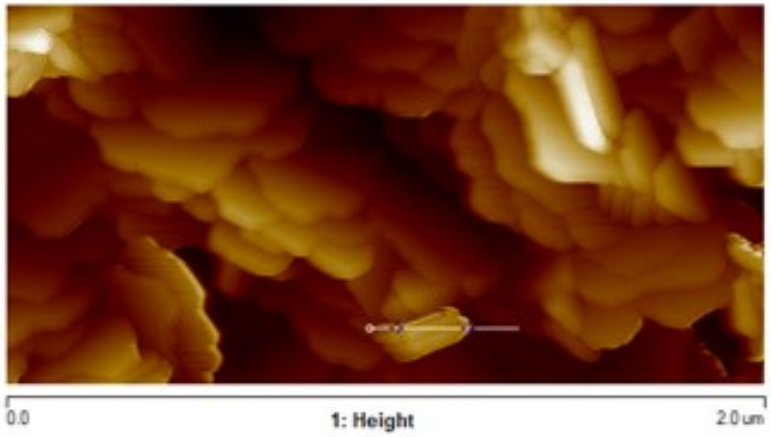
# Oscillation of a probe

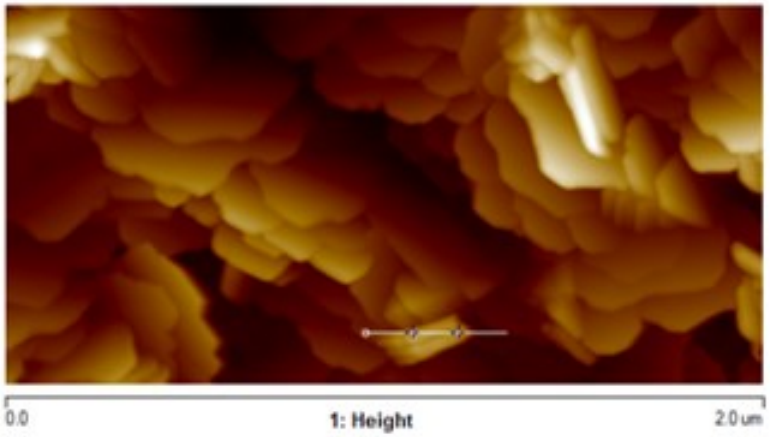


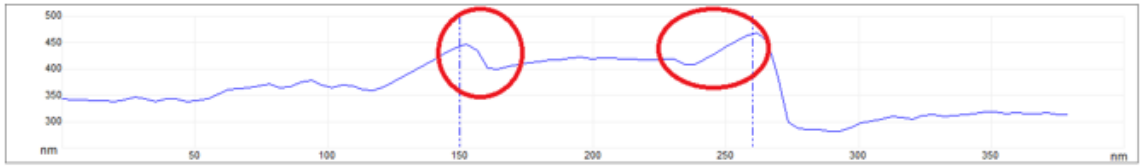
# Oscillation of a probe

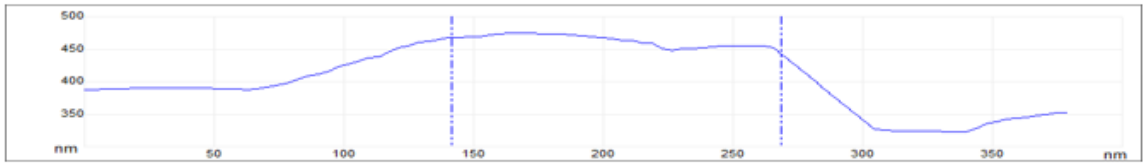


# Atractive or repulsive?

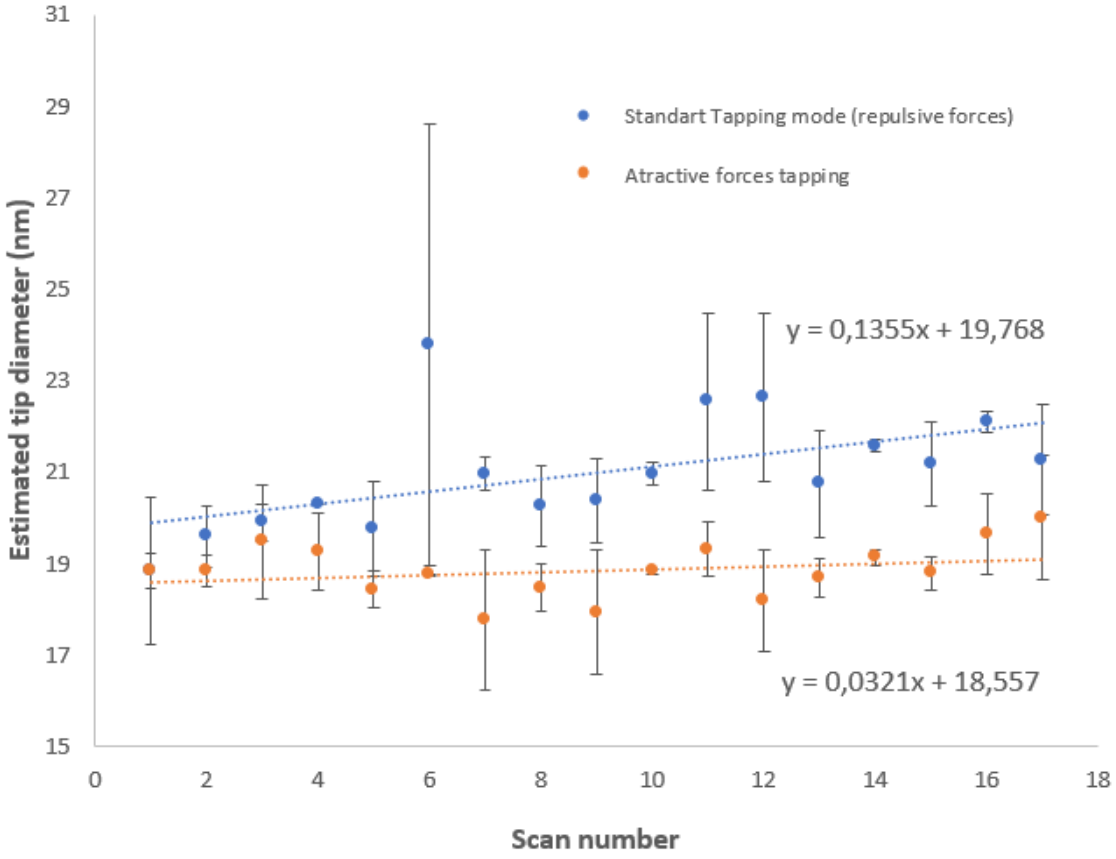


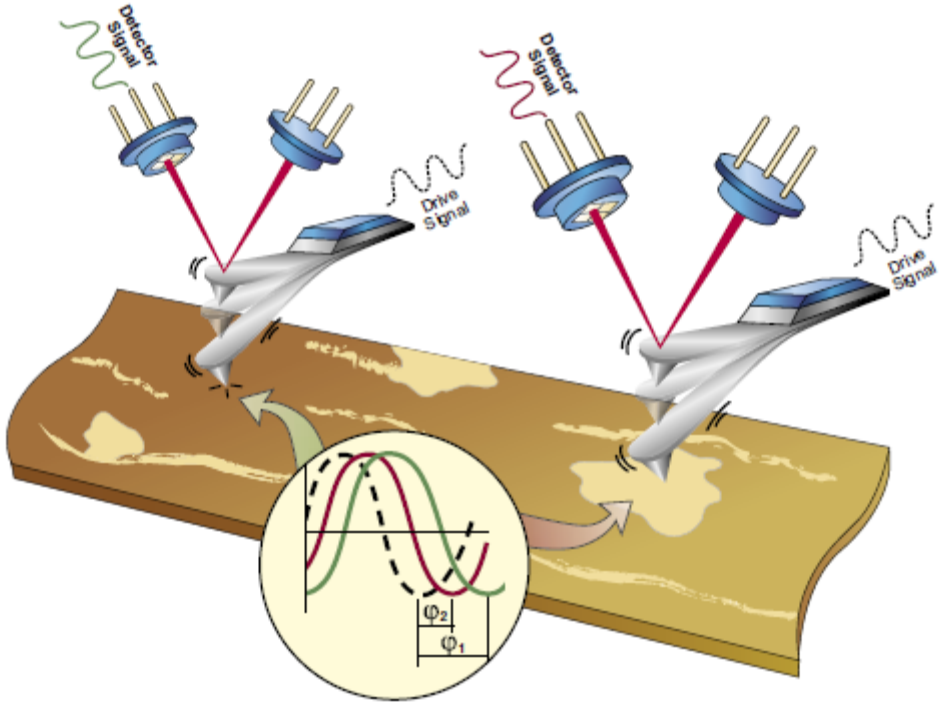



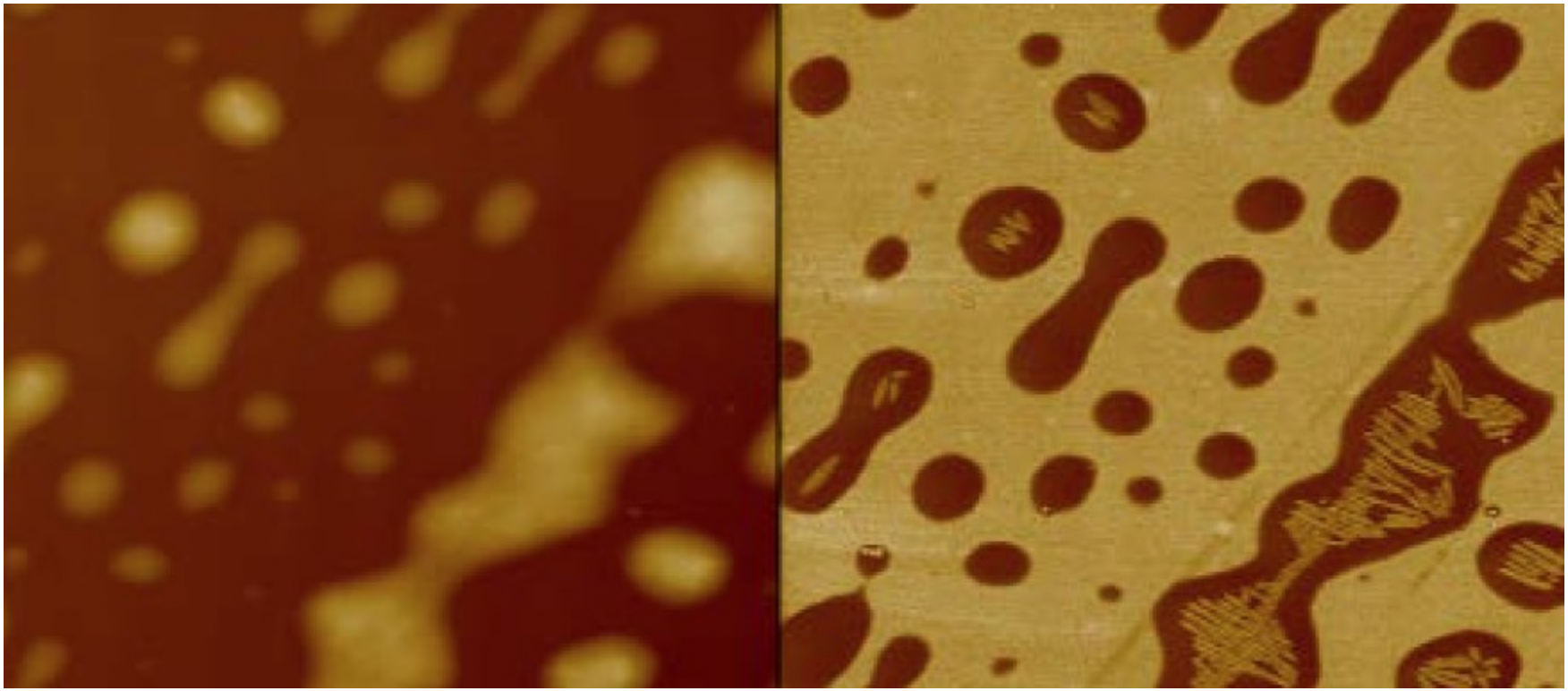
# Atractive or repulsive?



# Phase Imaging



# Phase Imaging



# Benefits of tapping mode

- Lower wear of a tip and a sample
- Better lateral resolution
- Phase imaging signal

# Disadvantage of tapping mode

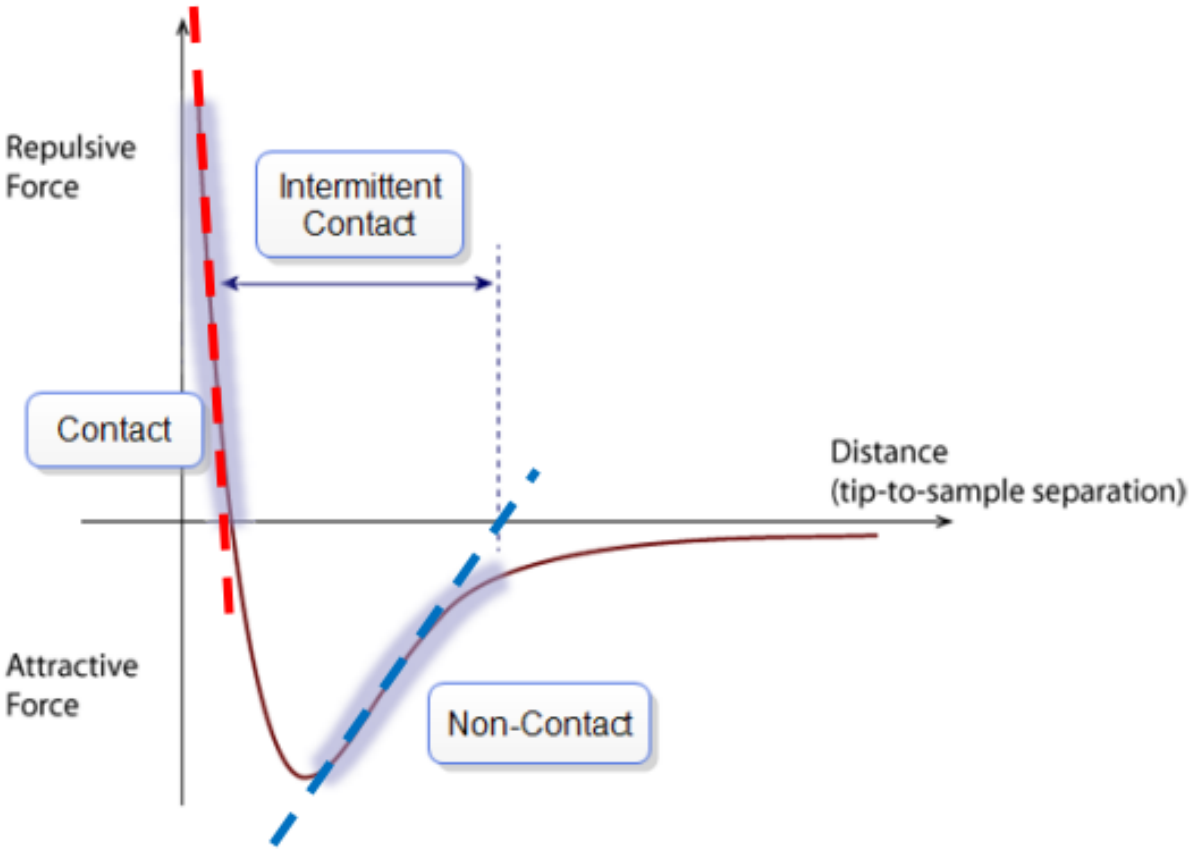
- More complicated mode
- Undirect force control
- Discontinuous contact with sample
- Need to tune the cantilever

# Thank you for your attention

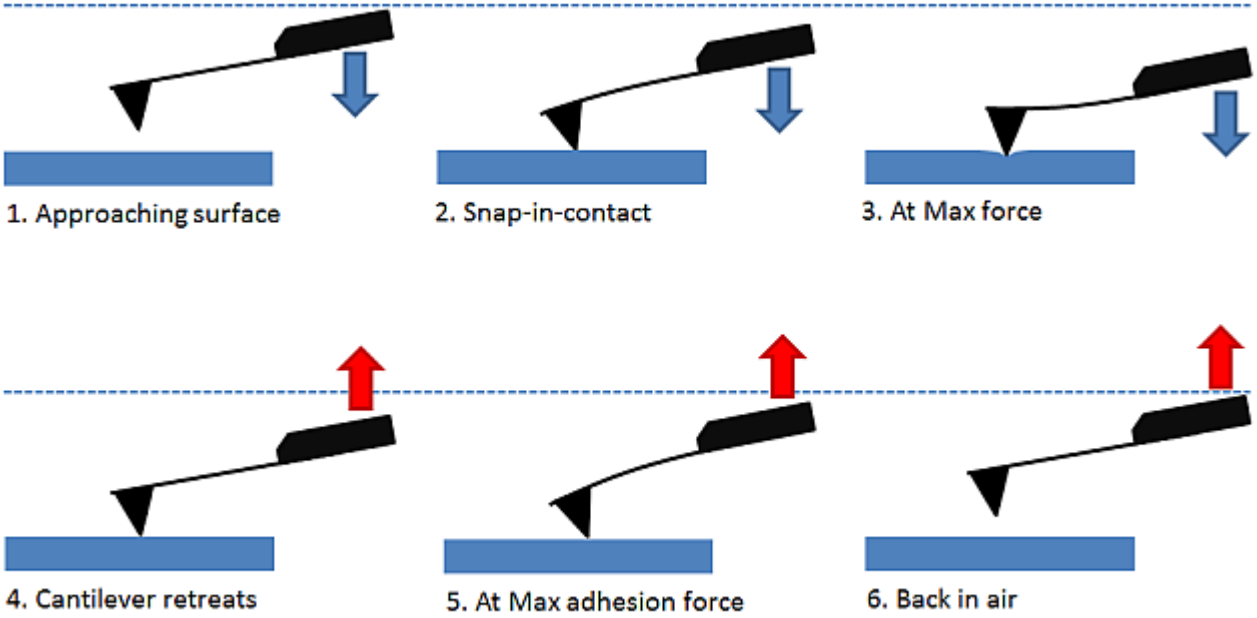
# Advance measuring modes

- **Force curves, Force volume/mapping**
- **PF QNM, QI**
- **Cytosurge**
- **High speed AFM**

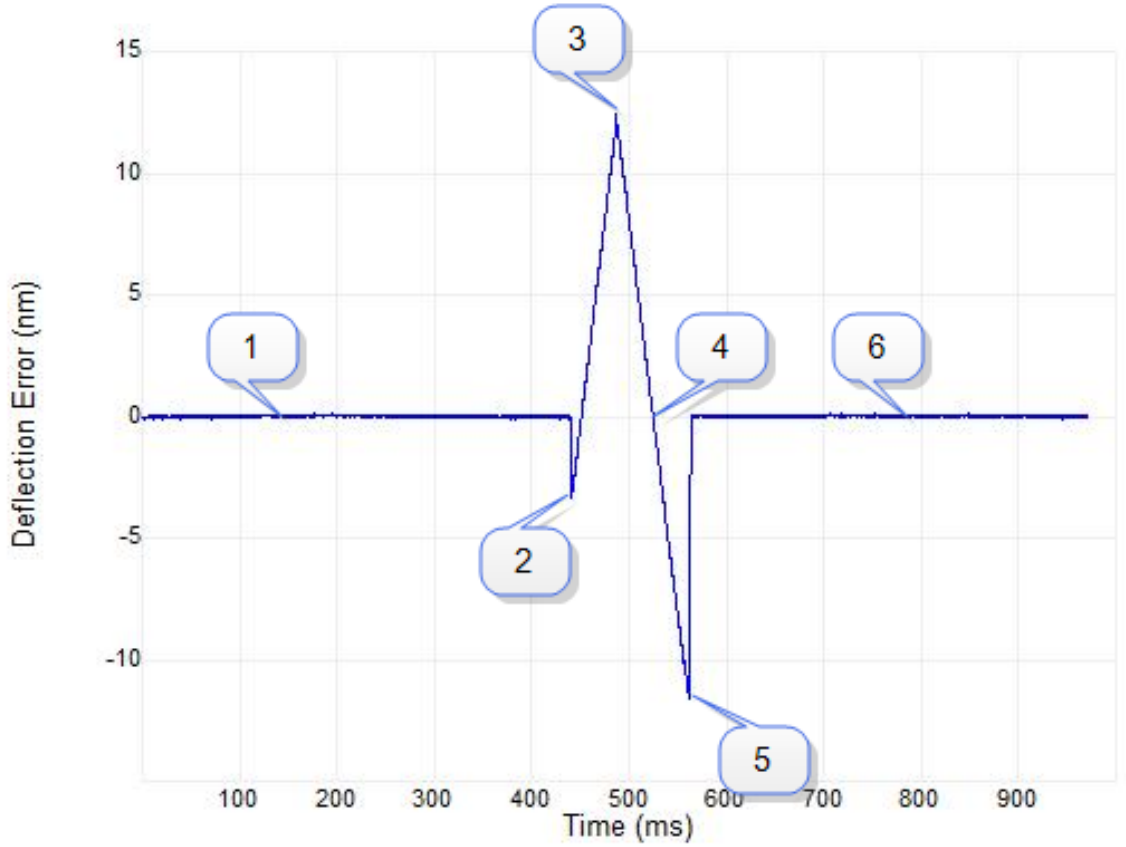
# Force curves



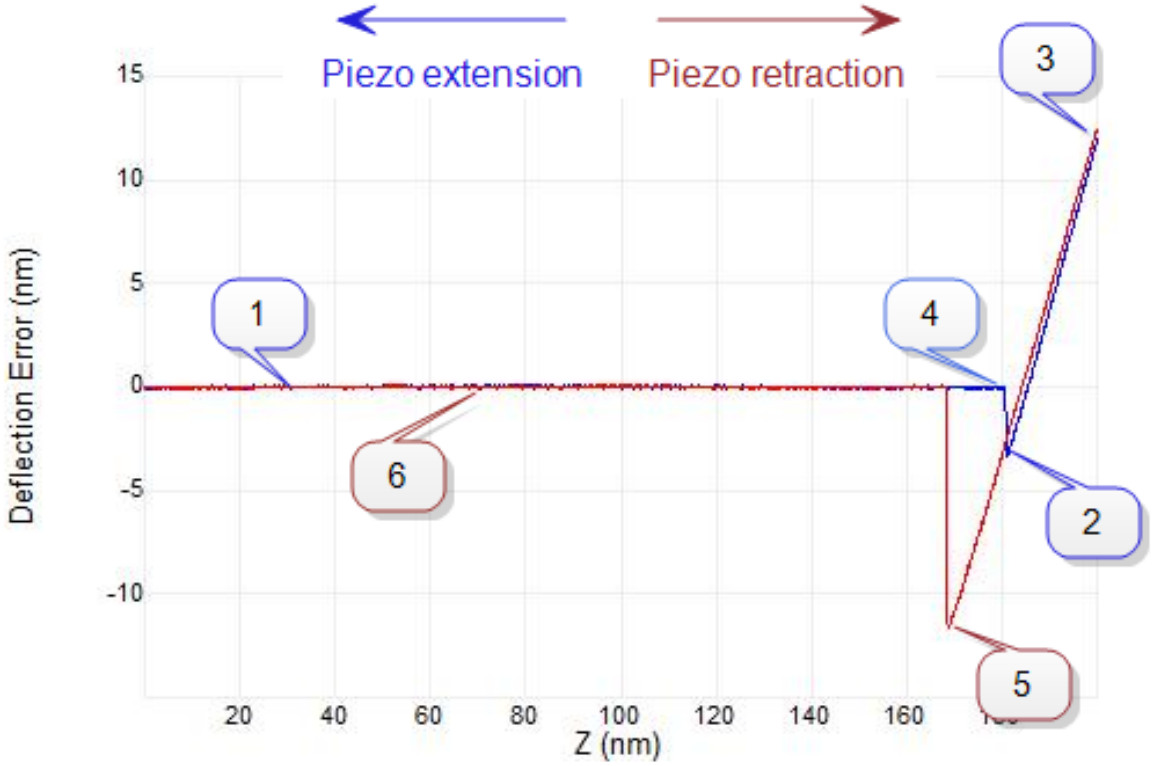
# Force curves



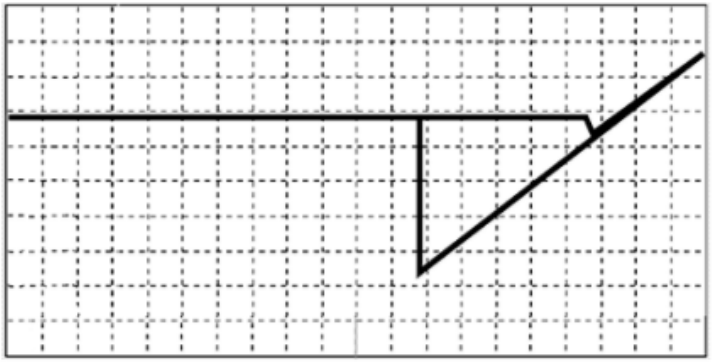
# Force curves



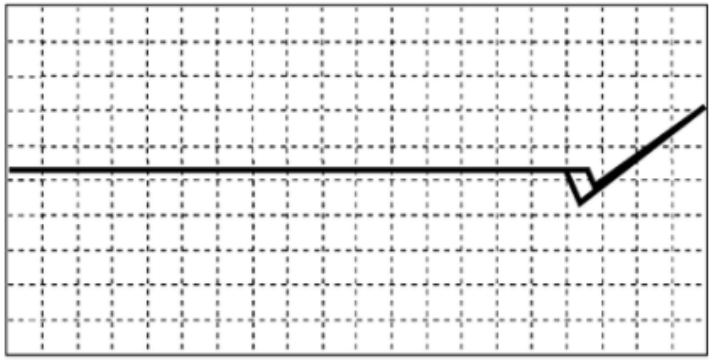
# Force curves



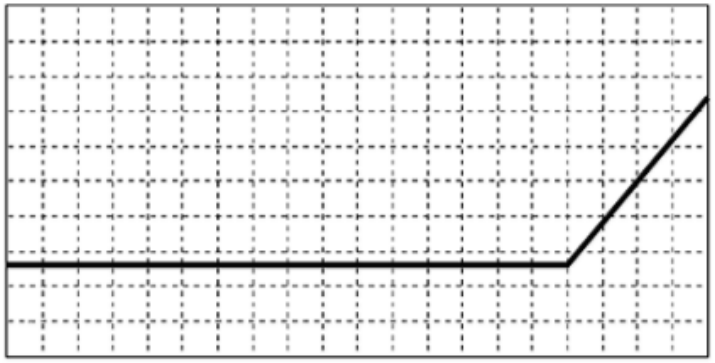
# Types



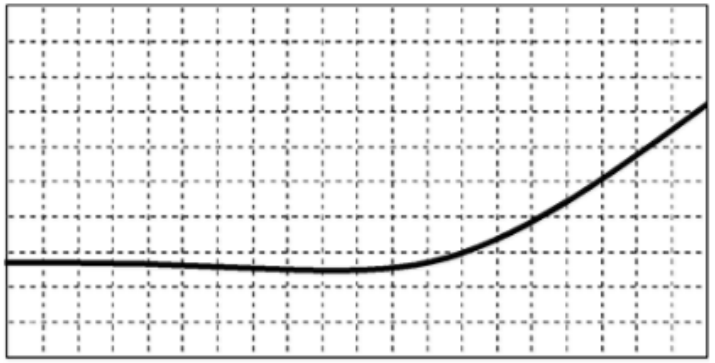
Large Adhesion



Small Adhesion

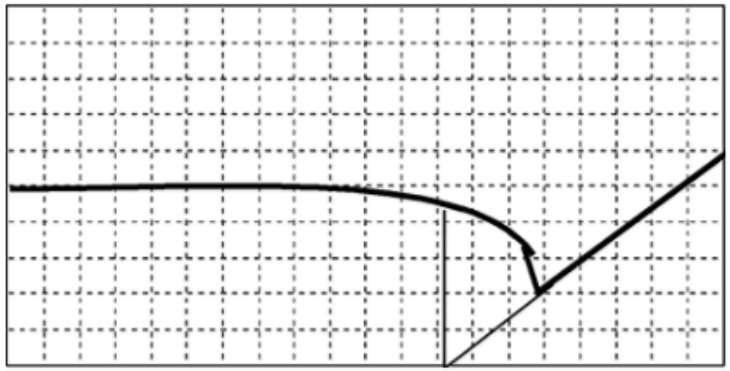


Stiff Sample

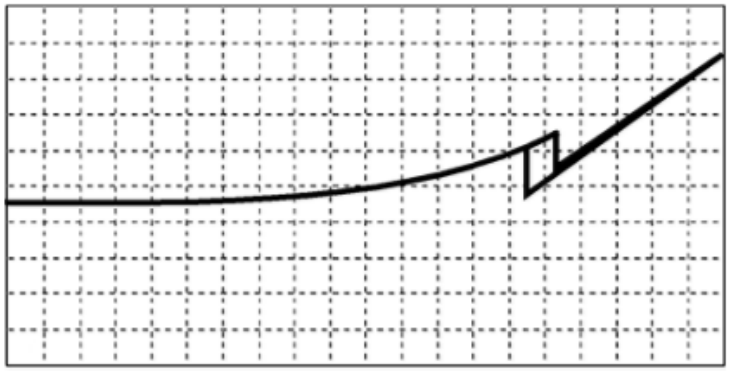


Soft Sample

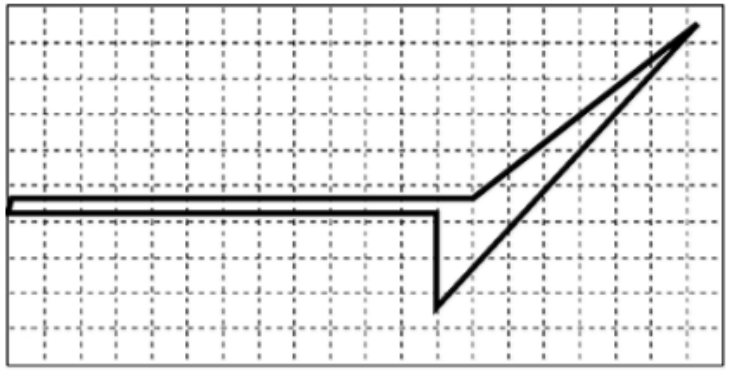
# Types



Long Range Attraction



Long Range Repulsion

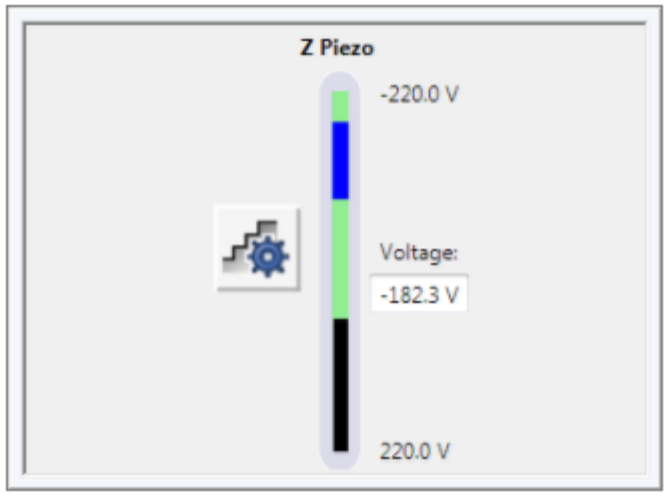


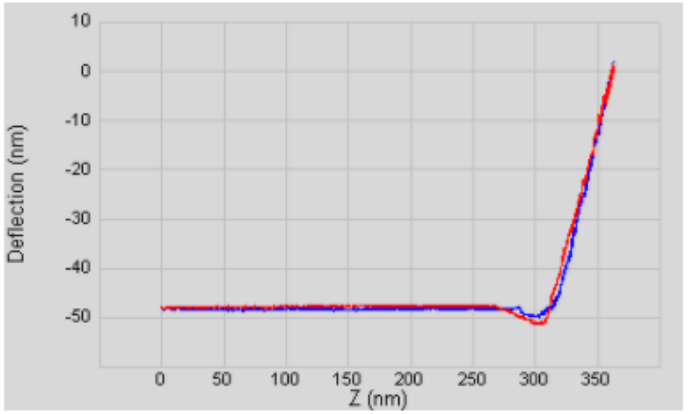
Plastic Deformation

# Measuring of force curves

a. In the Ramp panel set:
- **Ramp Output:** Z
- **Ramp size:** 1.00 µm
- **Ramp Position:** 0 nm
- **Ramp Rate:** 1.00 Hz
- **Number of samples:** 512

b. In the Channel 1 panel set:
- **Data Type:** Deflection Error
- **X Data Type:** Z
- **Display Mode:** Deflection Error vs. Z

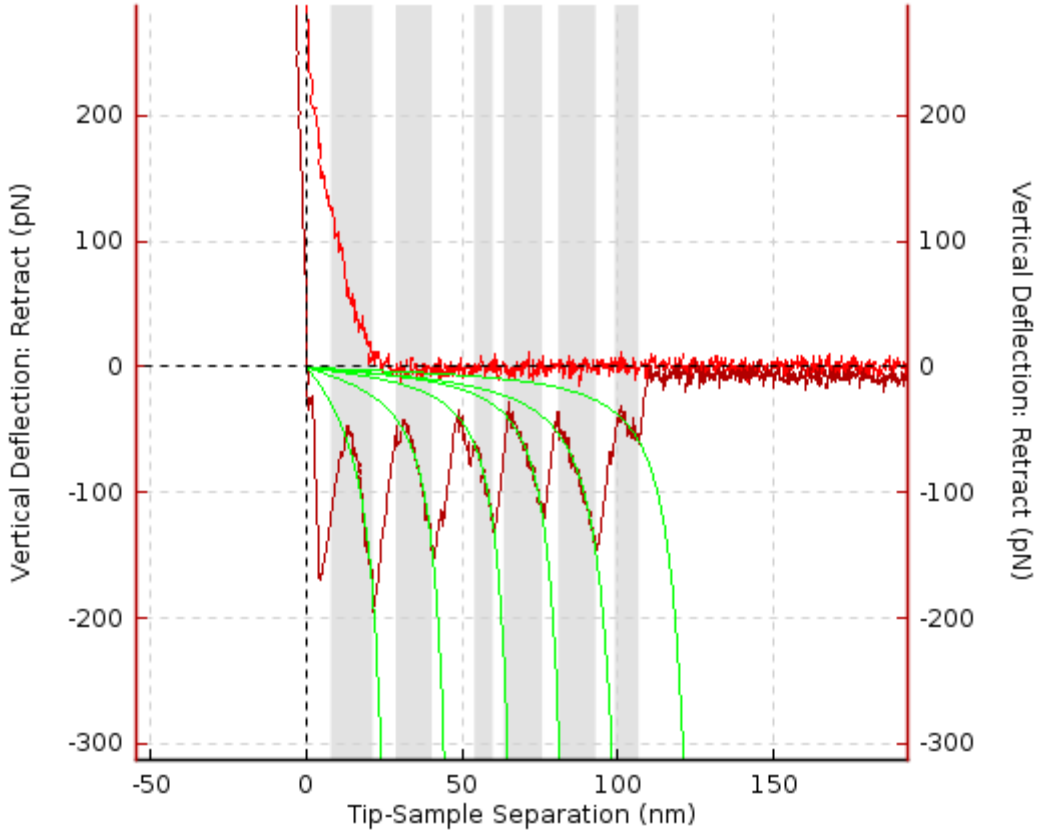


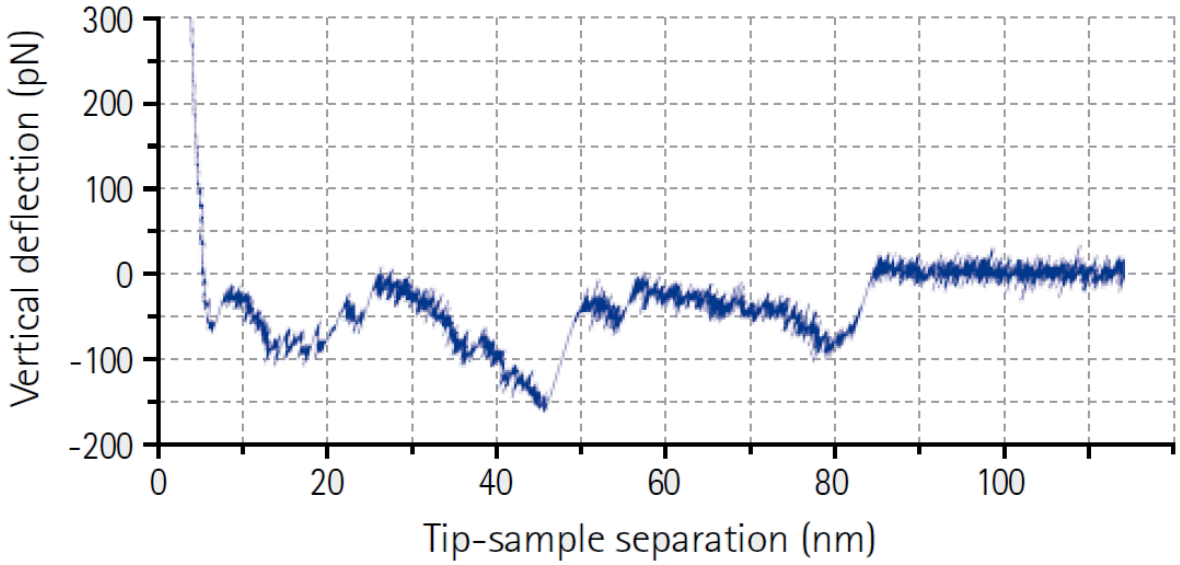
# Application

- Measuring of elasticity, adhesion, mechanical properties
- Long range forces measurements
- Force pulling investigation
  - Unwrapping of proteins
  - Molecule stretching
  - Molecule recognition

# Protein unfolding

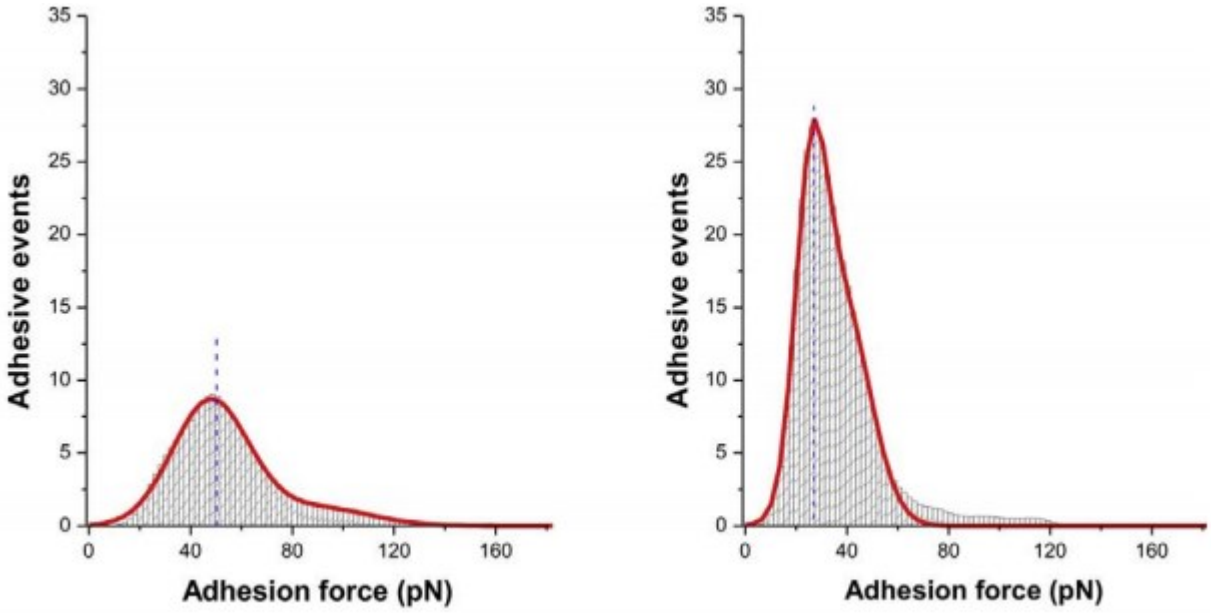


# Protein unfolding

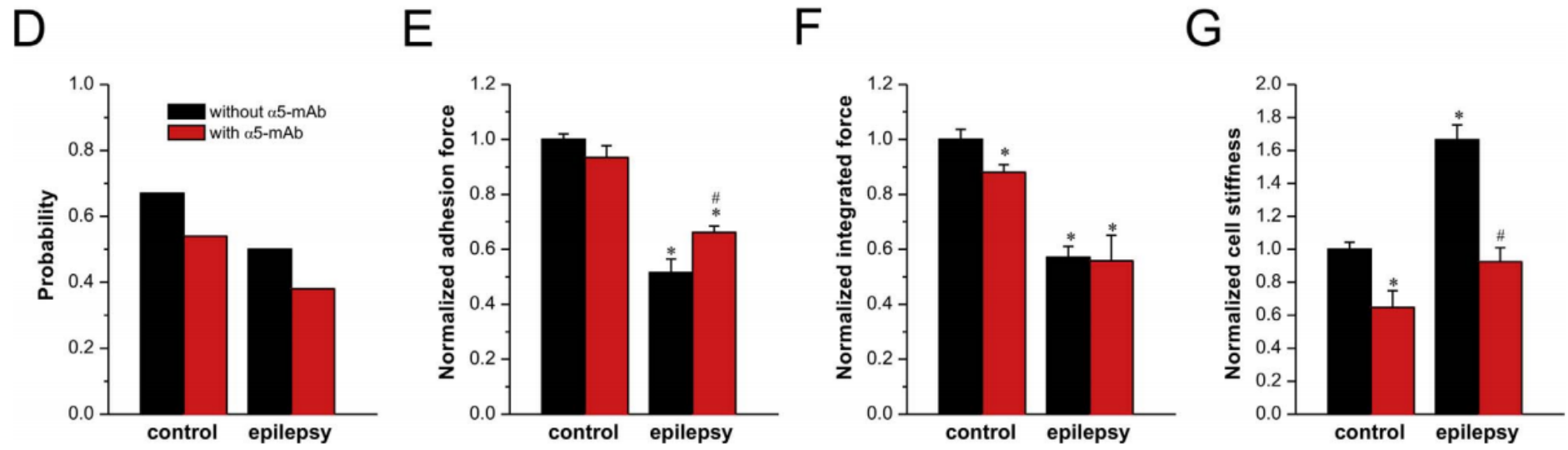


# Protein-integrin interaction on neurons

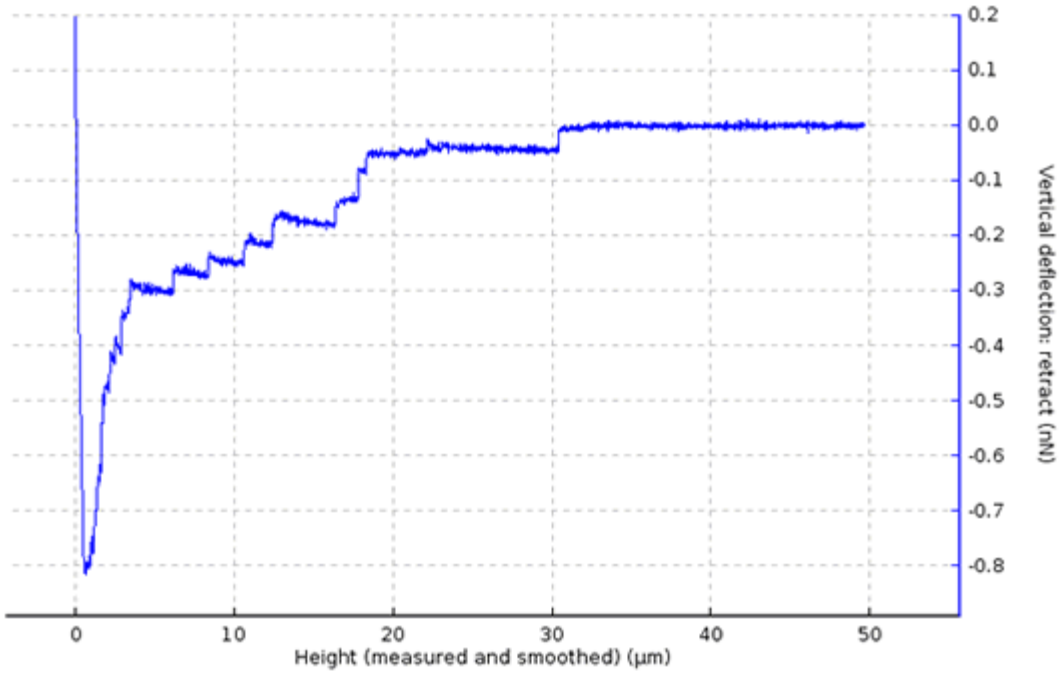
- **Atomic force microscopy investigations of fibronectin and α5β1-integrin signaling in neuroplasticity and seizure susceptibility in experimental epilepsy**
- **doi.org/10.1016/j.eplepsyres.2017.10.013**



# Protein-integrin interaction on neurons

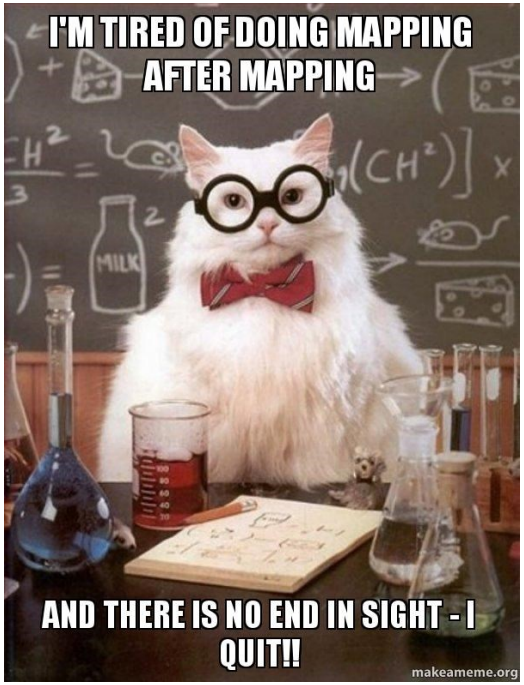


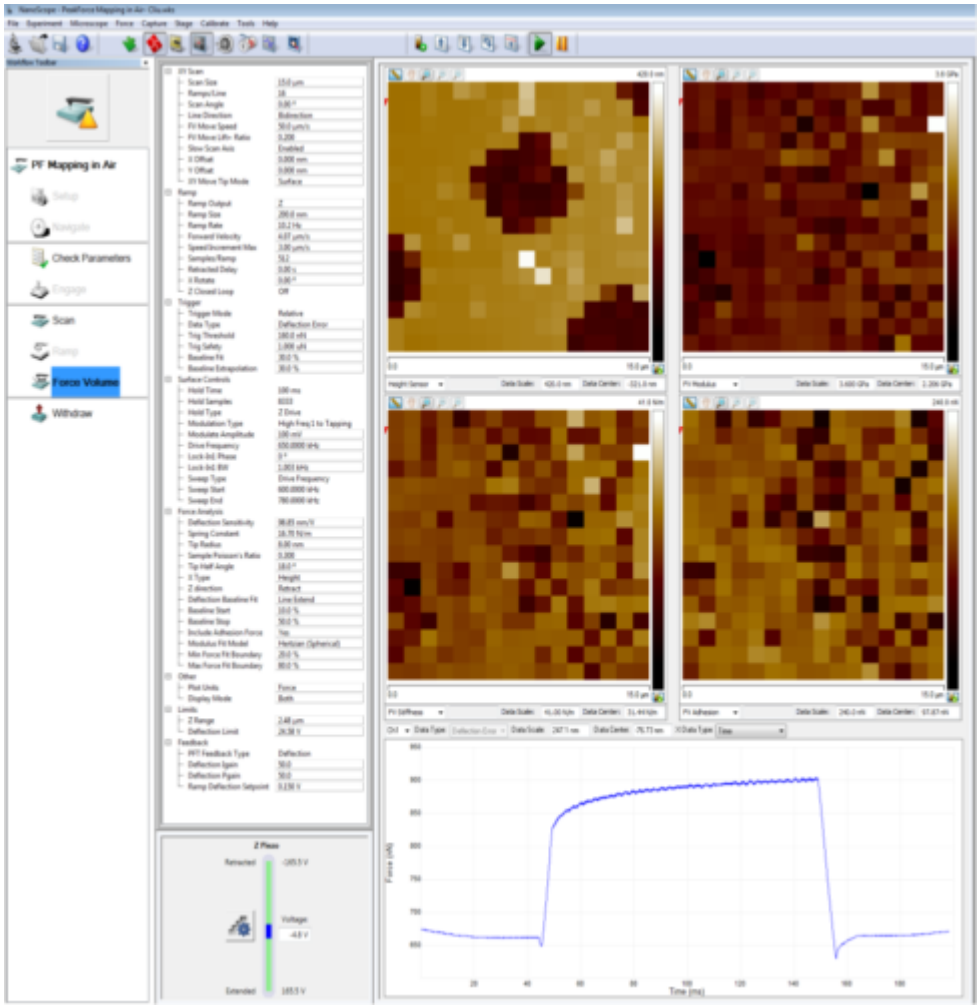
# Endothelial Cell – cell interaction



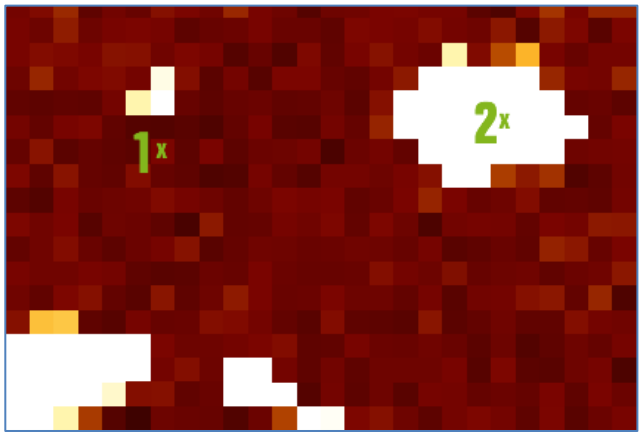
# Force map/ Force volume

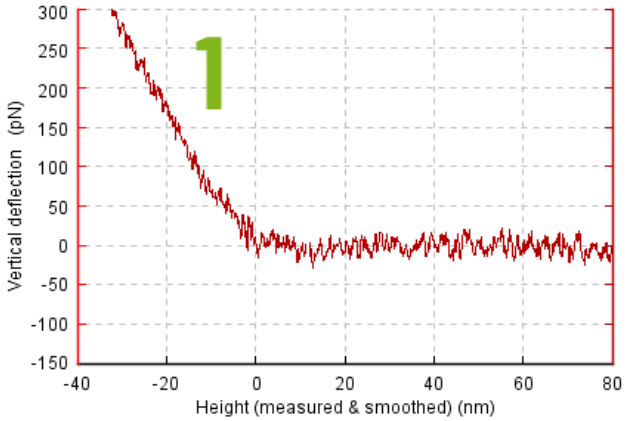
- **Matrix of force curves**

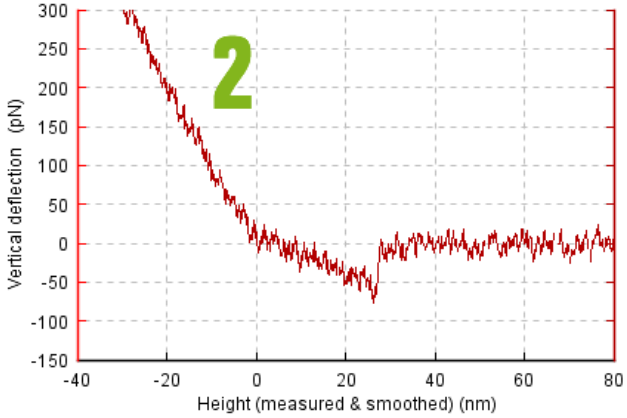


# Recognition map

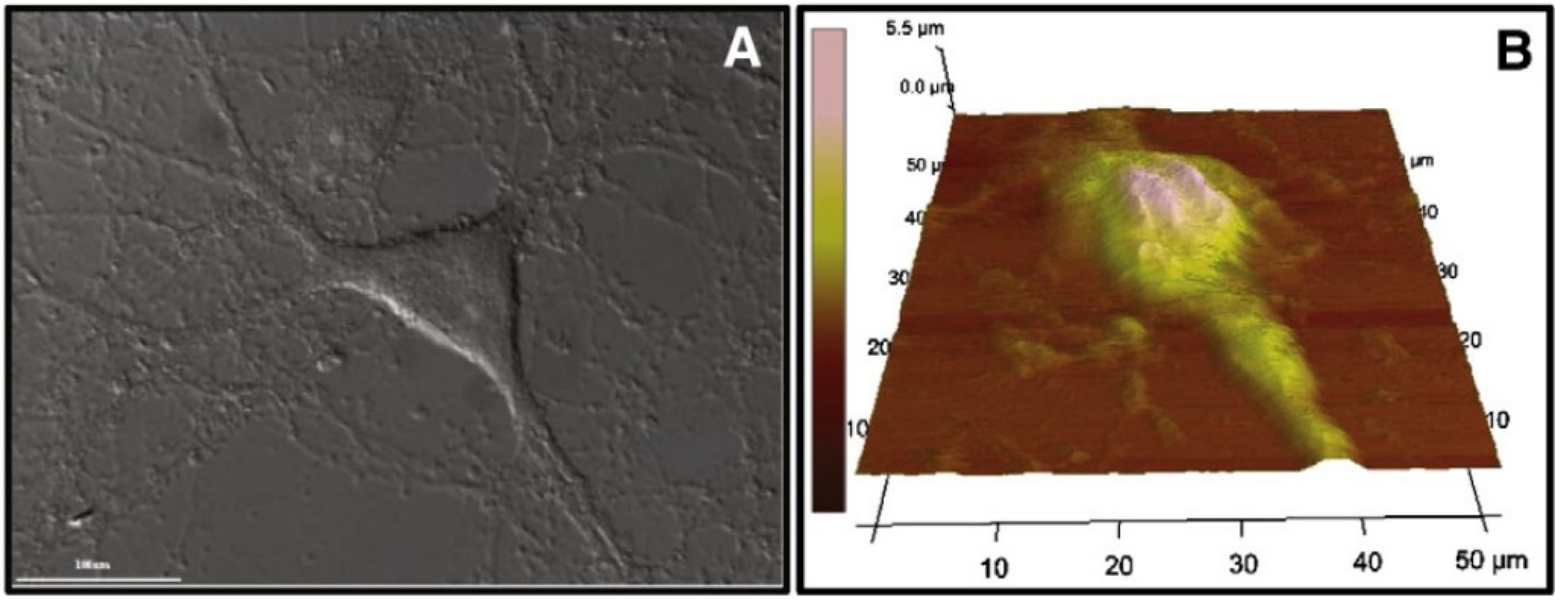


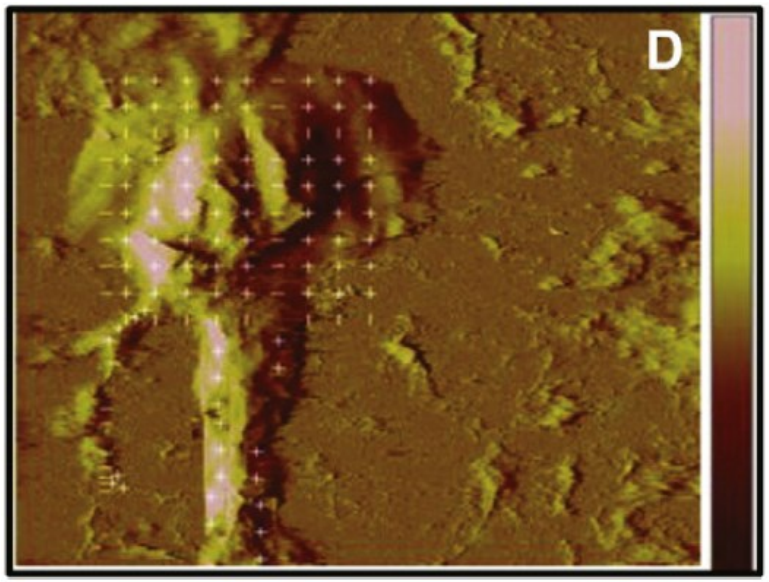



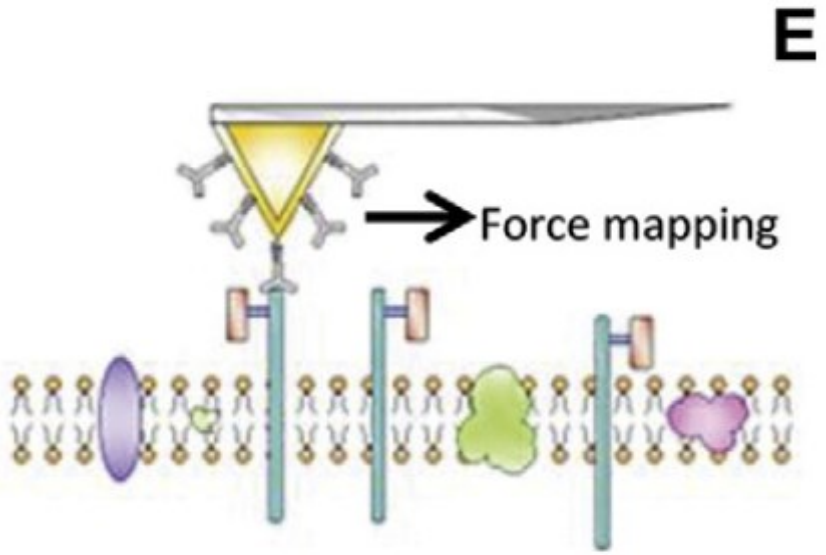
# MET receptors distribution

- Nanoscale mapping of the MET receptor on hippocampal neurons by AFM and confocal microscopy
- 10.1016/j.nano.2012.08.008

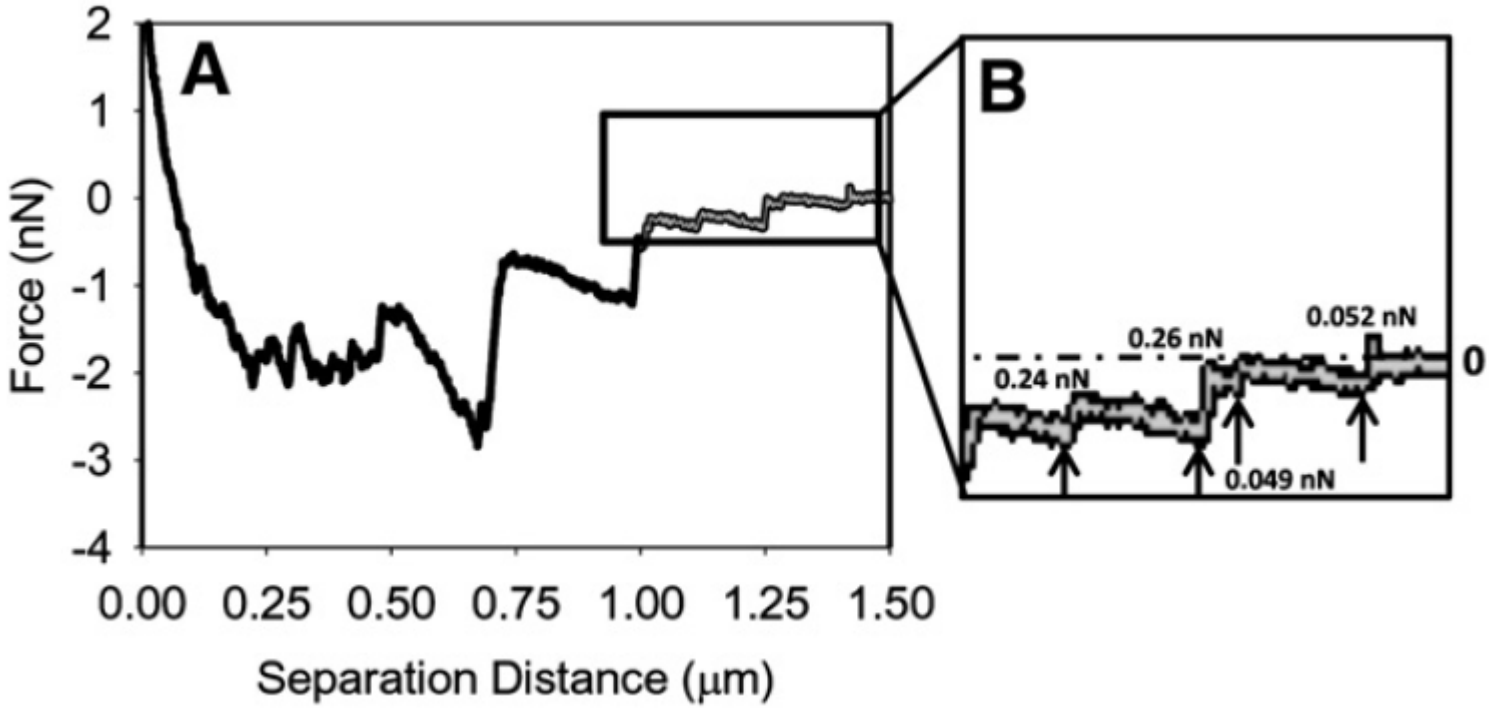


# MET receptors distribution

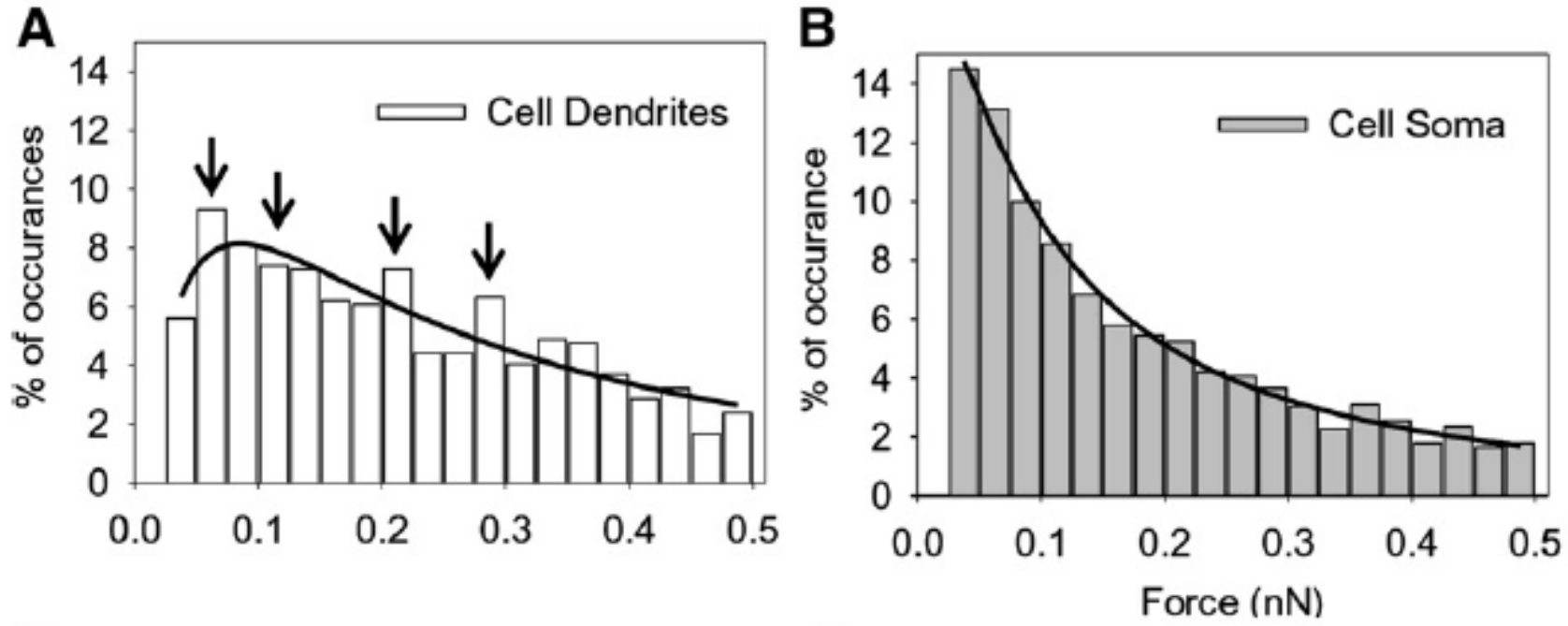


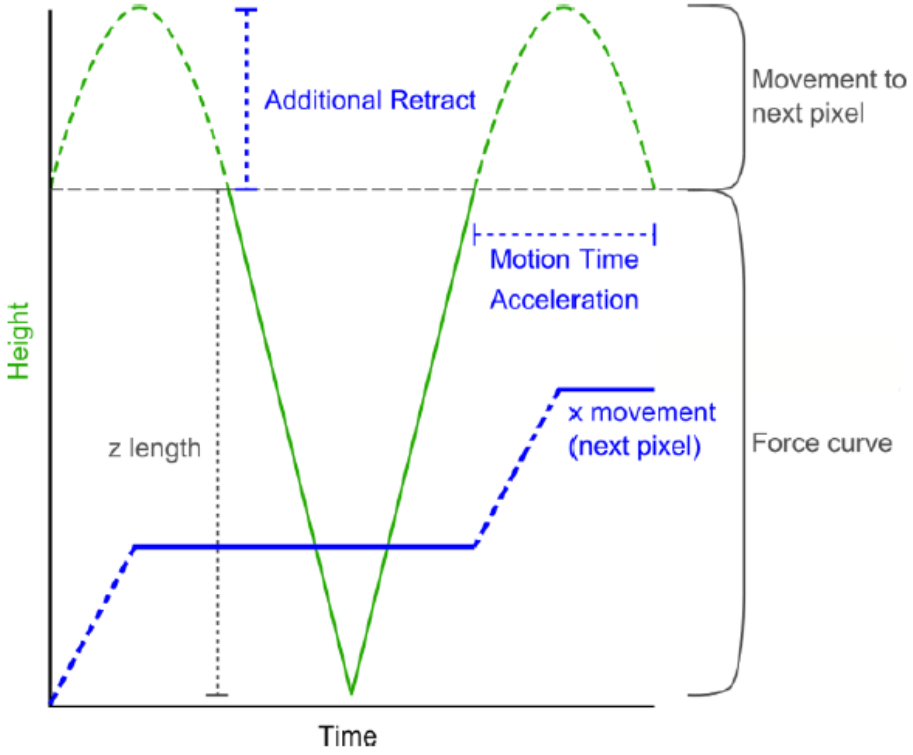
# MET receptors distribution

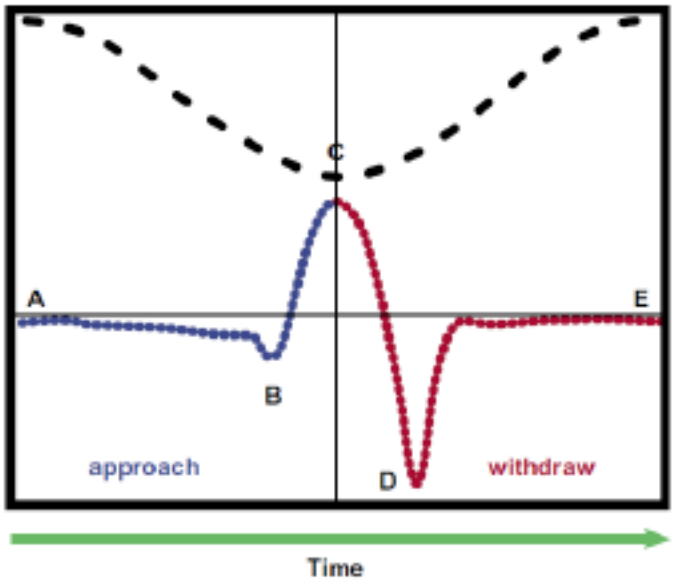


# MET receptors distribution



# QI and PF QNM

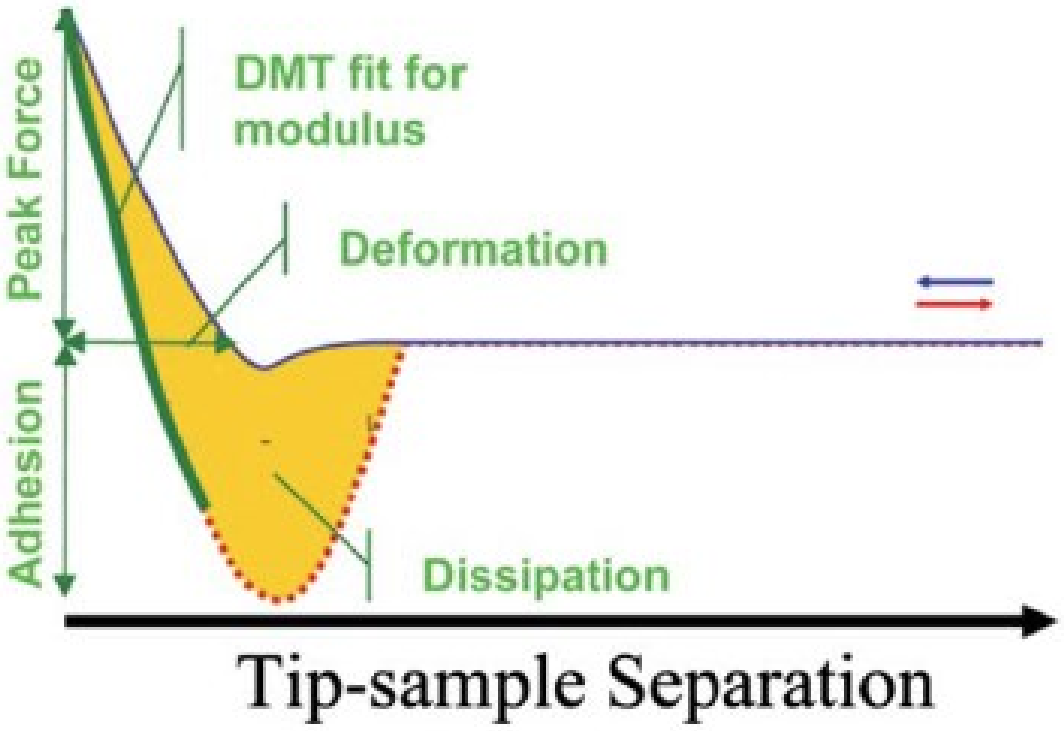

Additional Retract
Movement to next pixel
Motion Time
Acceleration
Height
z length
x movement (next pixel)
Force curve
Time


C
A
E
B
approach
withdraw
D
Time

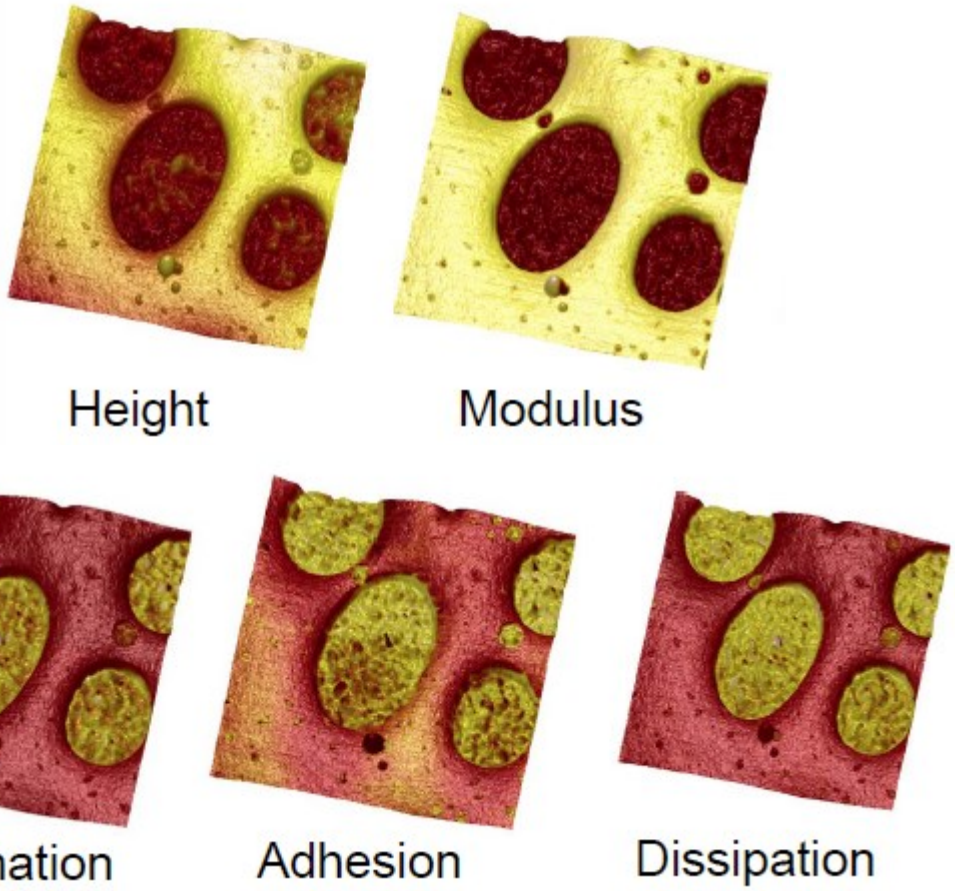
# Nomenclature

- Quantitative imaging – method linear z
- PeakForce tapping – method sinus z
- ScanAsyst – mode with self optimizing technology
- PF QNM – mode for mapping of mechanical properties
- ScanAsyst – probes

# Signals

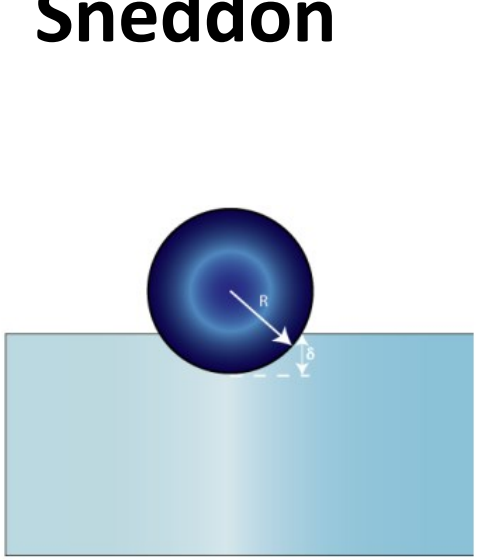

DMT fit for
modulus
Deformation
Peak Force
Adhesion
Dissipation
Tip-sample Separation

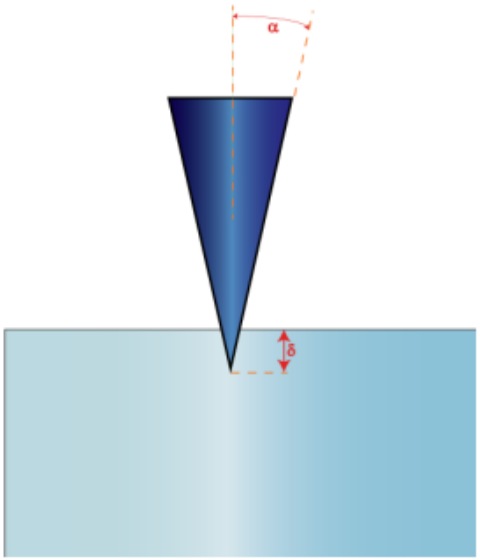
# Signals



# Indentation

- DMT (Derjaguin-Mueller-Toporov)
- Herzian
- Sneddon

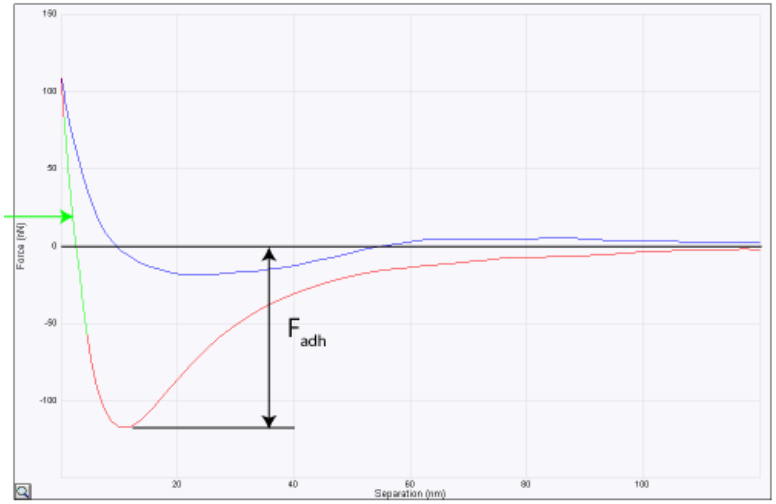


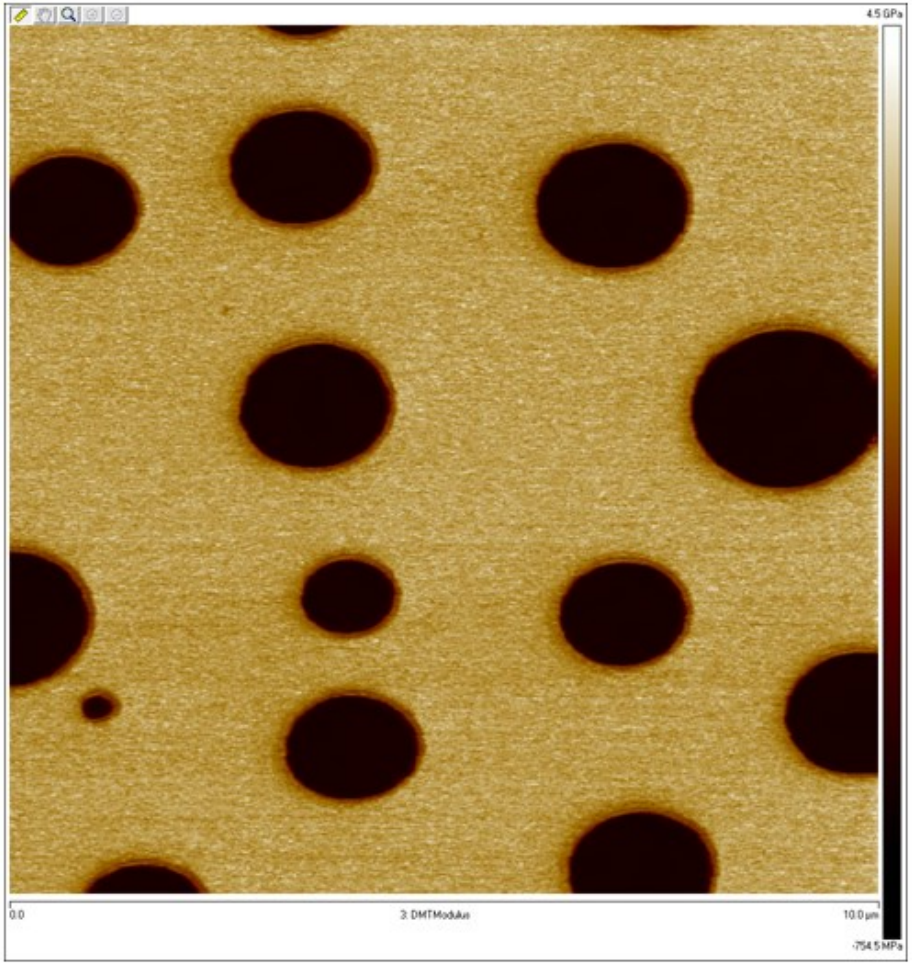



# Signals

## DMT modulus

- Young modulus

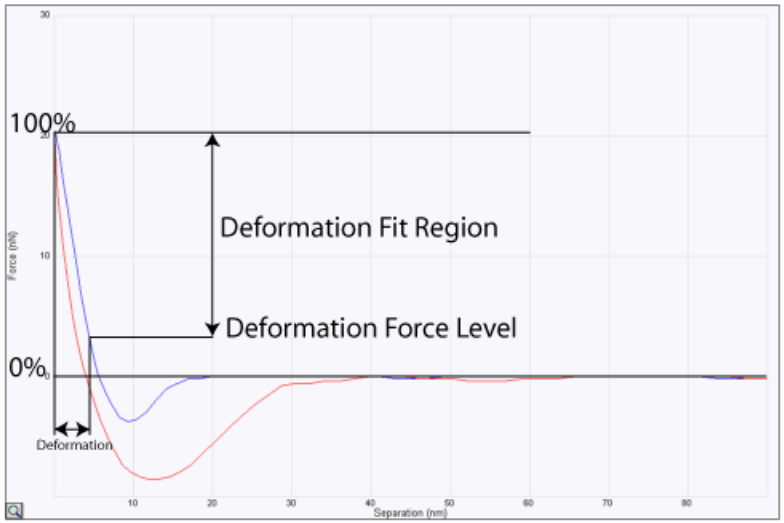


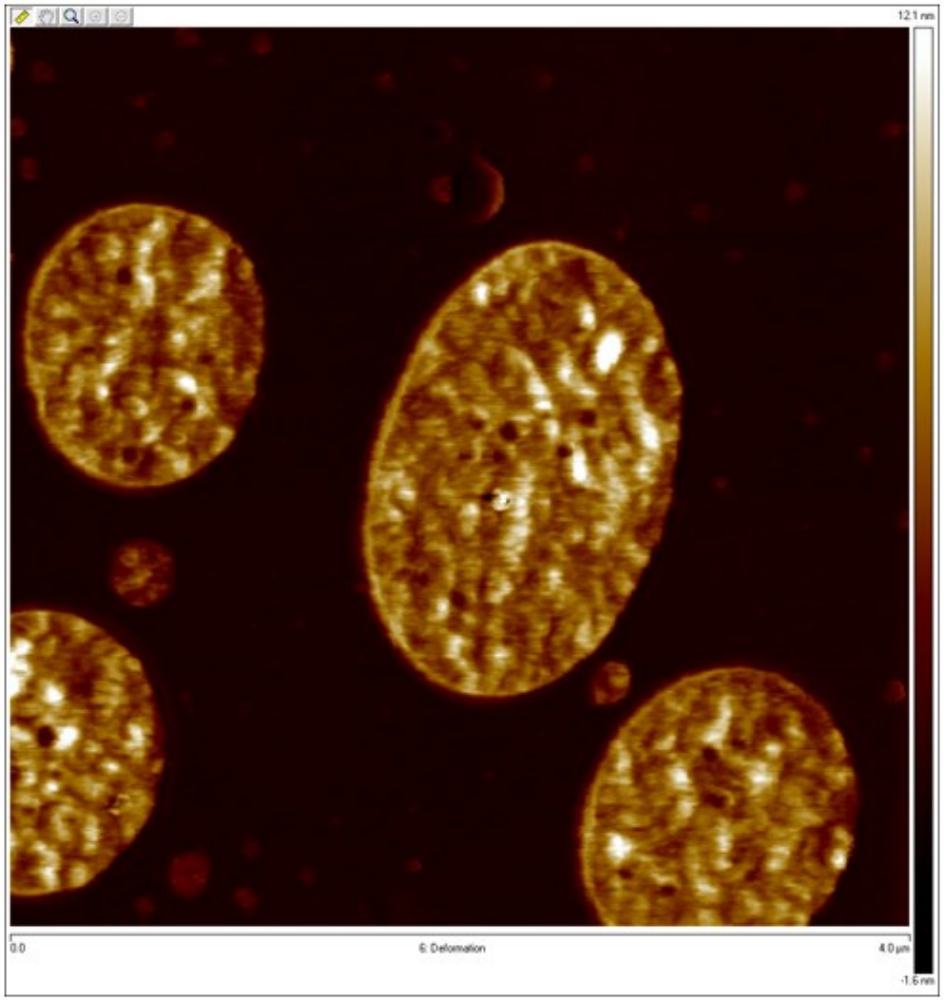



# Signals

## Deformation

- **Maximal deformation**

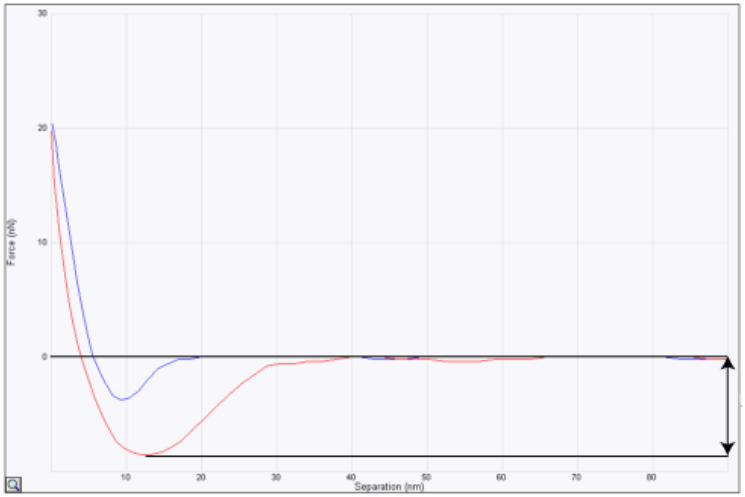


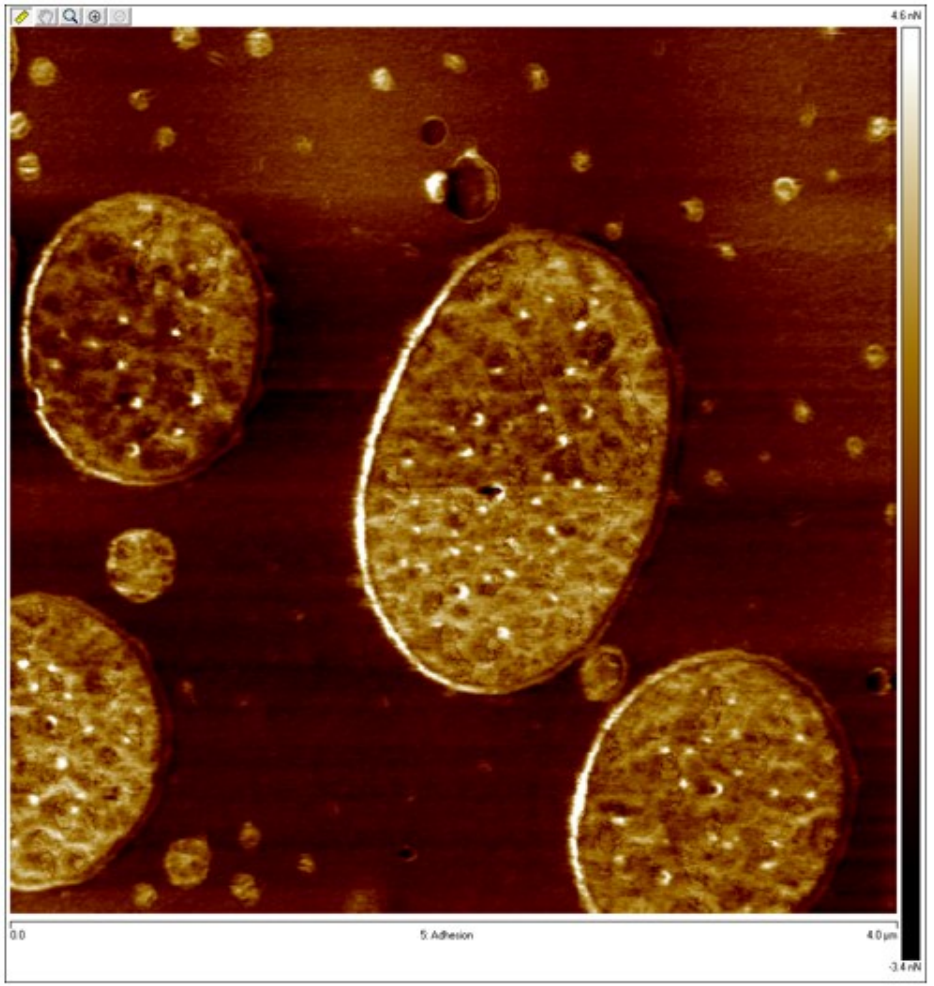



# Signals

## Adhesion

- **Maximal adhesion**

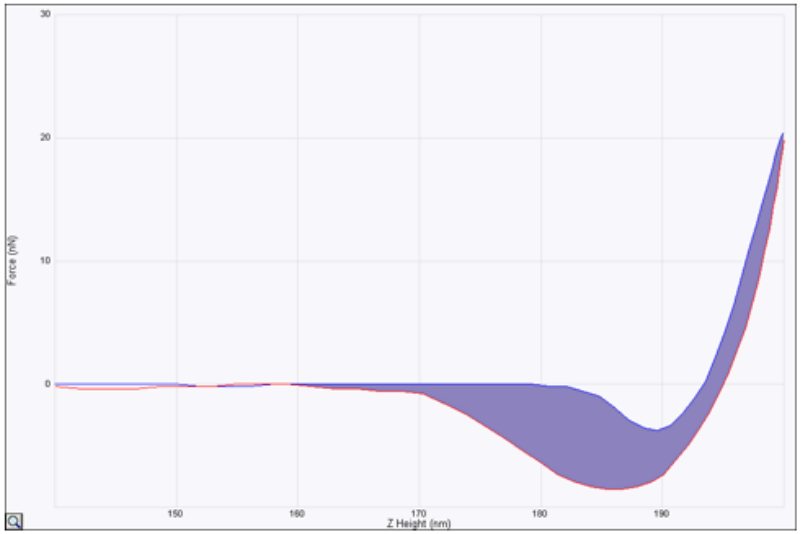


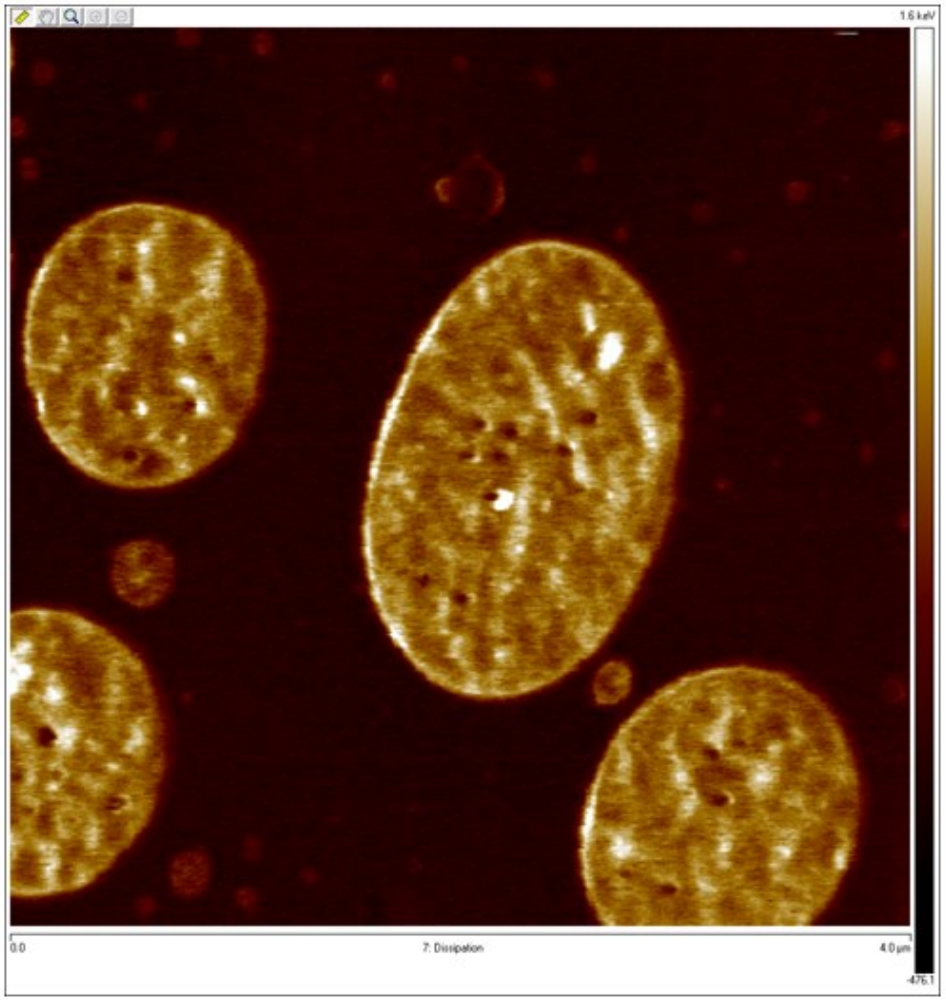



# Signals

## Dissipation

- Loss energy

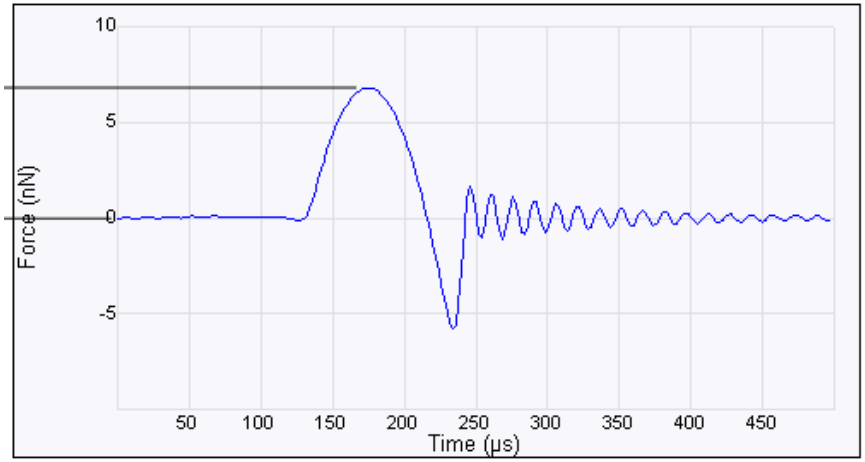


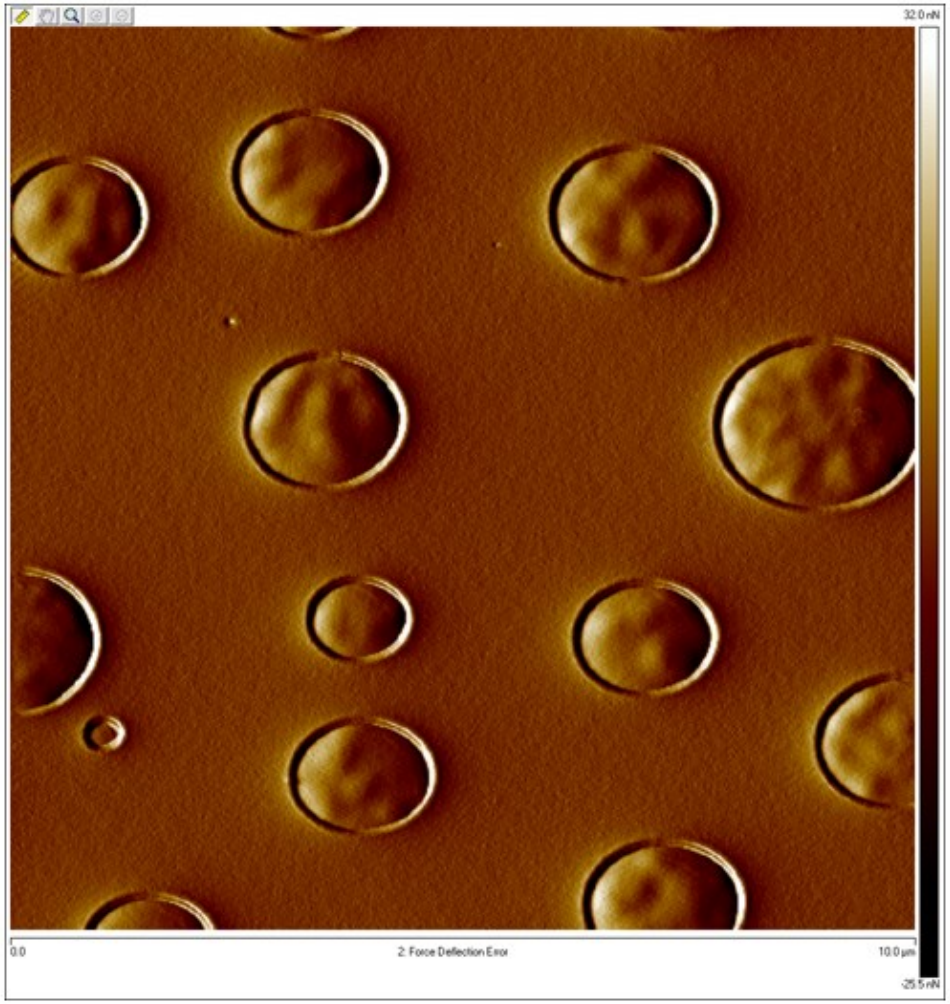



# Signals

## Peak Force

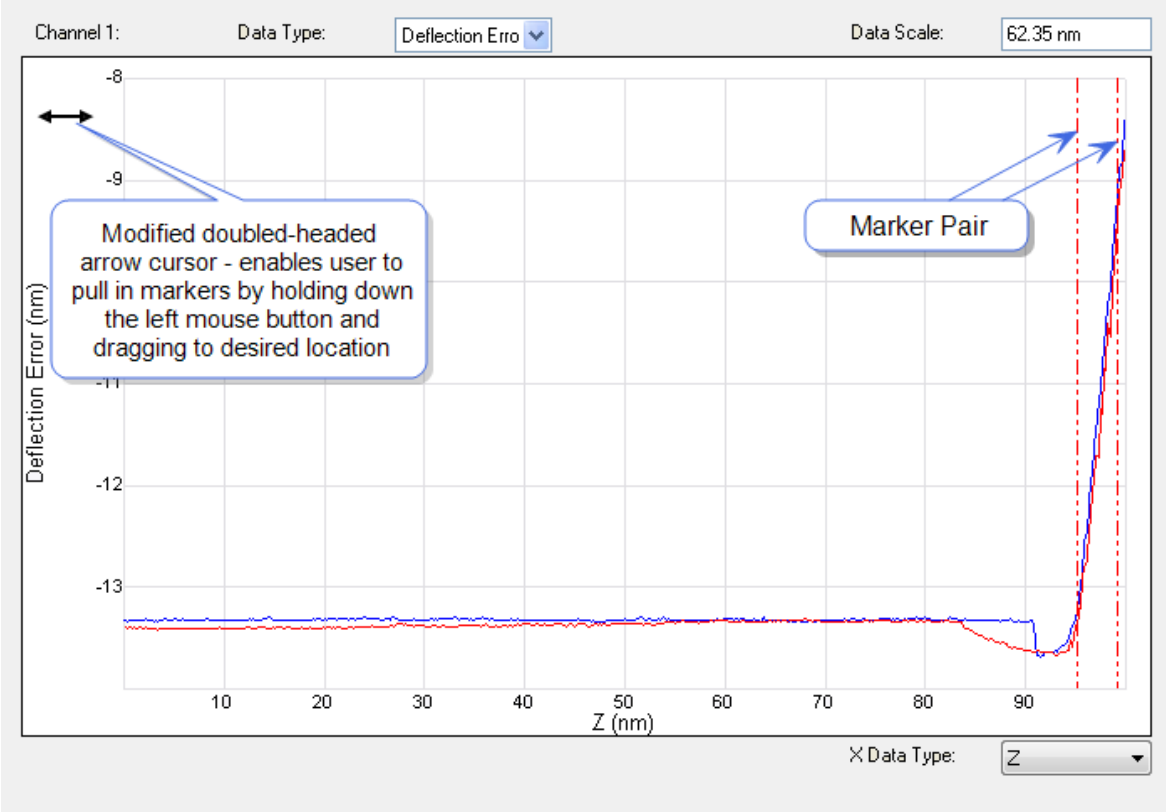
- Error signal with offset



# Calibration

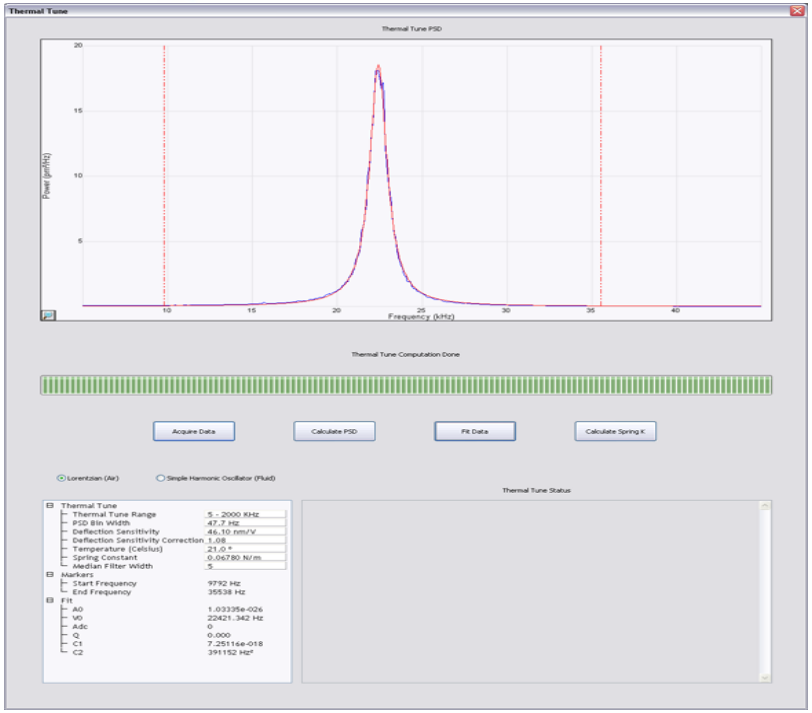
## Deflection sensitivity



# Calibration

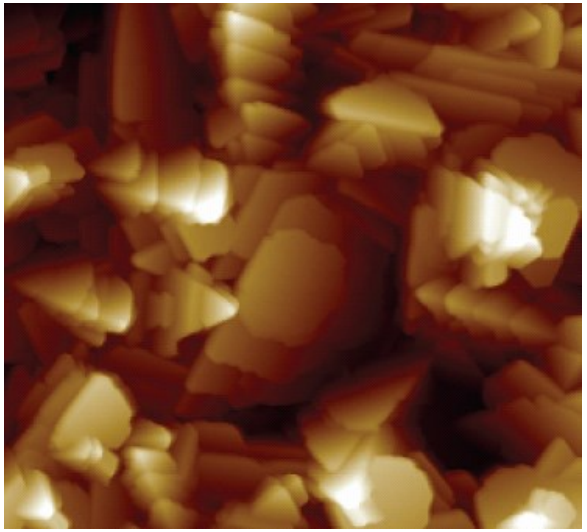
## Spring constant

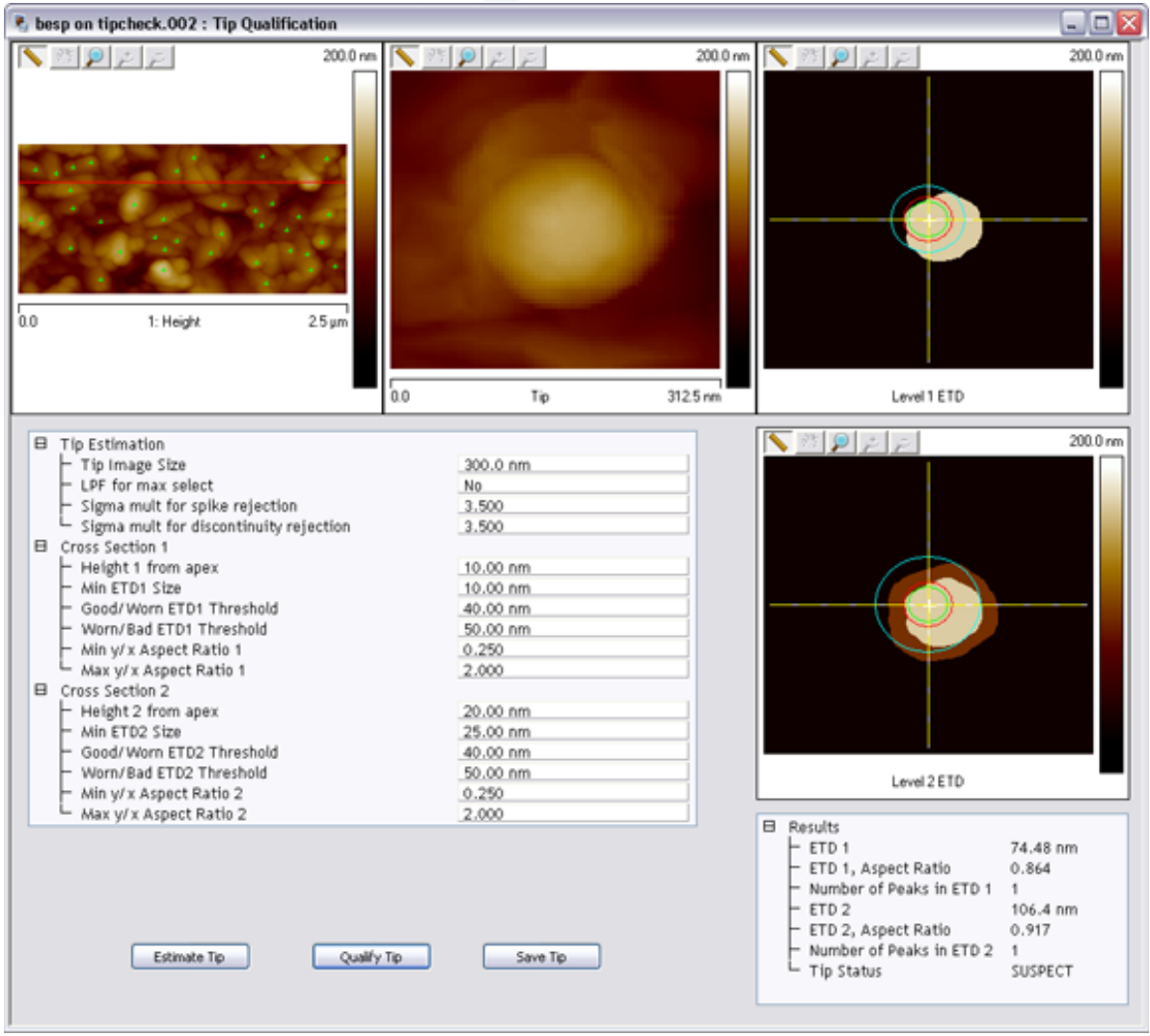
- Thermal tune
- Higher noise for stiff cantilevers




# Calibration

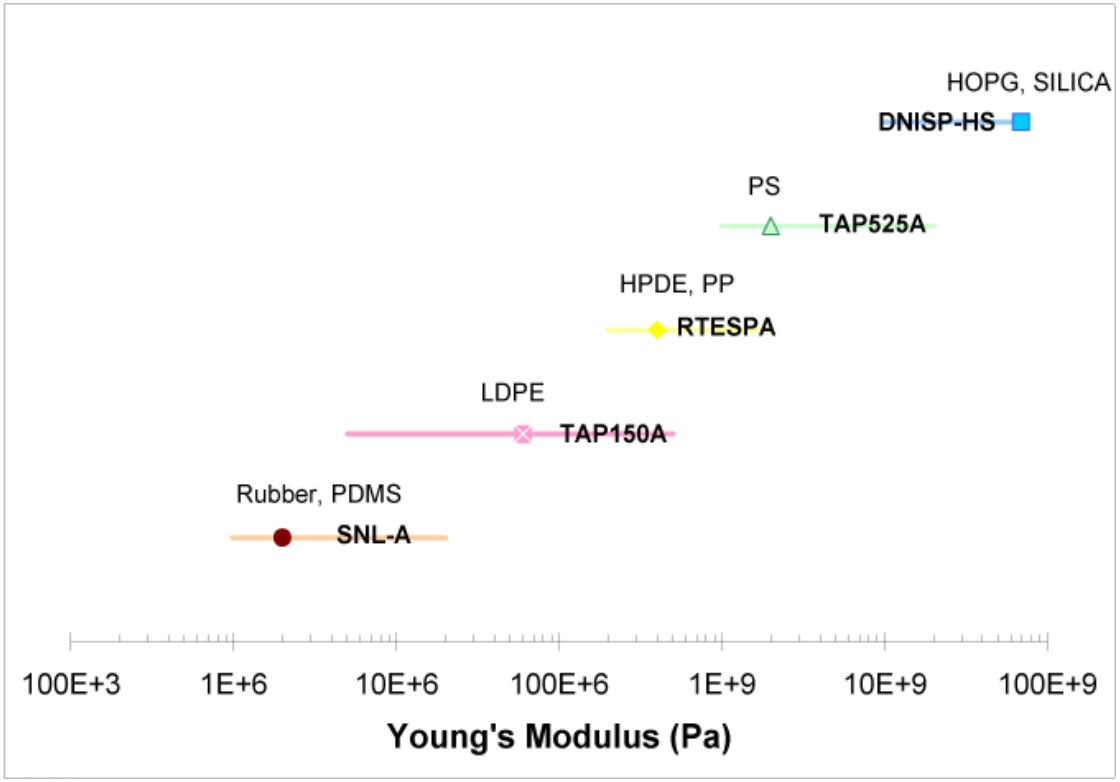
## Tip radius





# Probes for QNM

| Sample Modulus (E) | Probe | Nominal Spring Constant (k) |
|---|---|---|
| 1 MPa < E < 20 MPa | ScanAsyst-Air | 0.5 N/m |
| 5 MPa < E < 500 MPa | Tap150A, P/N MPP-12120-10 | 5 N/m |
| 200 MPa < E < 2000 MPa | Tap300A (RTESPA), P/N MPP-11120-10 | 40 N/m |
| 1 GPa < E < 20 GPa | Tap525A, P/N MPP-13120-10 | 200 N/m |
| 10 GPa < E < 100 GPa | DNISP-HS | 350 N/m |

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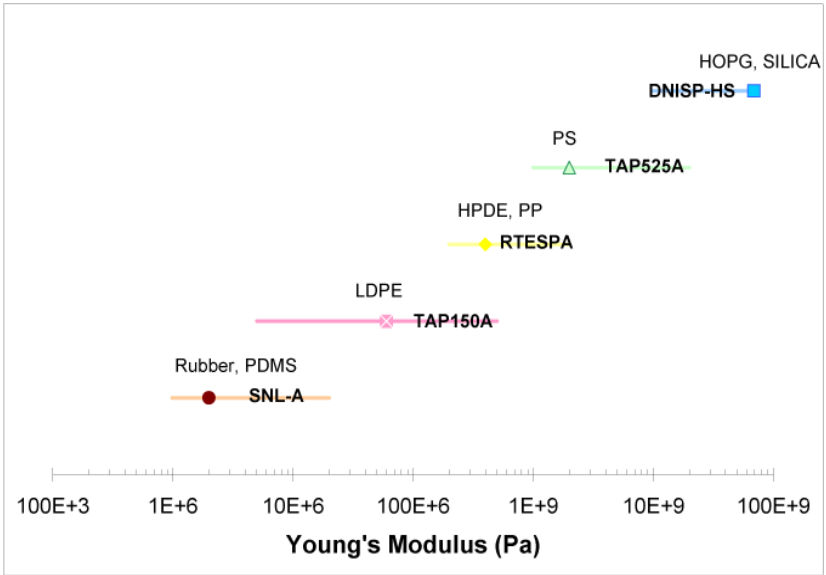
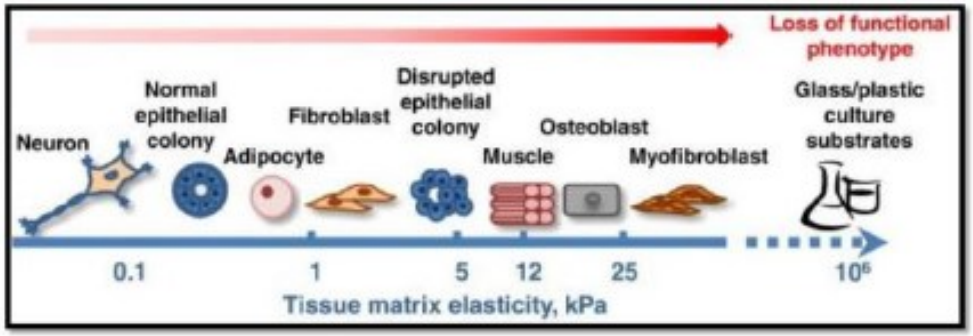
| 10 GPa < E < 100 GPa | DNISP-HS | 350 N/m |




# Relative method

## Advantages

- For soft and hard samples
- Avoids accumulated errors
- Faster calibration (no tip radius measurement)
- Spring constant not required for quantitative DMT modulus data

## Disadvantages

- Need to have reference sample with similar modulus to our unknow sample

1. Calibrate deflection sensitivity
2. Spring constant (Thermal tune) for Adhesion and Dissipation signal (optional)
3. Image reference sample and adjust Tip radius to make the measured Modulus equal the known value
4. Image sample. Adjust setpoint to match deformation depth used during imaging reference

# Absolute method

## Advantages

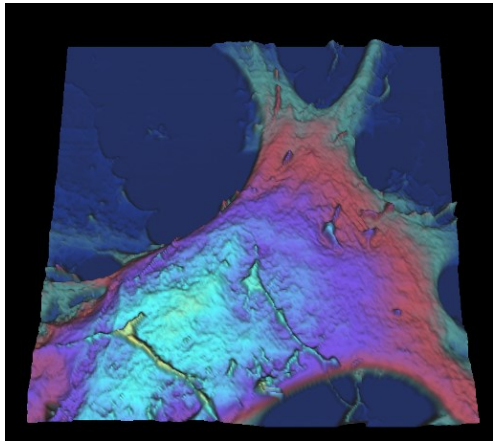
- No need for a reference sample

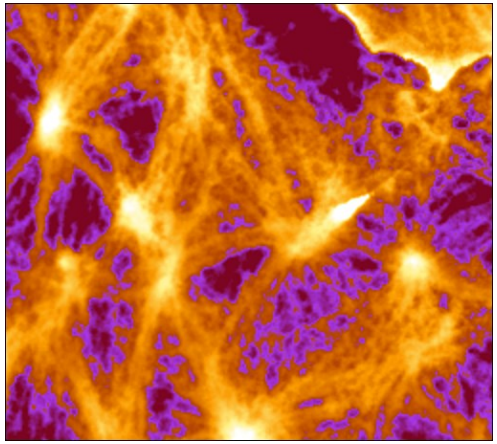
## Disadvantages

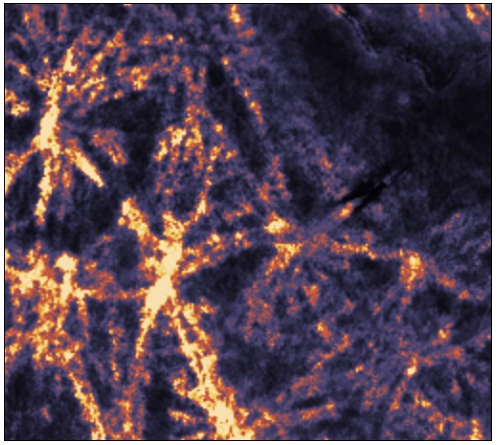
- Susceptible to accumulated errors
- Requires accurate tip radius and spring constant measurement
- Slower method

1. Calibrate deflection sensitivity
2. Spring constant (Thermal tune) for Adhesion and Dissipation signal
3. Image a rough sample to evaluate tip radius
4. Image a sample

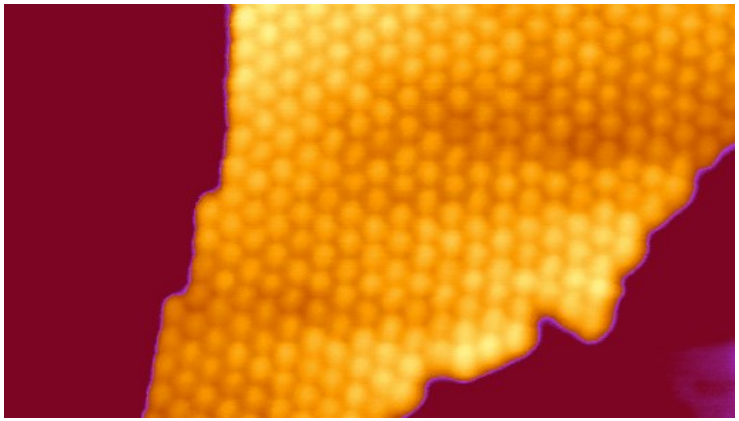
# Mechanical properties

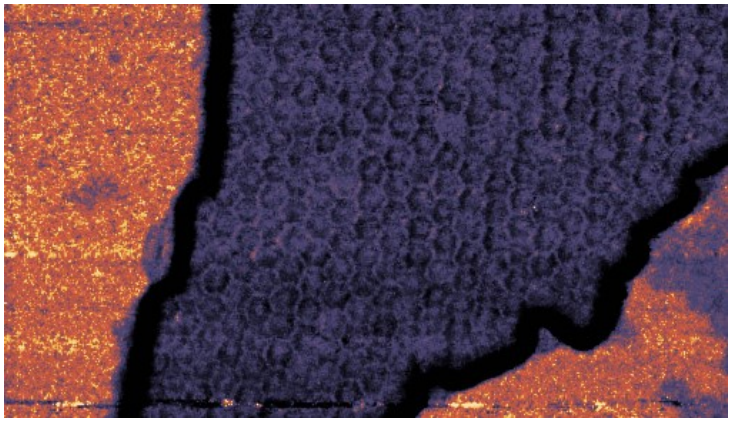


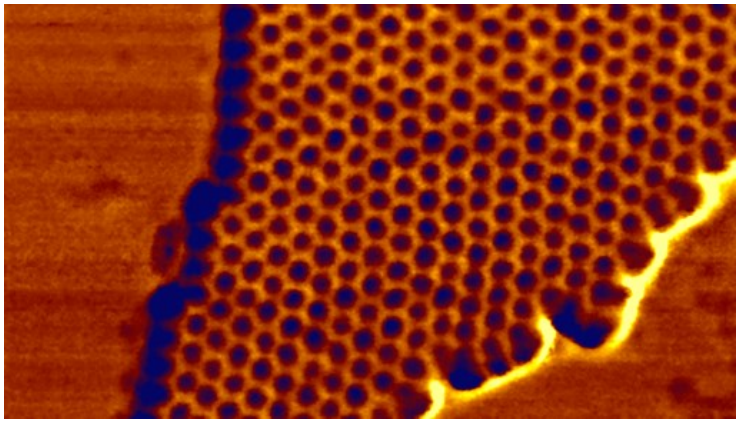




# Mechanical data







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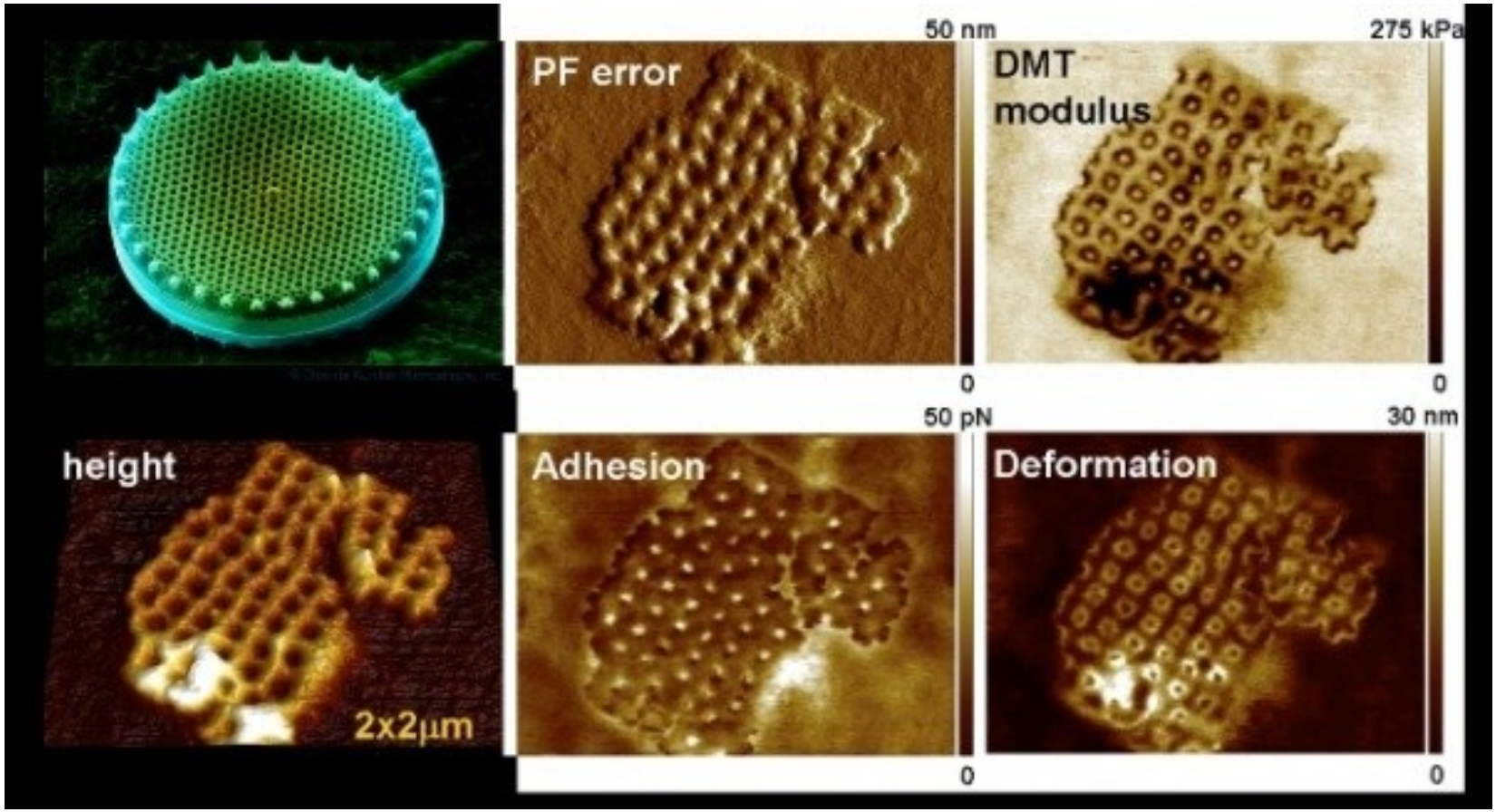
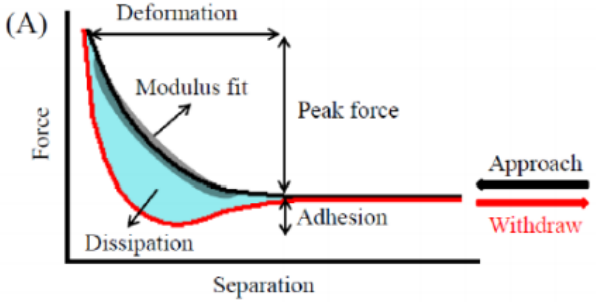
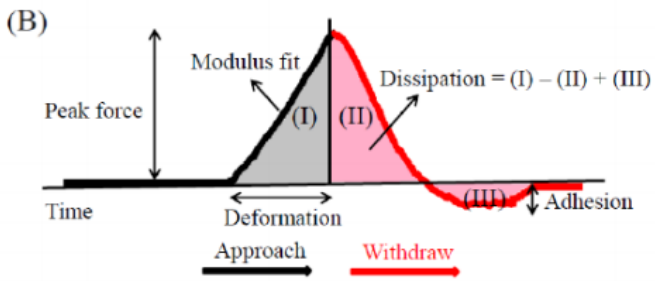
# Phytoplankton

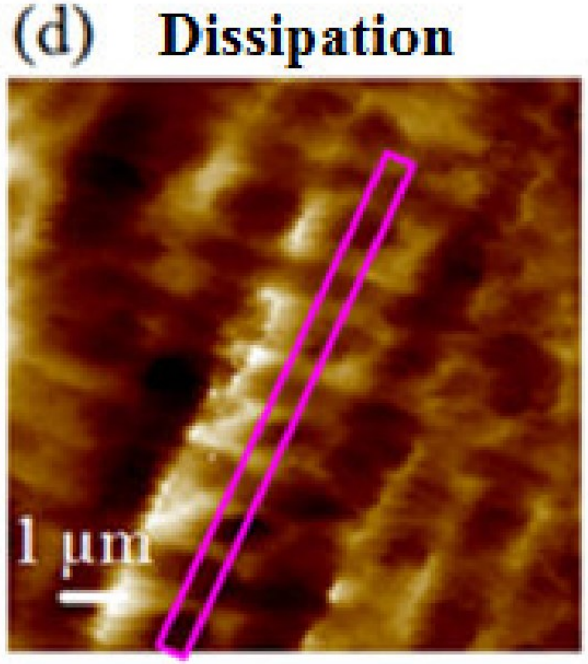


# PF QNM on cardyomyocytes

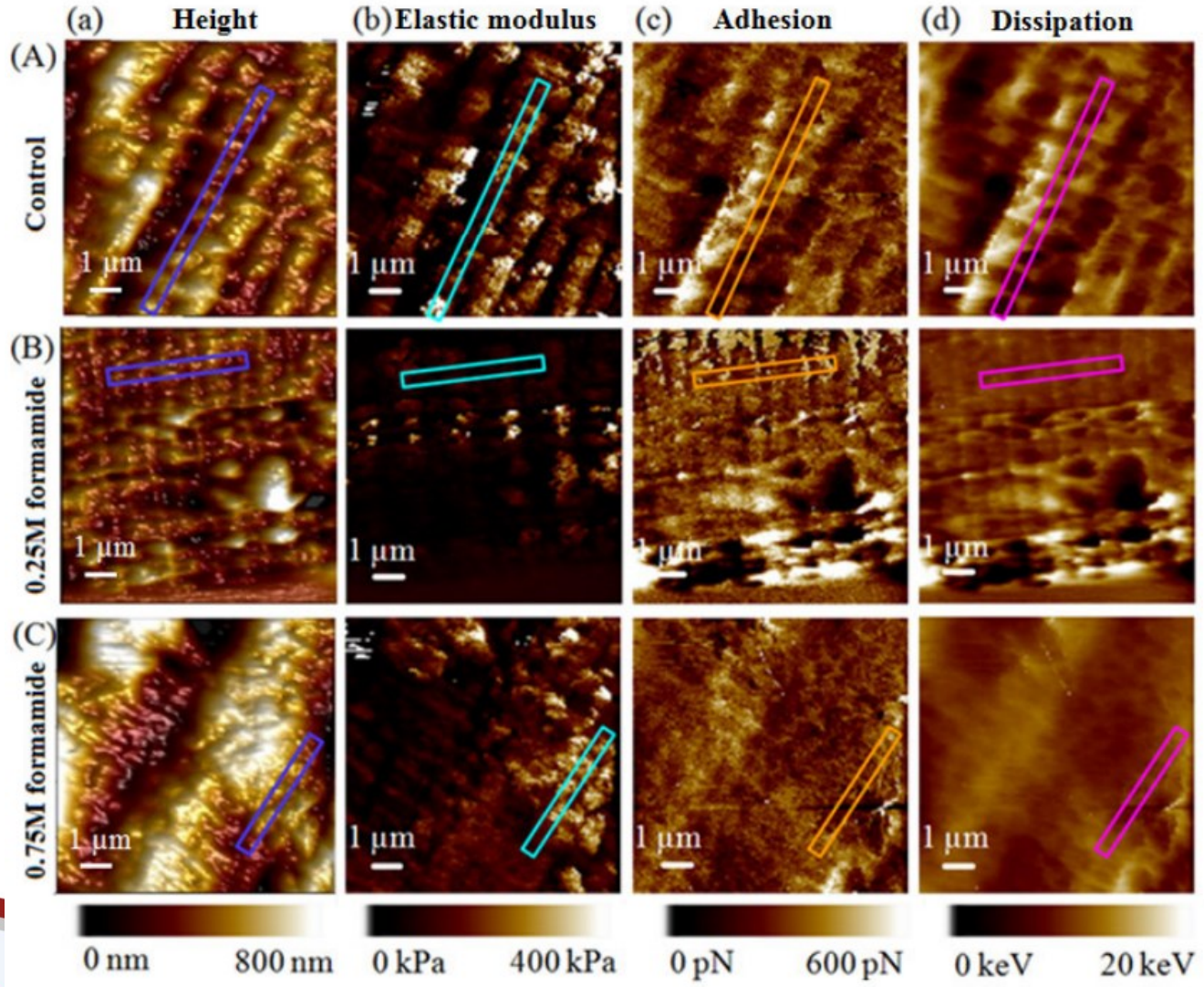
- **Biophysical properties of cardiomyocyte surface explored by multiparametric AFM**
- **dx.doi.org/10.1016/j.jsb.2017.03.001**



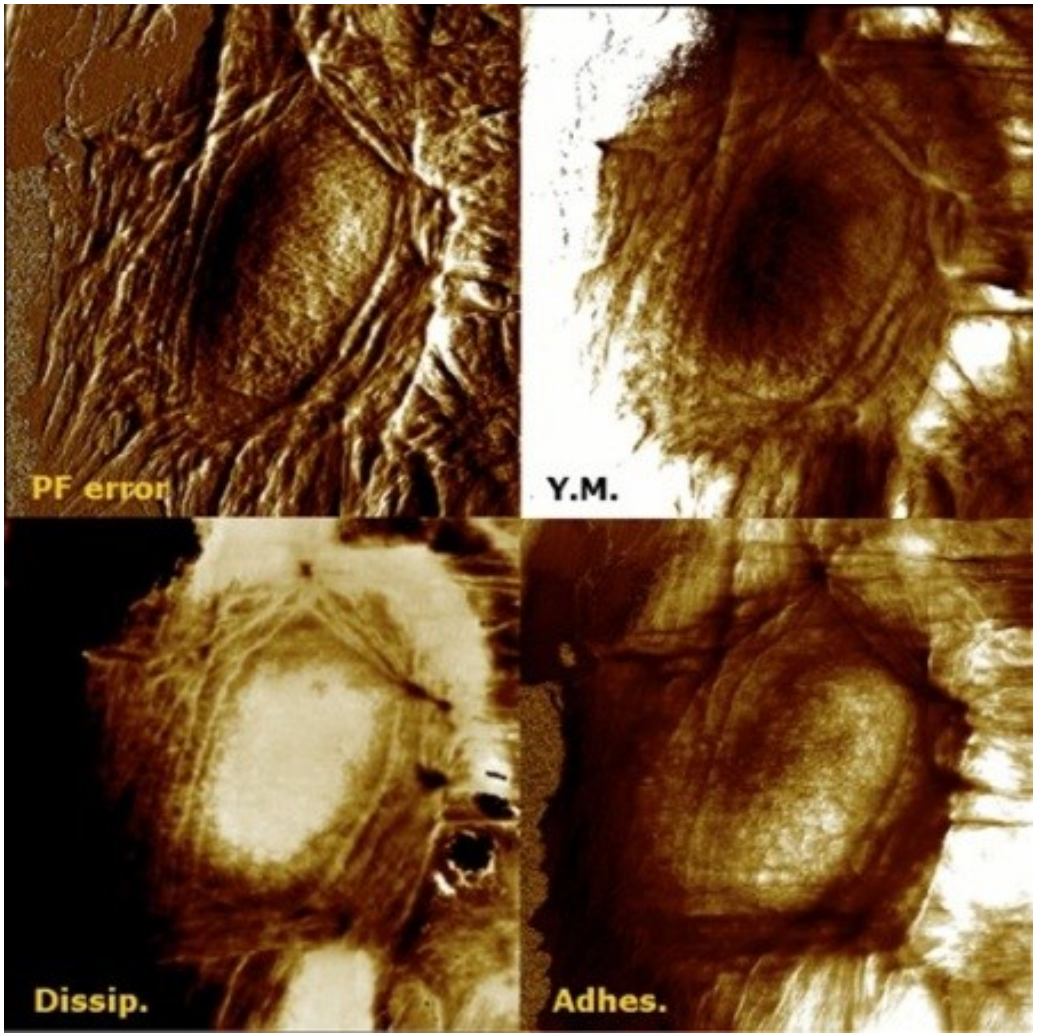



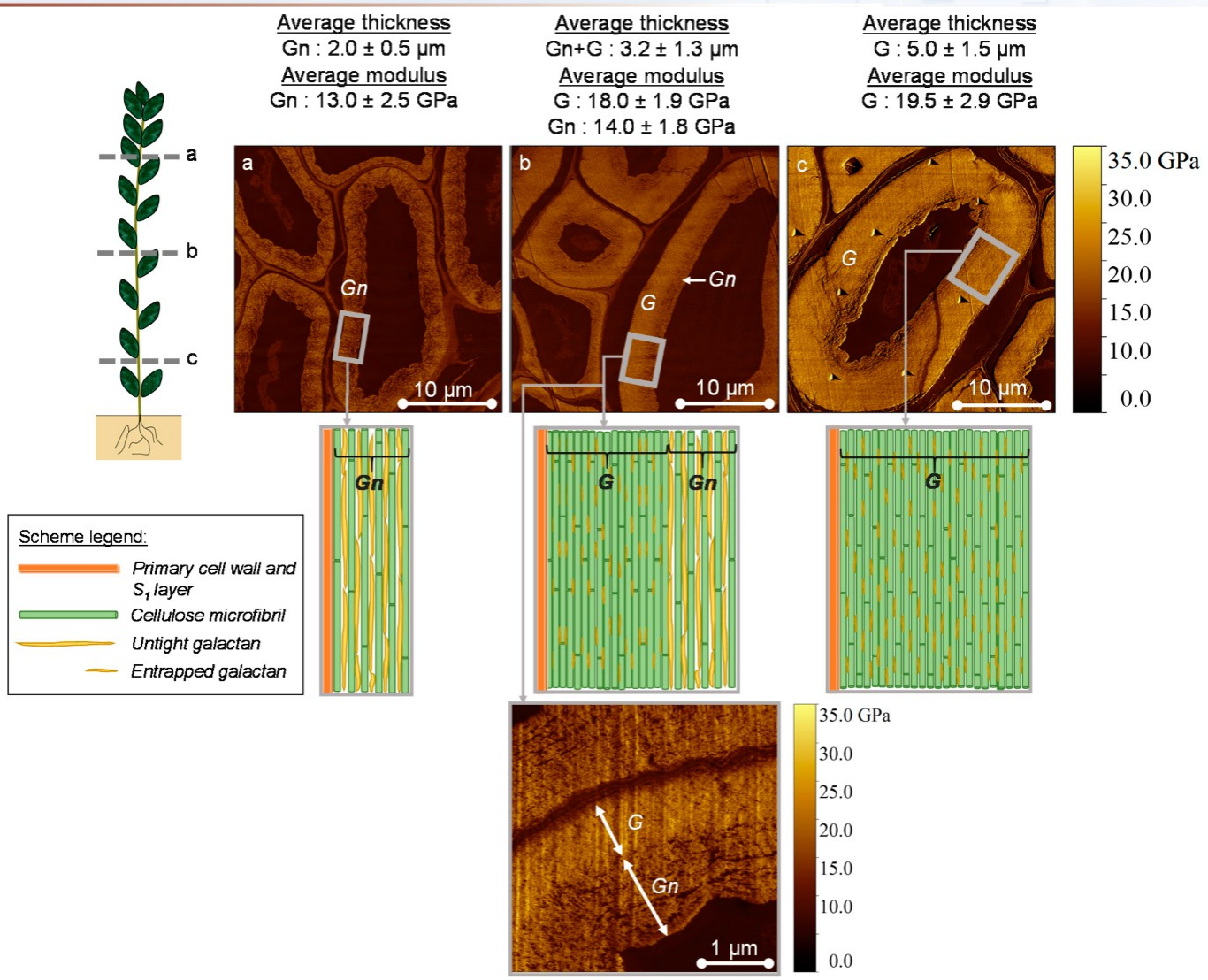
# PF QNM on cardyomyocytes



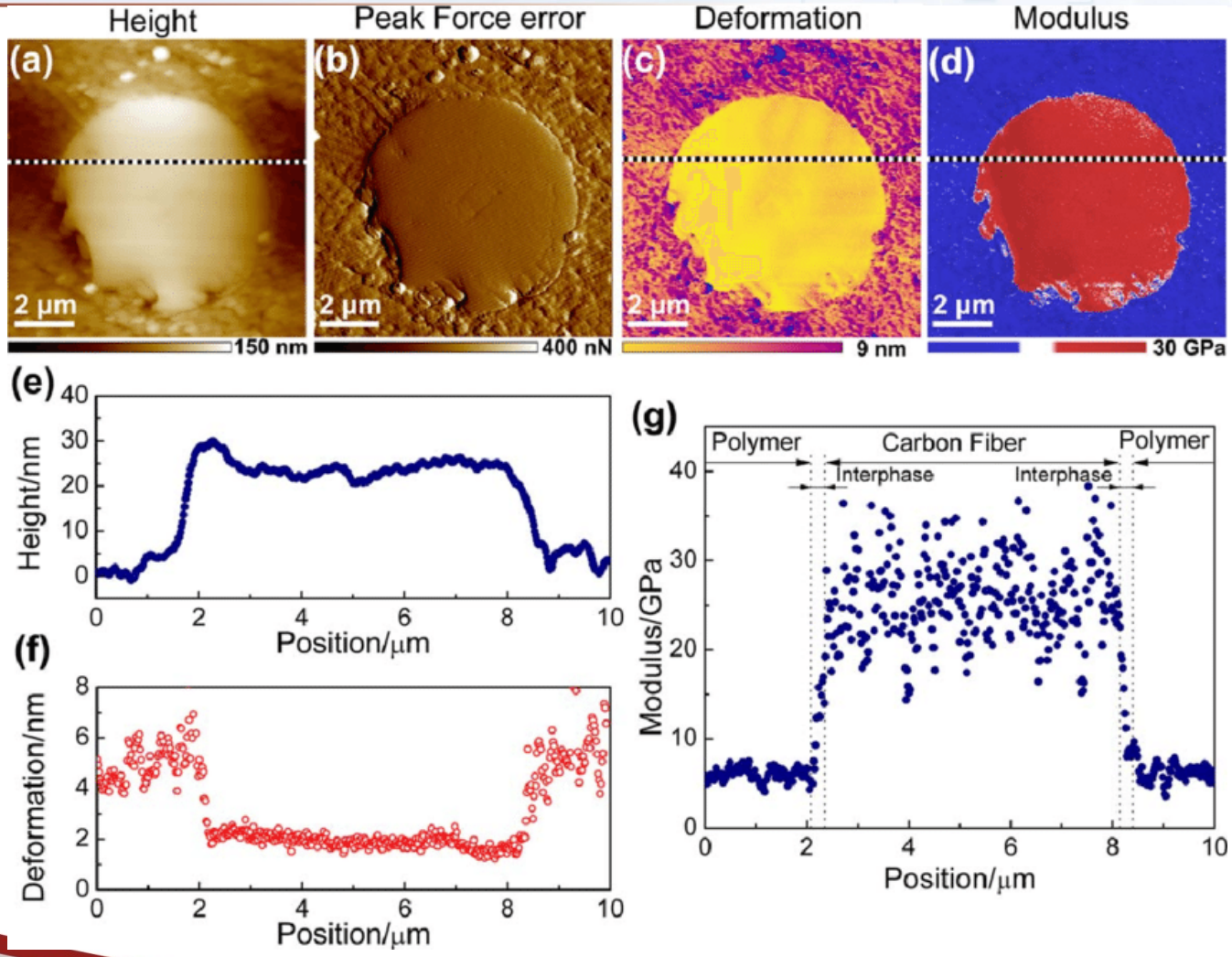
# Living HaCat cells



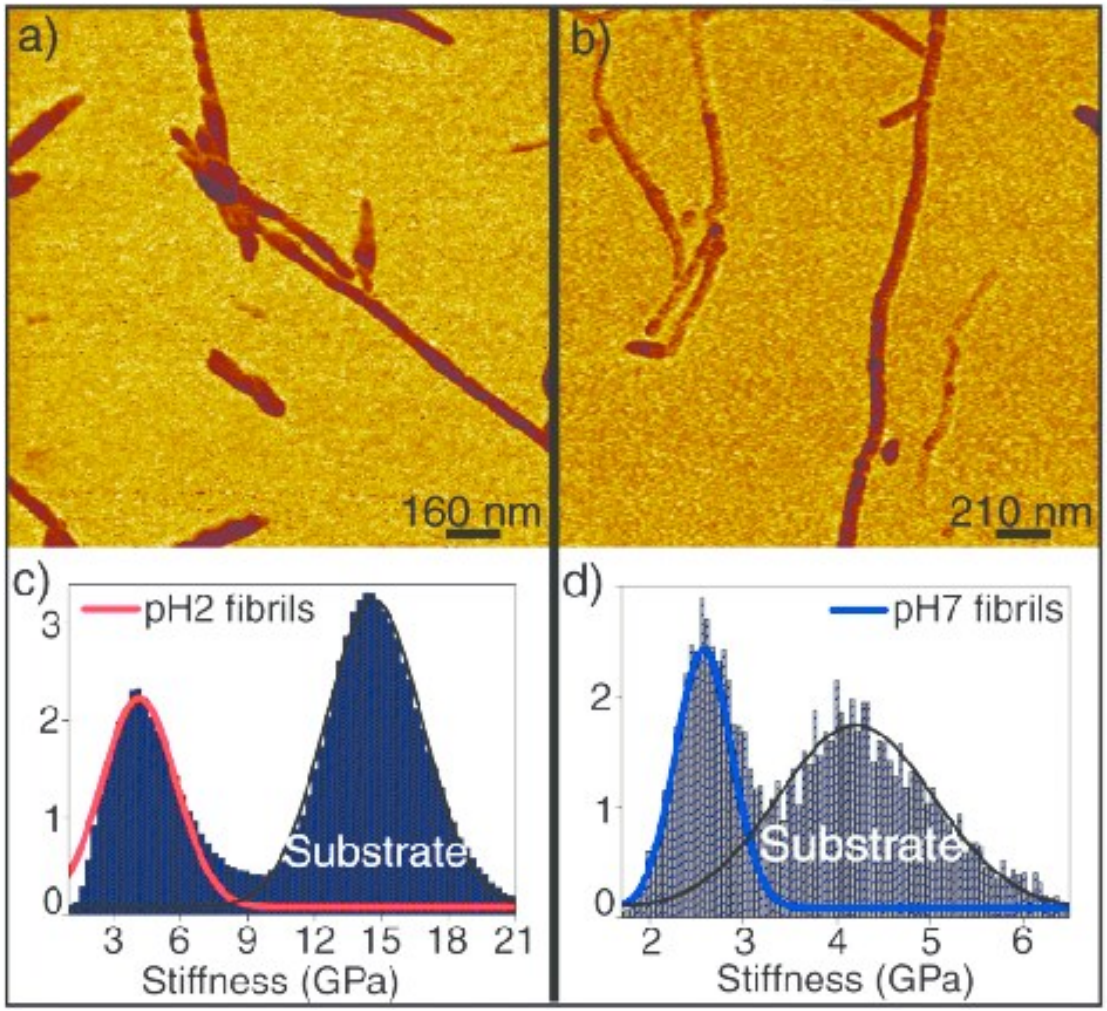
# Flax fibers


Average thickness
Gn : 2.0 ± 0.5 µm
Average modulus
Gn : 13.0 ± 2.5 GPa
Average thickness
Gn+G : 3.2 ± 1.3 µm
Average modulus
G : 18.0 ± 1.9 GPa
Gn : 14.0 ± 1.8 GPa
Average thickness
G : 5.0 ± 1.5 µm
Average modulus
G : 19.5 ± 2.9 GPa
a
b
c
Gn
G
10 µm
35.0 GPa
30.0
25.0
20.0
15.0
10.0
0.0
Scheme legend:
Primary cell wall and S1 layer
Cellulose microfibril
Untight galactan
Entrapped galactan
1 µm

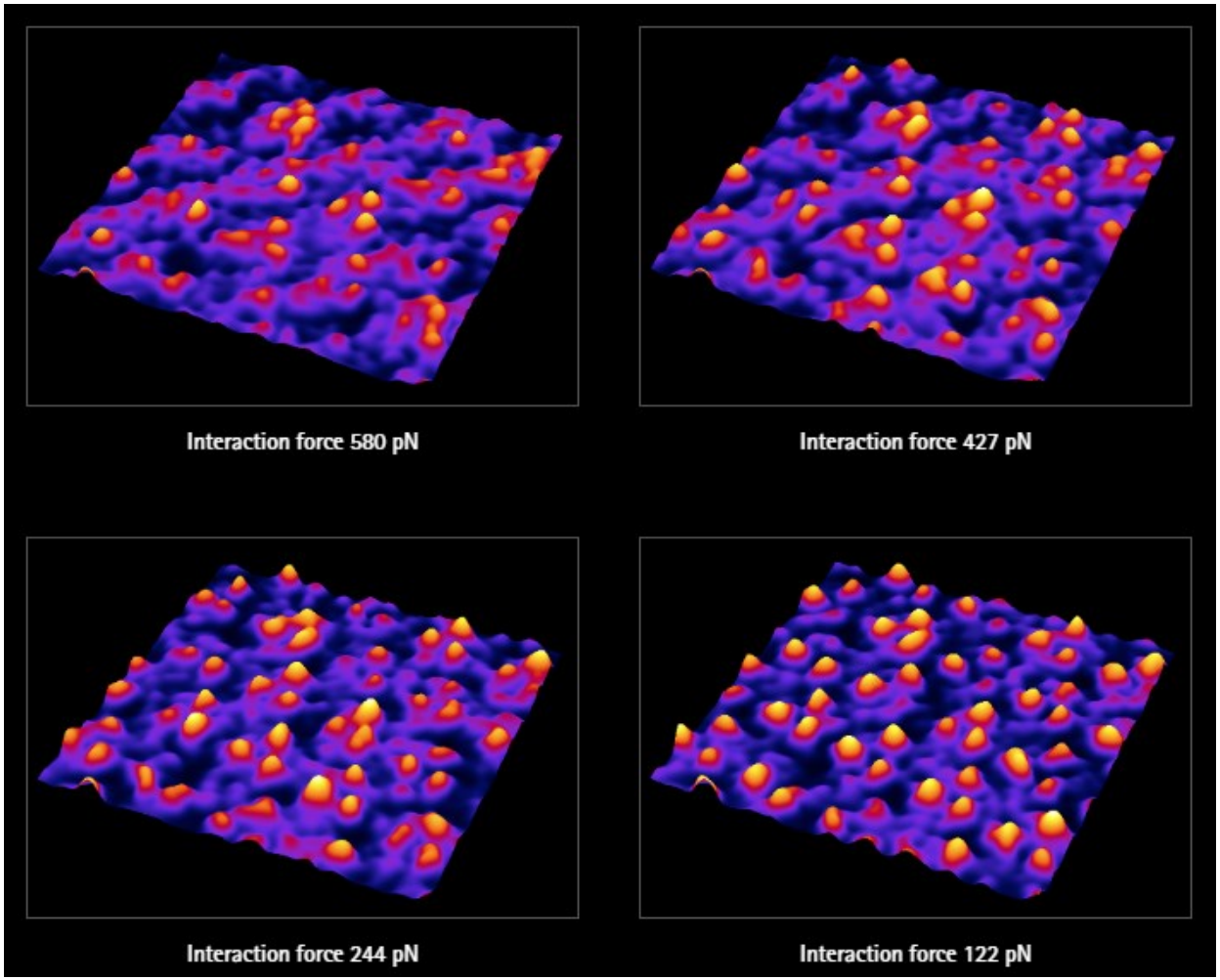
# Carbon fiber



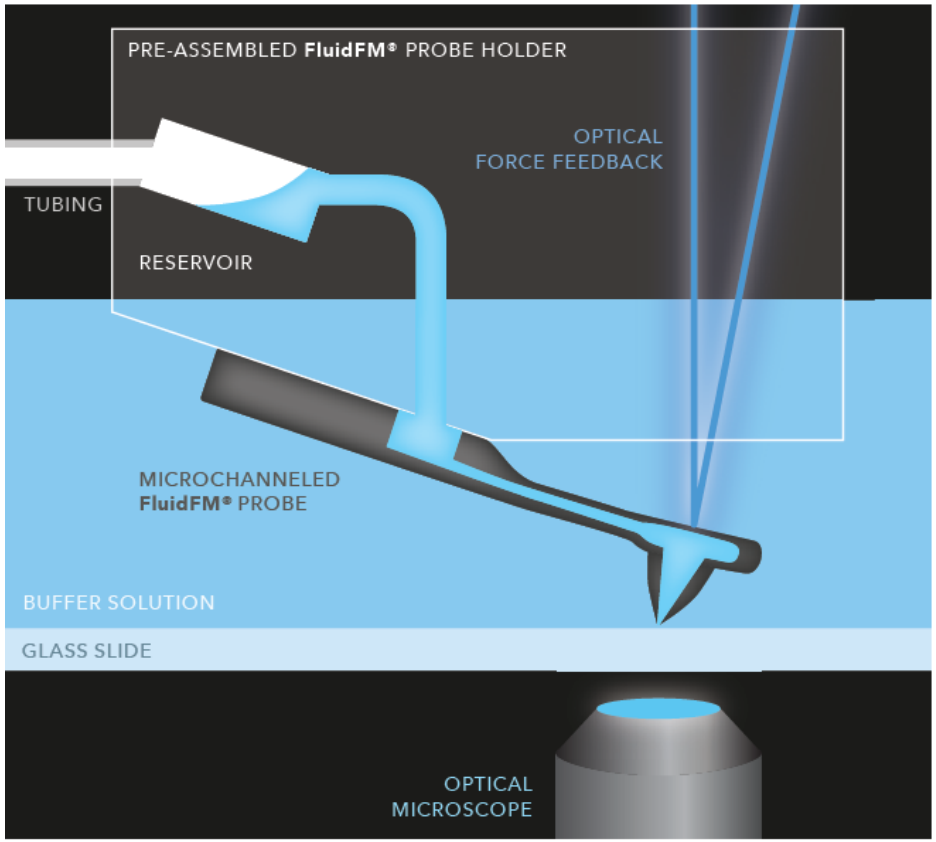
# Fibrils


a)
160 nm
b)
210 nm
c)
pH2 fibrils
Substrate
Stiffness (GPa)
d)
pH7 fibrils
Substrate
Stiffness (GPa)

# Effect of force for topography

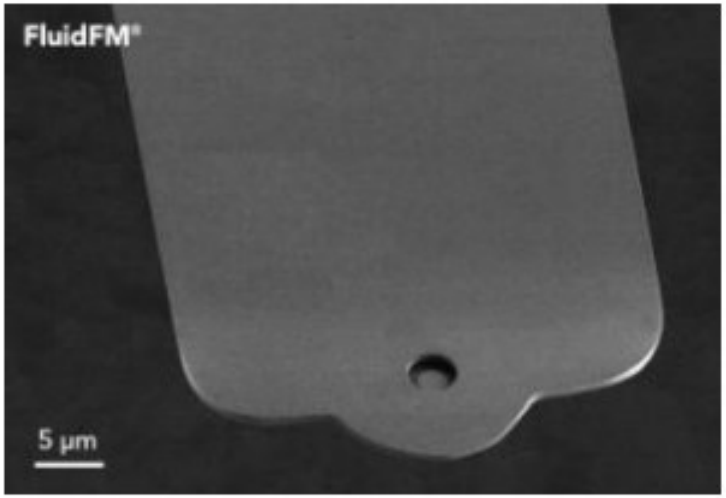


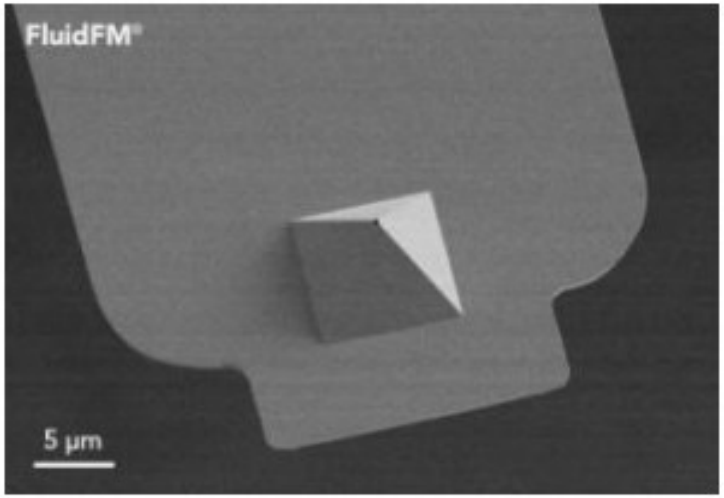
# FluidFM

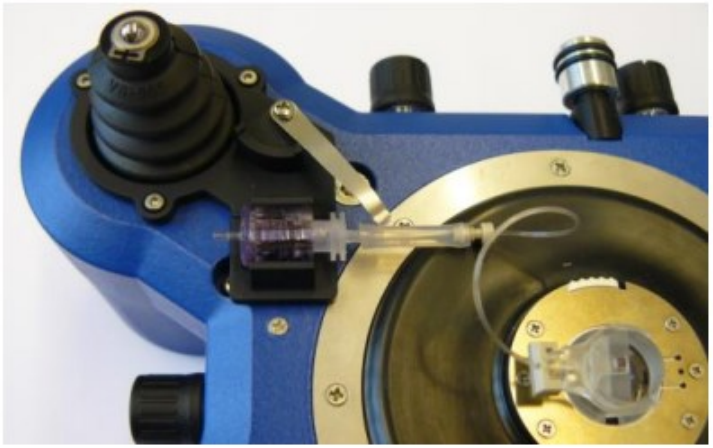



**REINVENTING THE MICROPIPETTE WITH THE FluidFM® TECHNOLOGY.**
Symbiosis of nanofluidics and force microscopy.

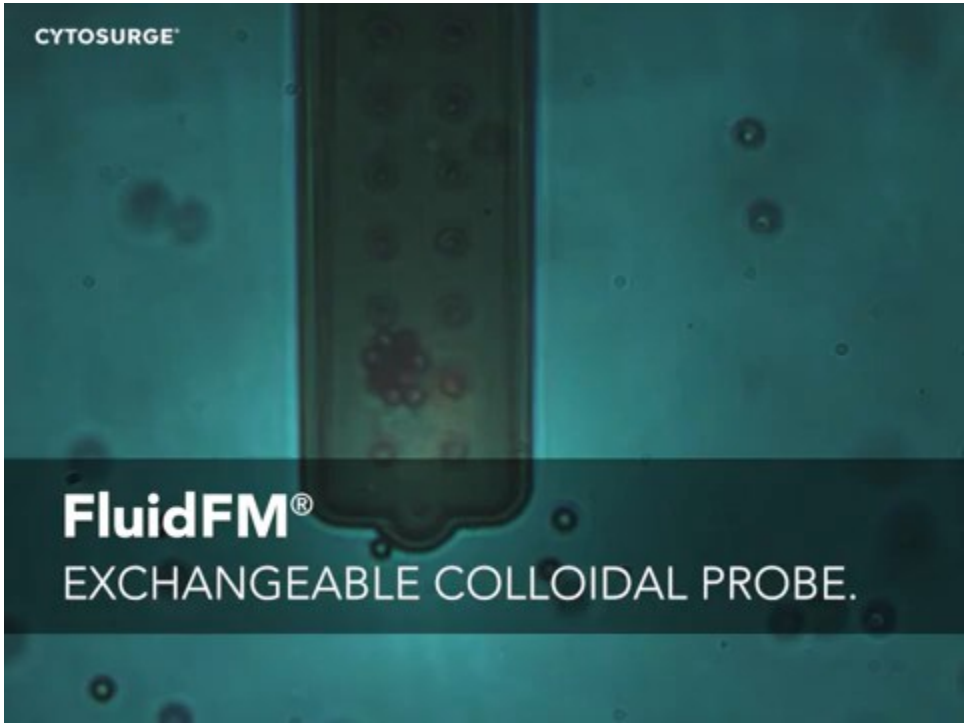
# FluidFM



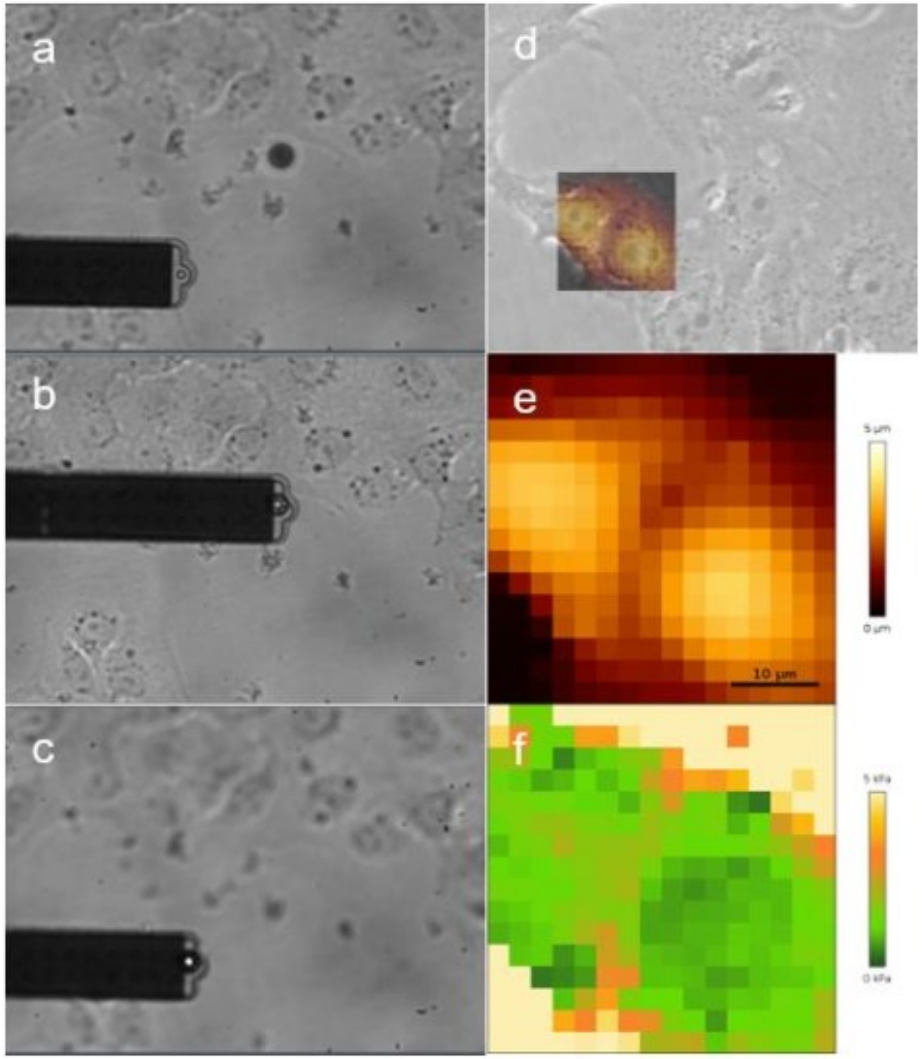



# FluidFM



# FluidFM

***Fig. 5: Pick-up of a bead for Force Mapping on living cells.***
*(a,b,c): Phase contrast images of living vero cells, the FluidFM micropipette and an 11 µm polystyrene bead in front of it (a), during (b) and after pickup (c). (d) Overlay of optical image with Height map using the polystyrene bead. (e, f) Height and apparent stiffness map of two cells.*



# FluidFM

# FluidFM

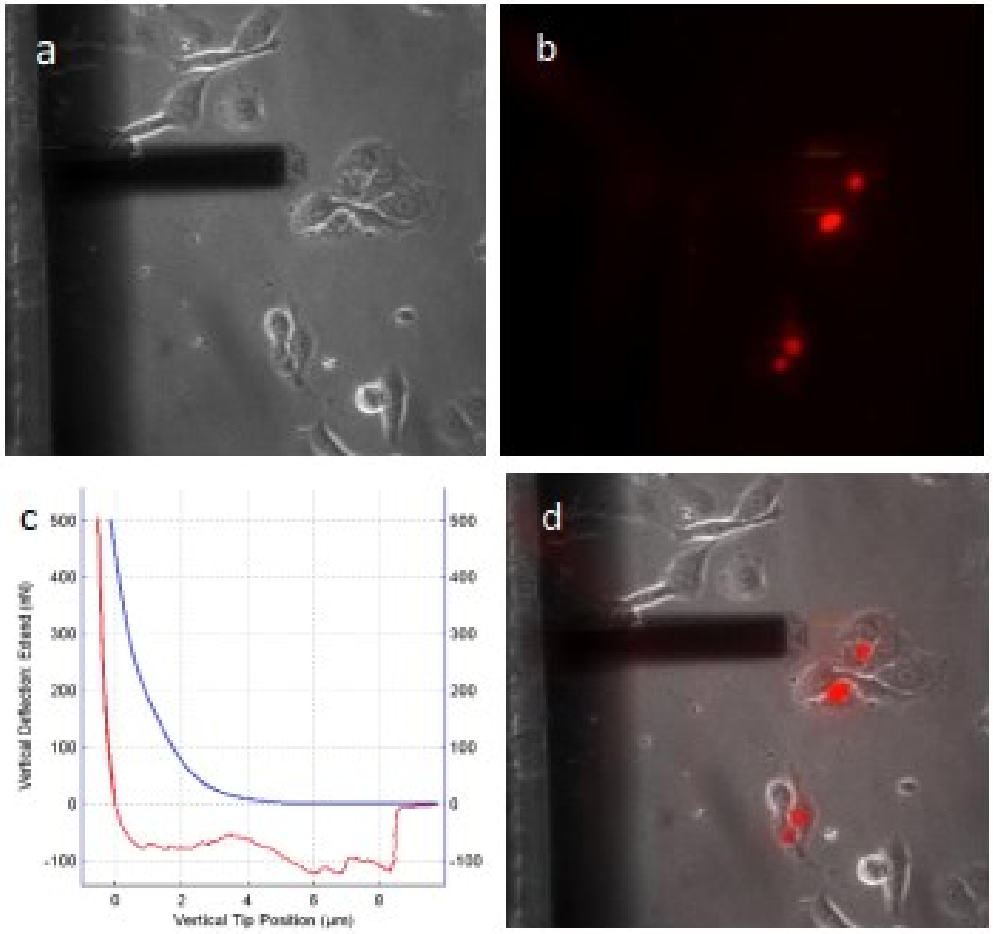
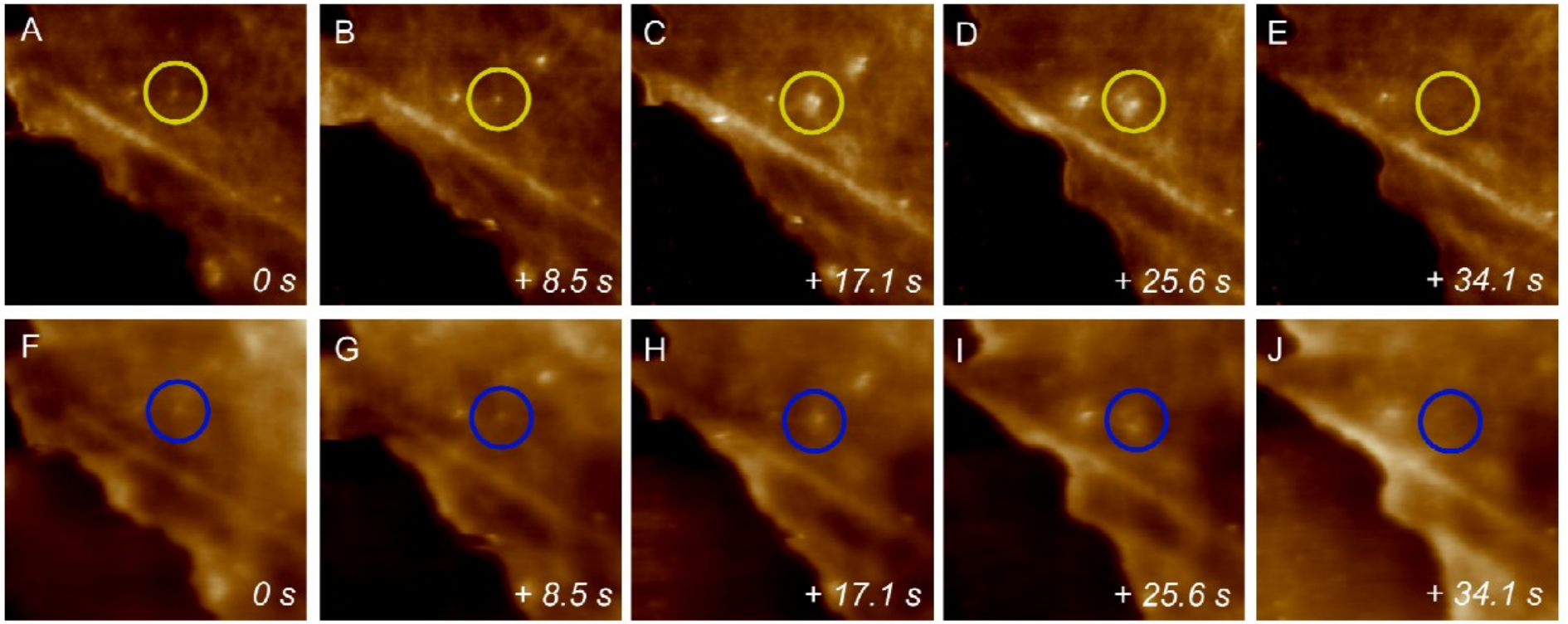



***Fig. 6: Injection experiment*** *(a) Phase contrast image (b) Fluorescence image after injection of propidium iodide (c) Force distance curve during injection (d) Overlay of phase and fluorescence image to demonstrate nucleus position*

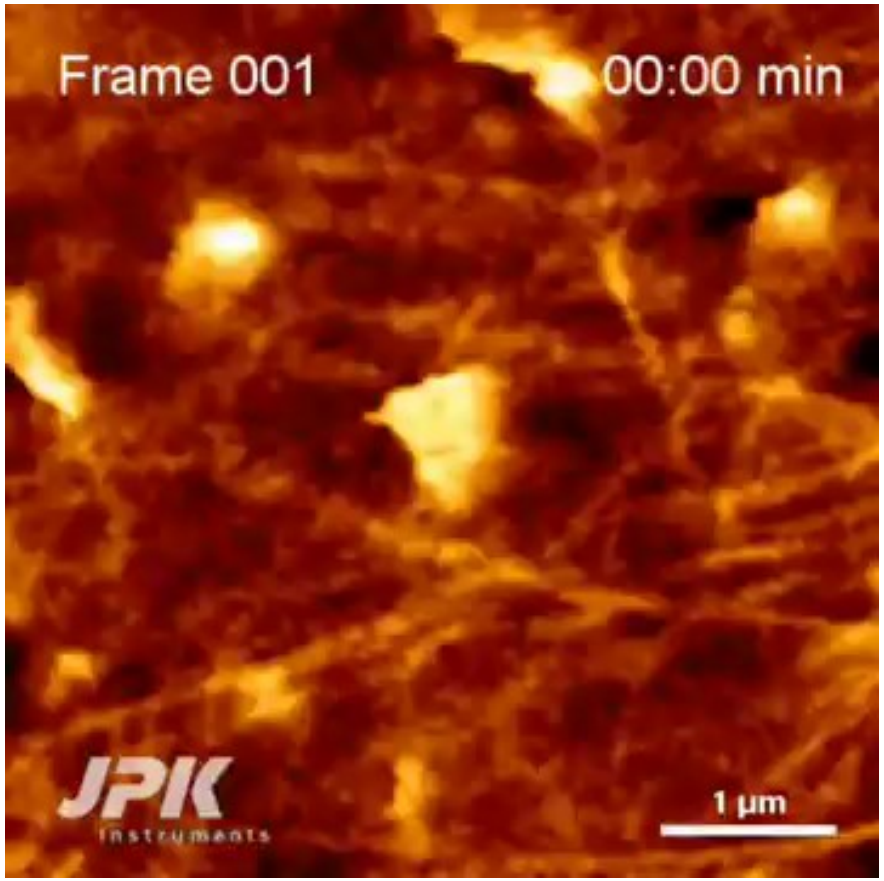
# High speed AFM

- Molecular (self)assembly
- Molecular conformational changes
- Enzymatic interaction
- Diffusion processes
- Life cells
- Thermal effects

# High speed AFM

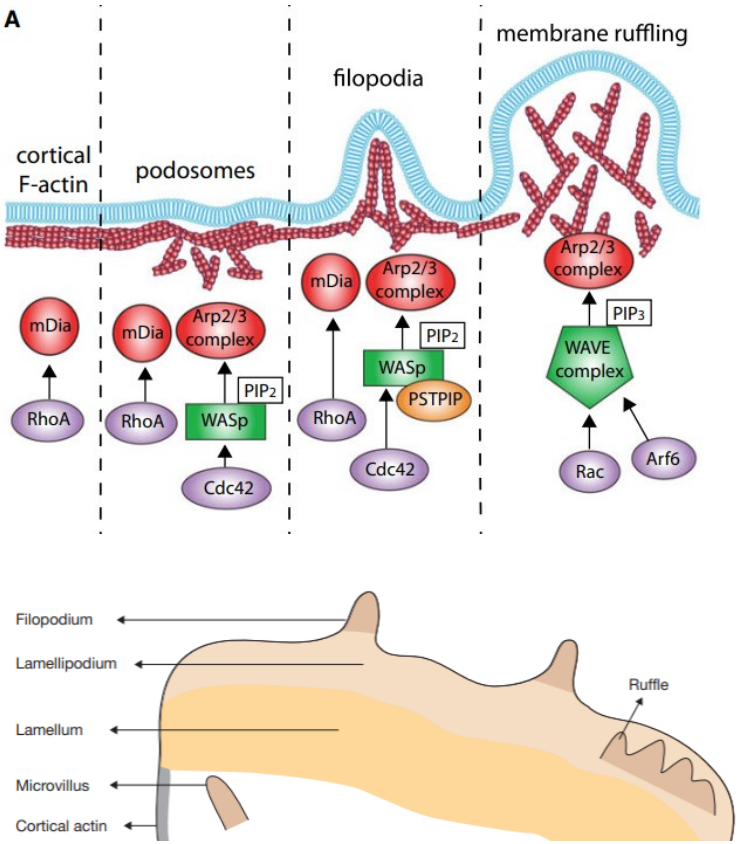


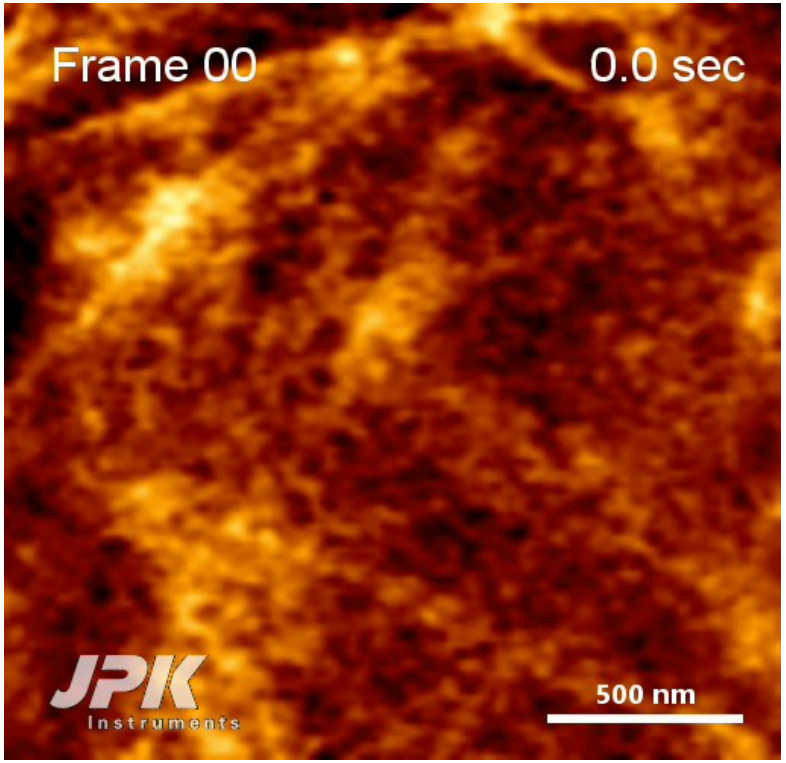
# Cell dynamics in living CHO cells




Phase; 5×5µm²; 256×256 Pixels; DMEM medium (37°C)
Linerate: 48 lines/s; Playback factor 10x

# Cell dynamics in living CHO cells

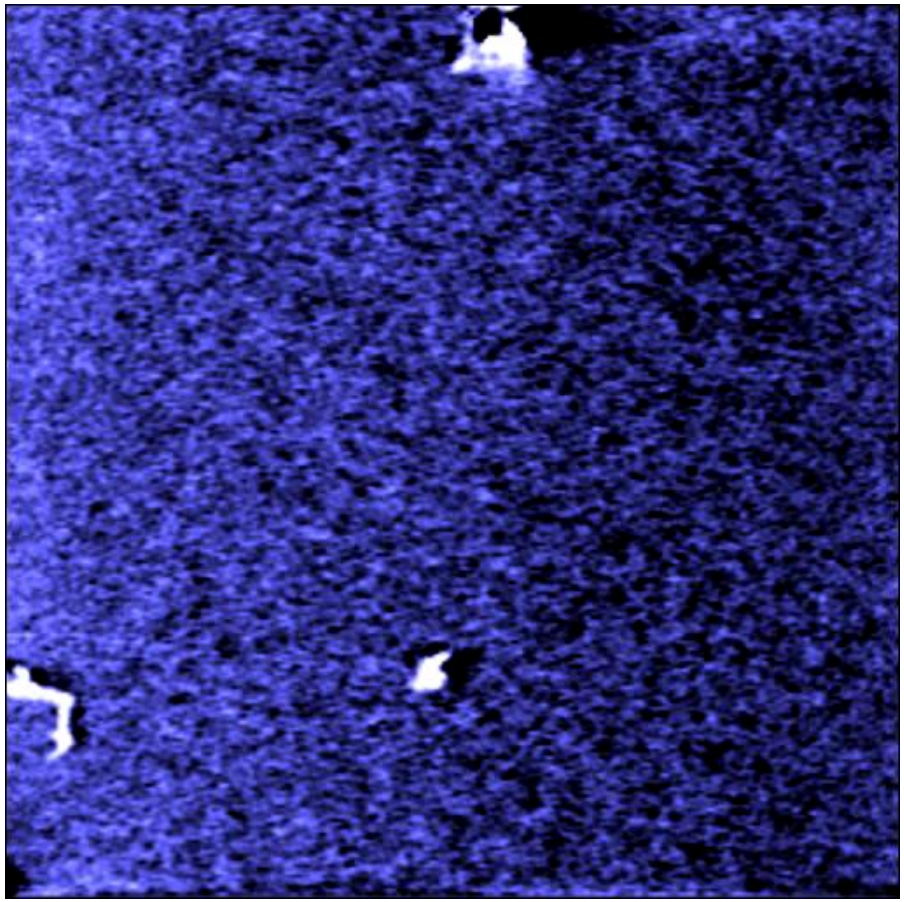



Phase; 2×2µm²; 128×128 Pixels; DMEM medium (37°C)
Linerate: 120 lines/s; Playback factor 5x

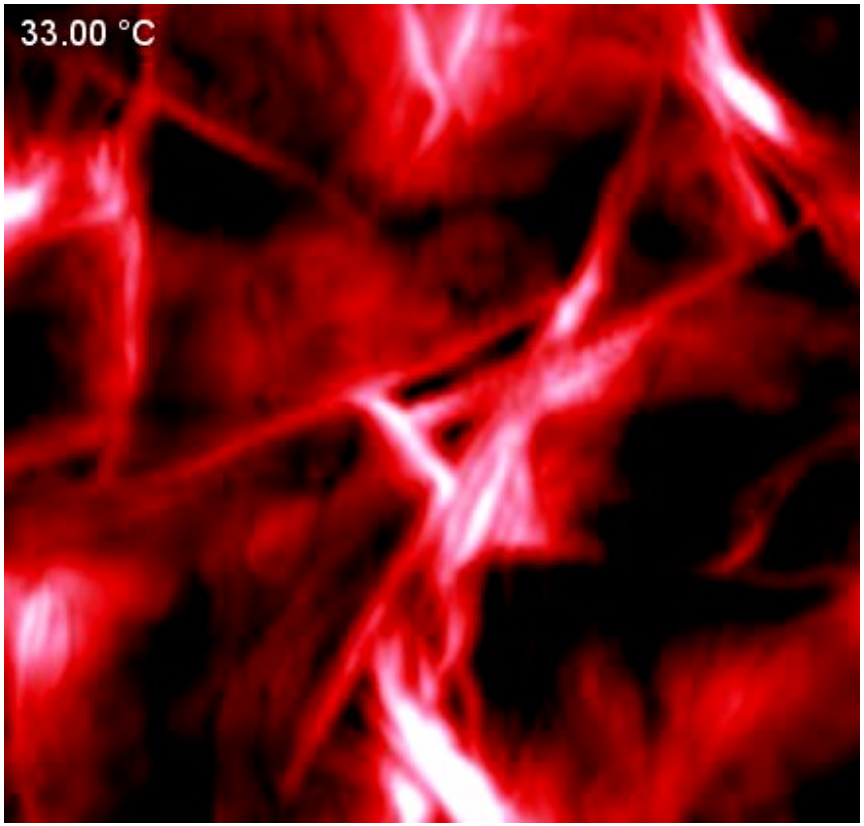
# High speed AFM

- **Real time crystallization of polymer film PHB/V**



# High speed AFM

- **Phase transition in polycaprolactone**



# Thank you for your attention