A direct and at nanometer scale study of electrical charge distribution on membranes of alive cells.

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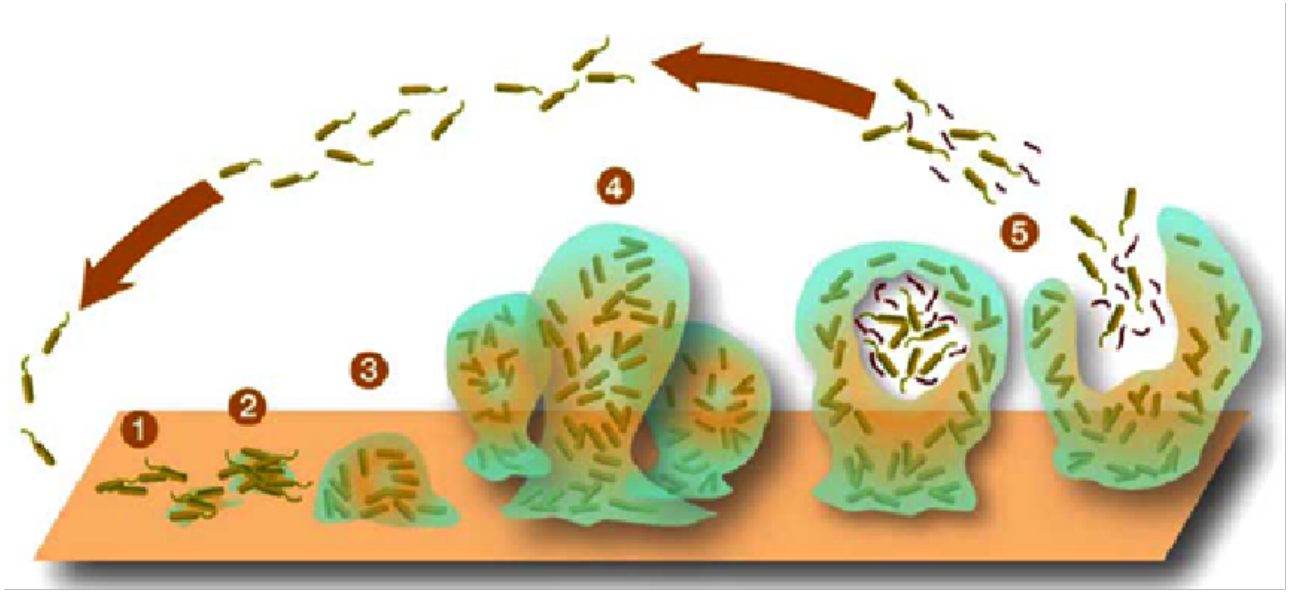
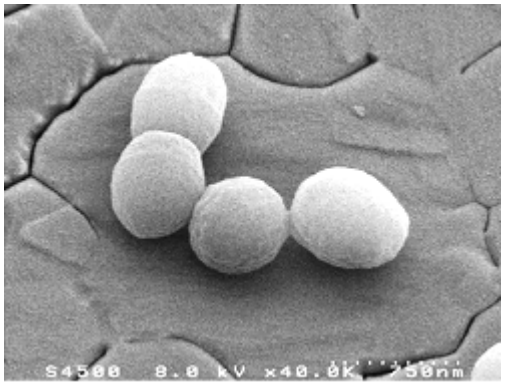
ANR
cnrs
UNIVERSITÉ PARIS SUD
Comprendre le monde, construire l'avenir
université PARIS-SACLAY

Outline :

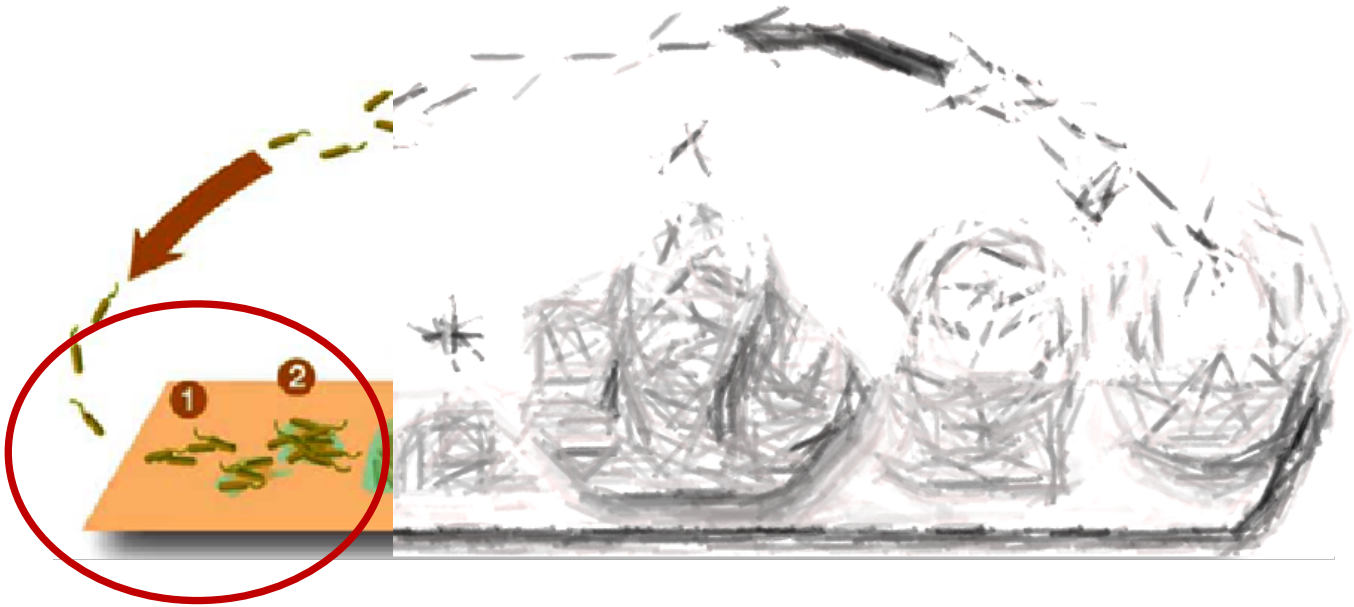
- ✓ Introduction
- ✓ Principle of measurement
- ✓ Experimental
- ✓ Results #1: ‘’*fast*’’ regime
- ✓ Results #2: ‘’*slow*’’ regime
- ✓ Next future
- ✓ Conclusion

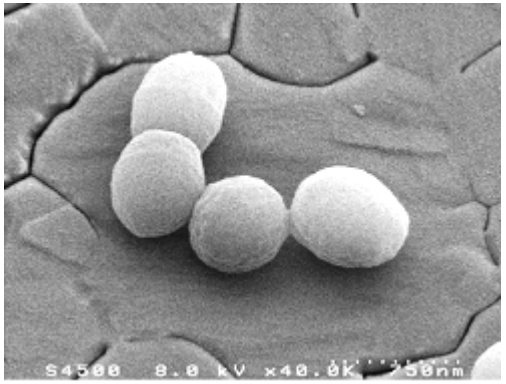
Bacterial adhesion and formation of biofilms



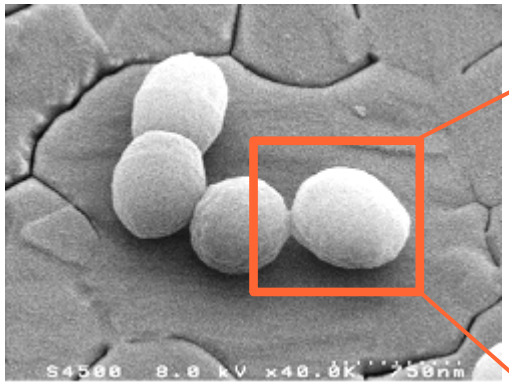


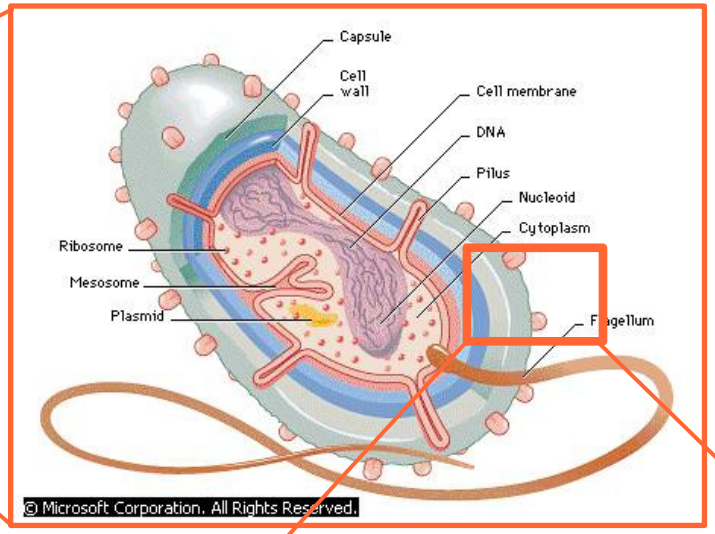
Bacterial adhesion and formation of biofilms

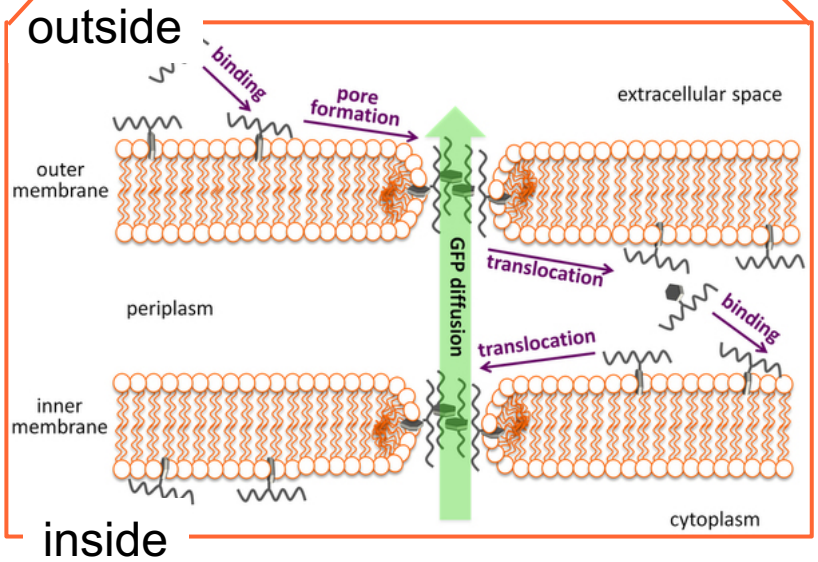




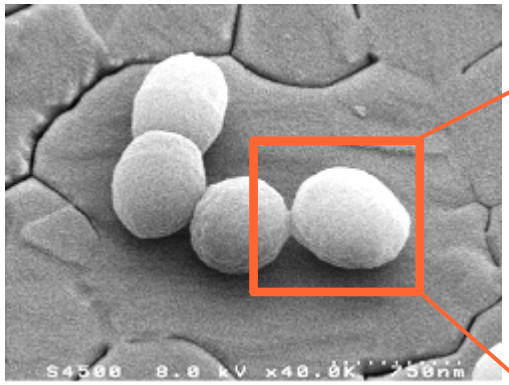
Introduction

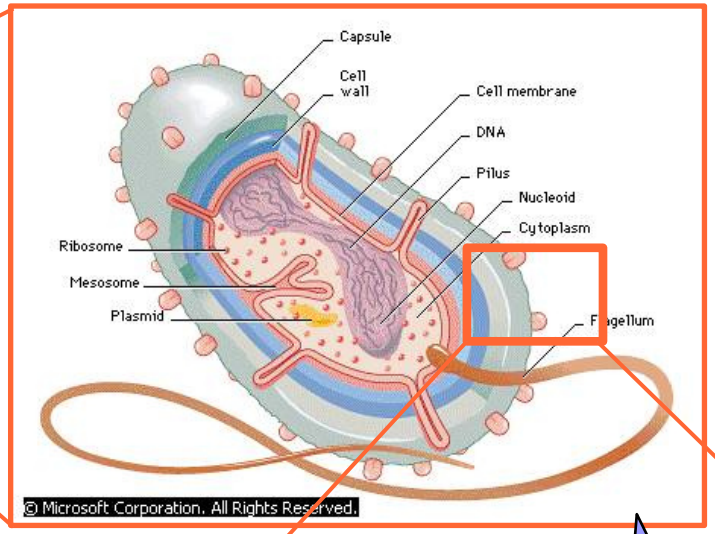




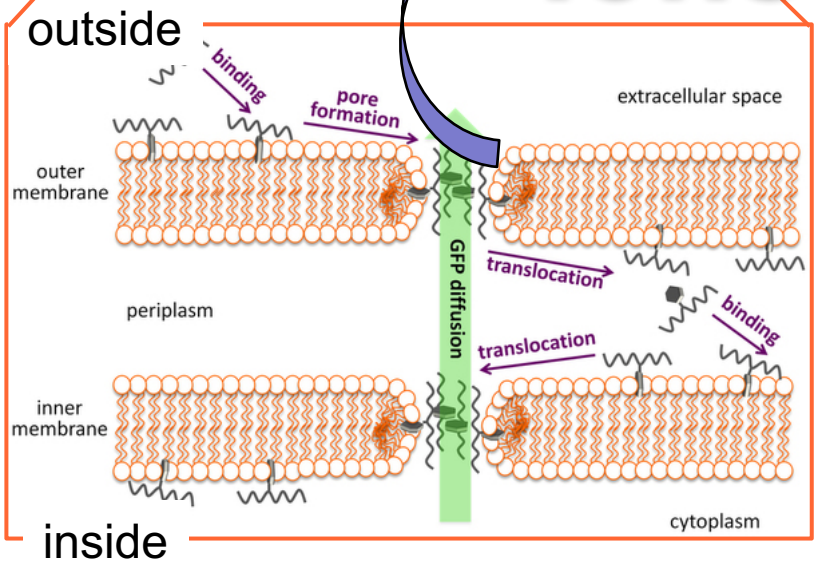


Introduction

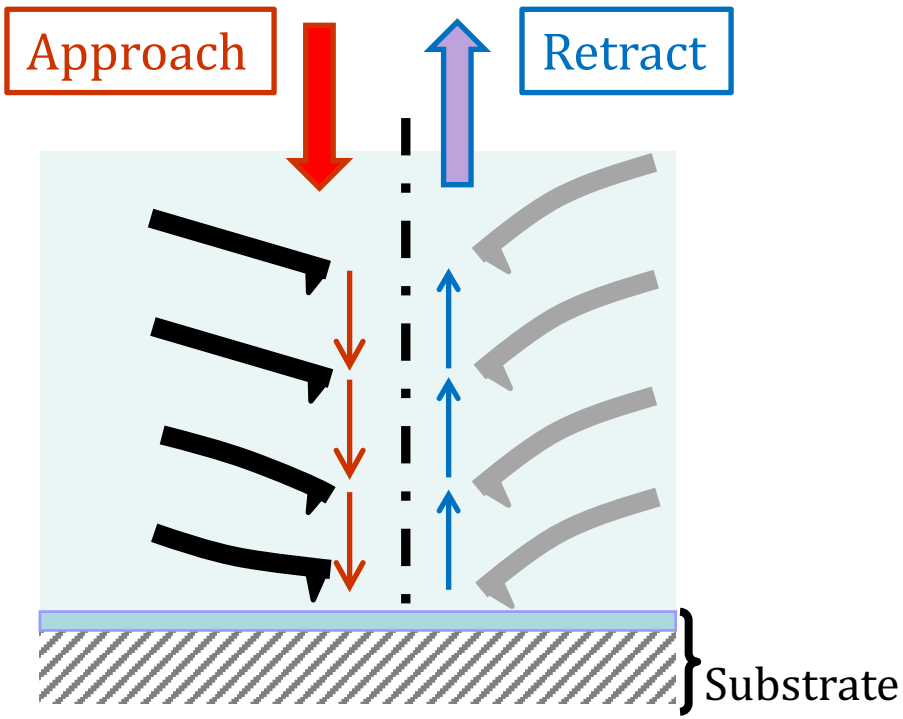


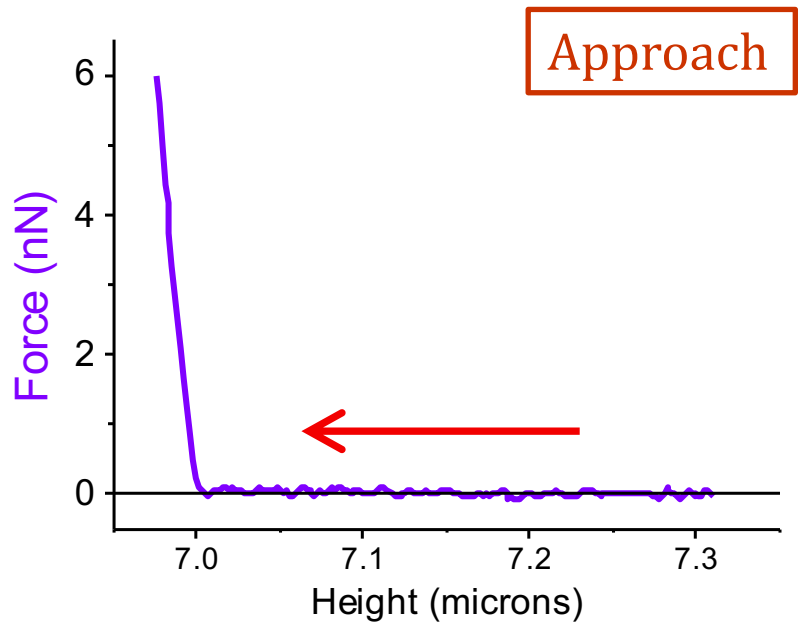


Ions ?



AFM: Atomic Force Microscopy





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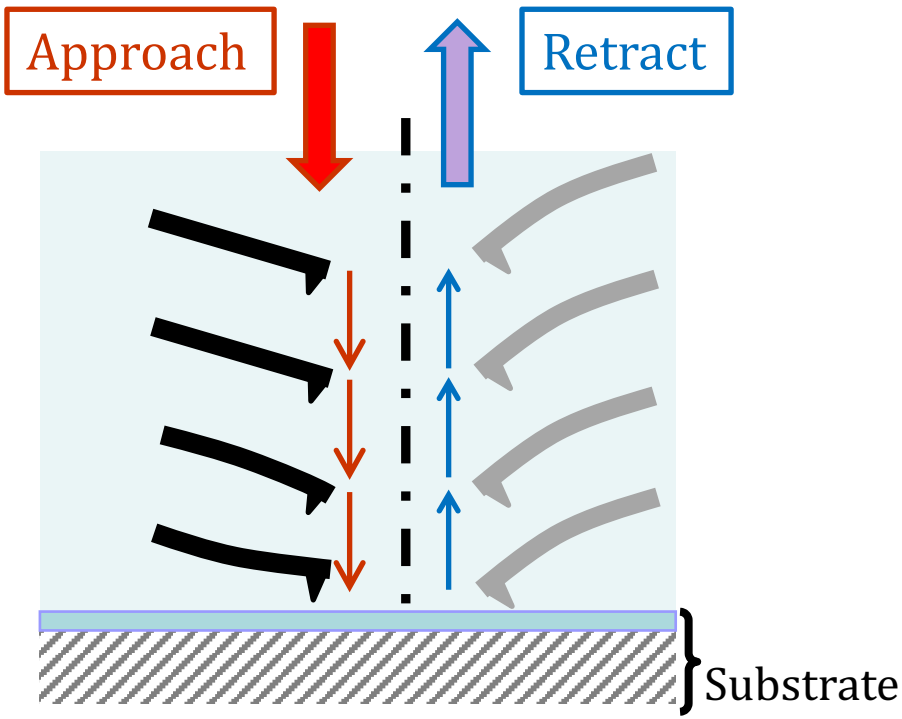

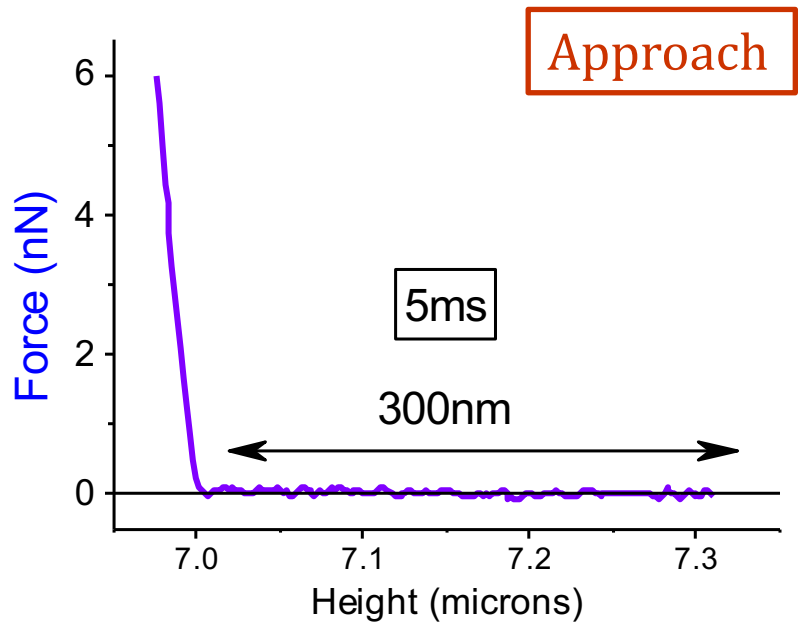

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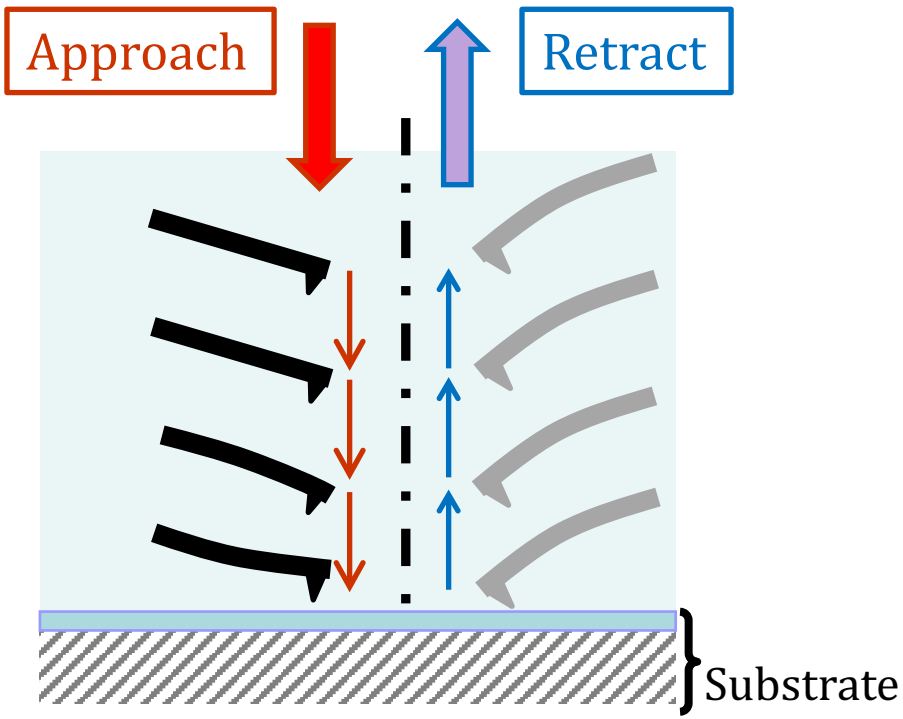

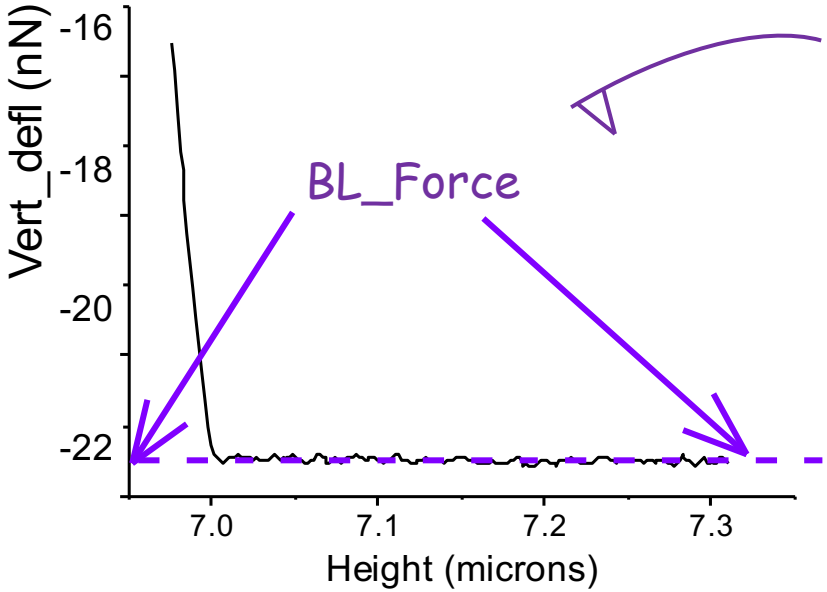

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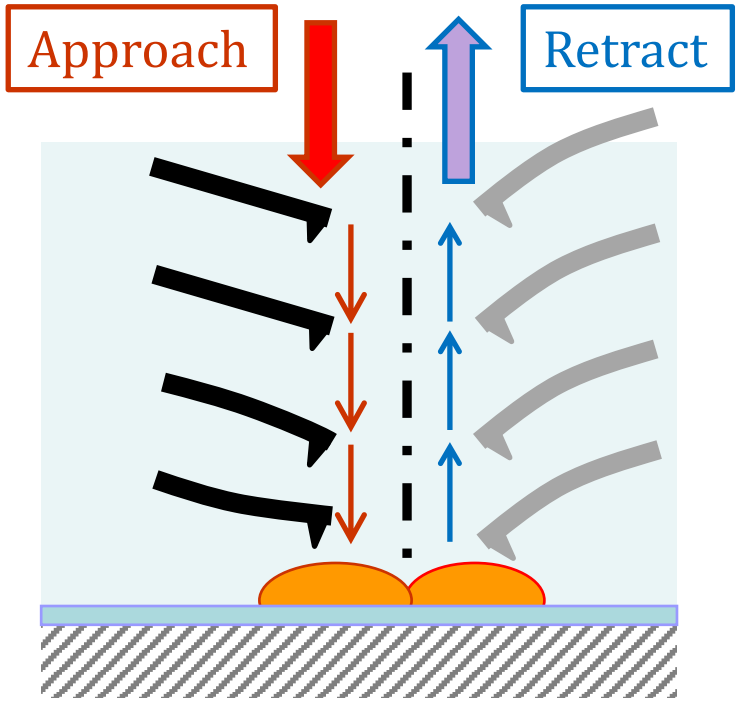
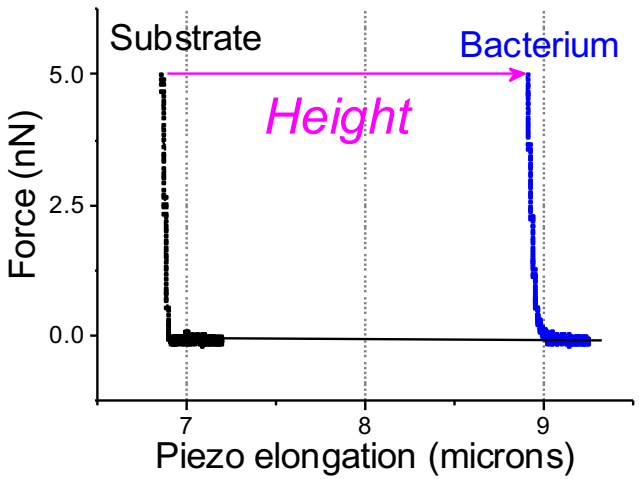
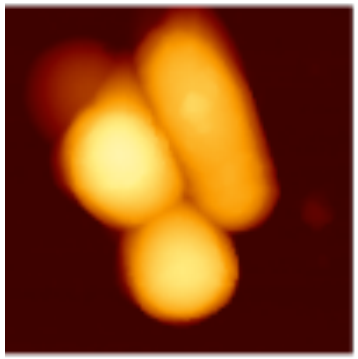




Height imaging



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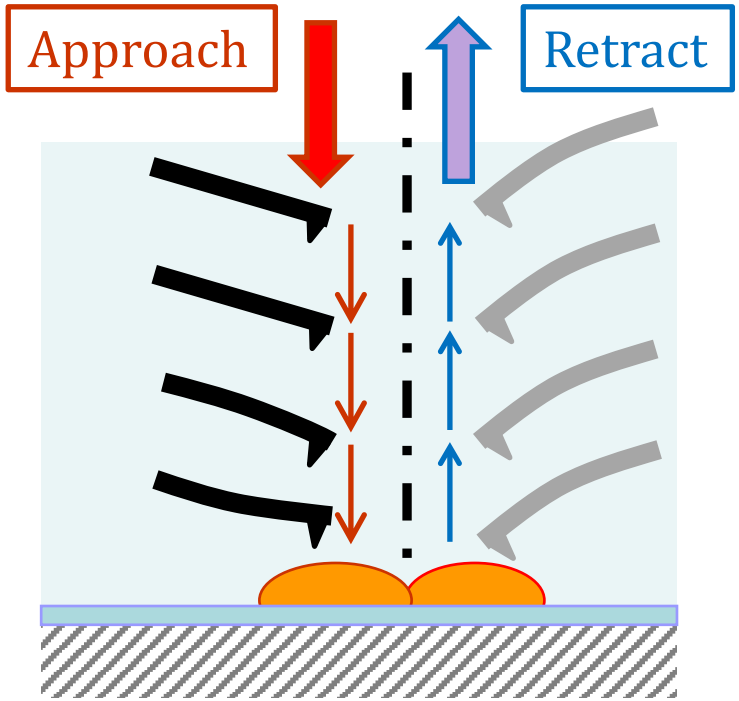

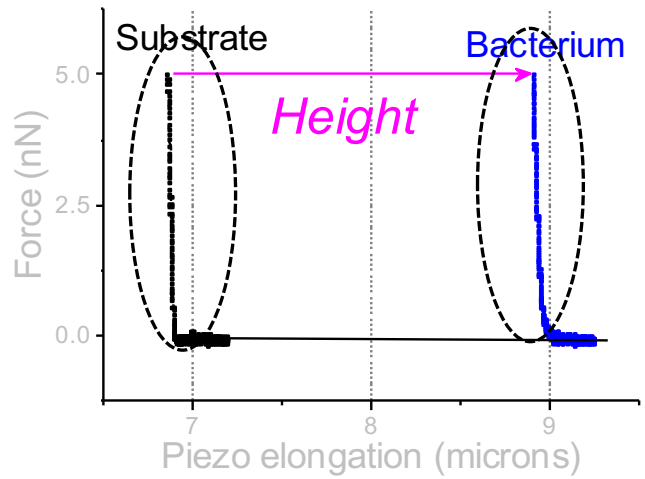

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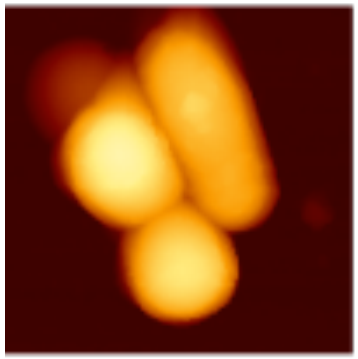
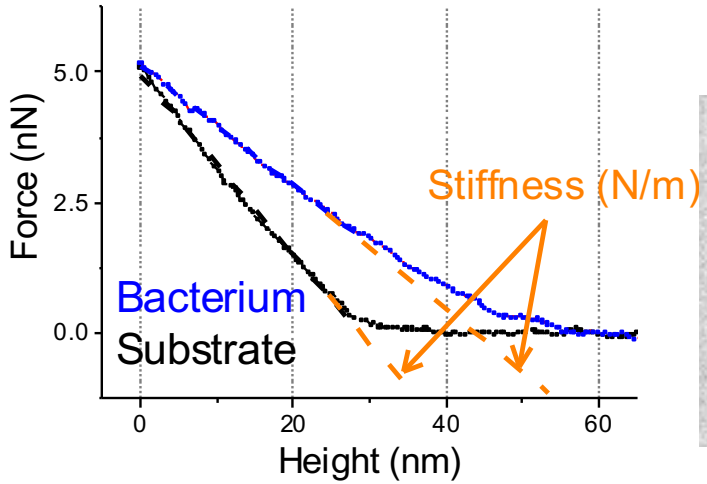


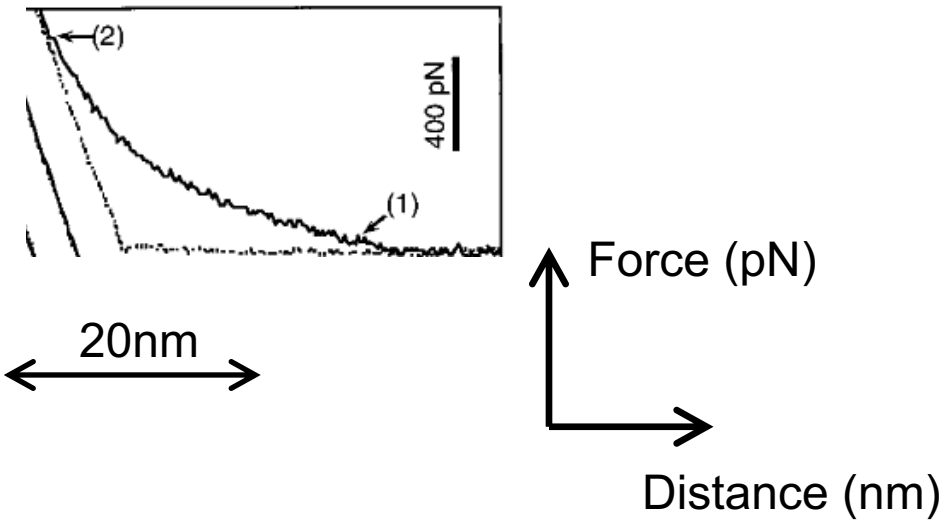


Mechanical imaging

Electrostatic forces

In air / under vacuum

$$F_{DLVO}(z) = -\frac{H_a R_{glo}}{6(z + R_{loc})^2}$$

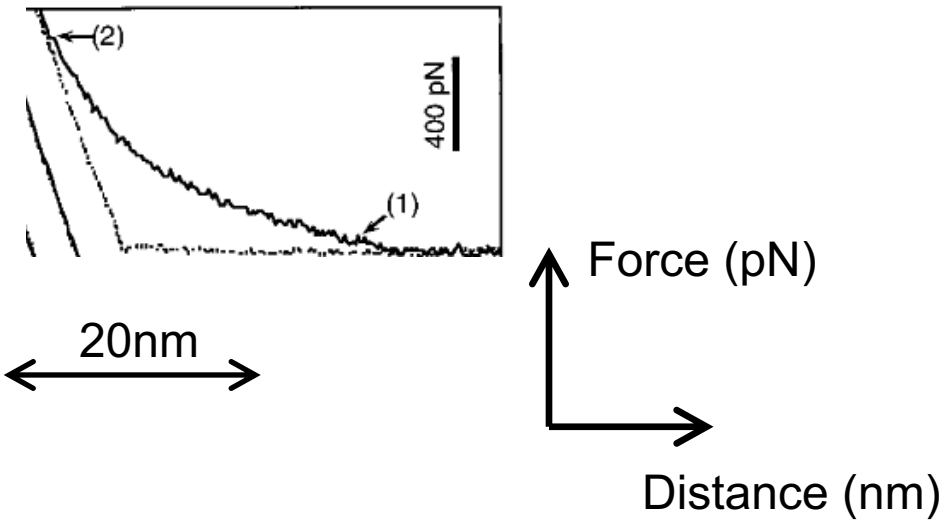


Biophys_J_1999_Muller et al.

Electrostatic forces

Aqueous medium at *low* Ionic Strength

$$F_{DLVO}(z) = -\frac{H_a R_{glo}}{6(z + R_{loc})^2}$$

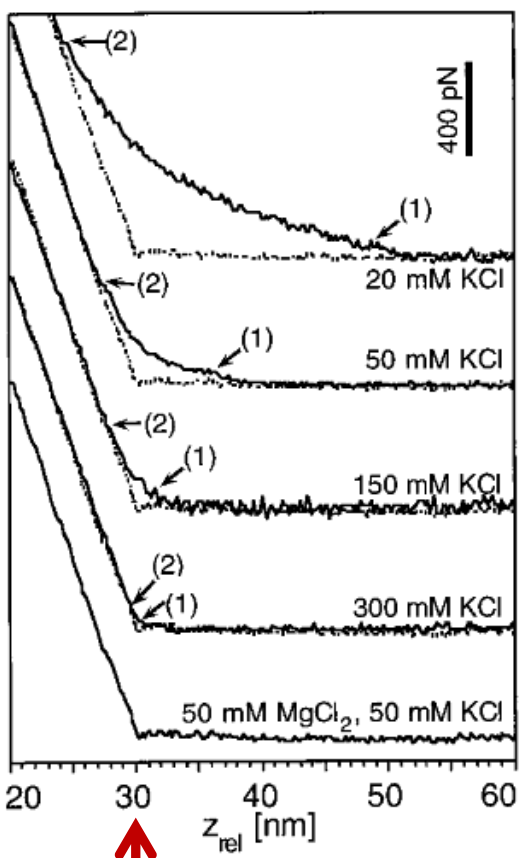


Biophys_J_1999_Muller et al.

Electrostatic forces

Aqueous medium with *higher* Ionic Strength

Increasing Ionic Strength



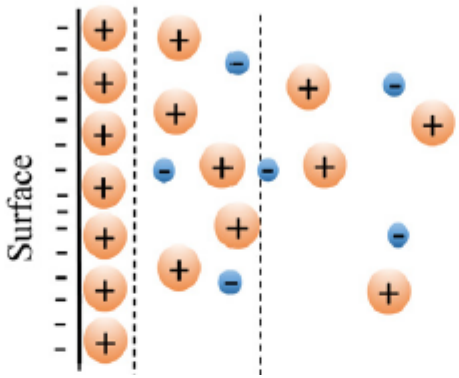
membrane

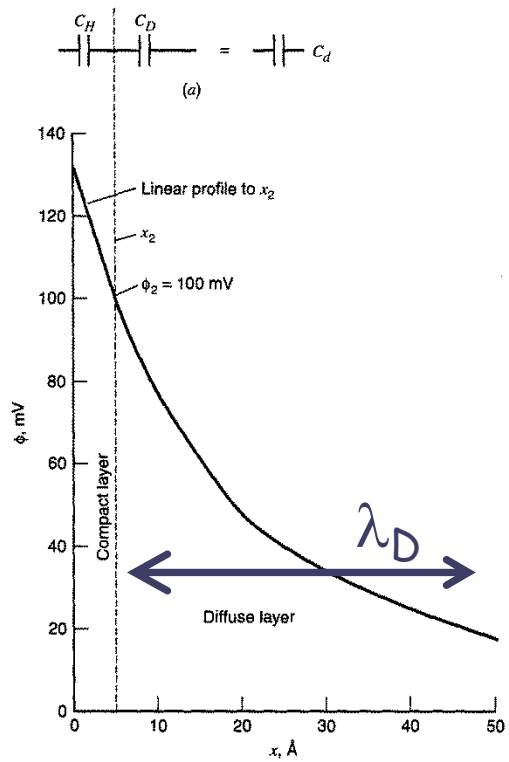
$$F_{DLVO}(z) = \frac{4\pi\sigma_s\sigma_t R_{glo}\lambda_D}{\epsilon_e\epsilon_0} e^{-(z+R_{loc})/\lambda_D} - \frac{H_a R_{glo}}{6(z+R_{loc})^2}$$

$$\lambda_D = \sqrt{\frac{\epsilon_0\epsilon_e kT}{e^2\sum_i c_i q_i^2}}$$

Biophys_J_1999_Muller et al.

~~Electrostatic forces~~





Ionic Strength ~150mM → $\lambda_D \approx 1nm$

Bard et Faulkner (2000), Electrochemical Methods

Principle of measurement

Nanoscale

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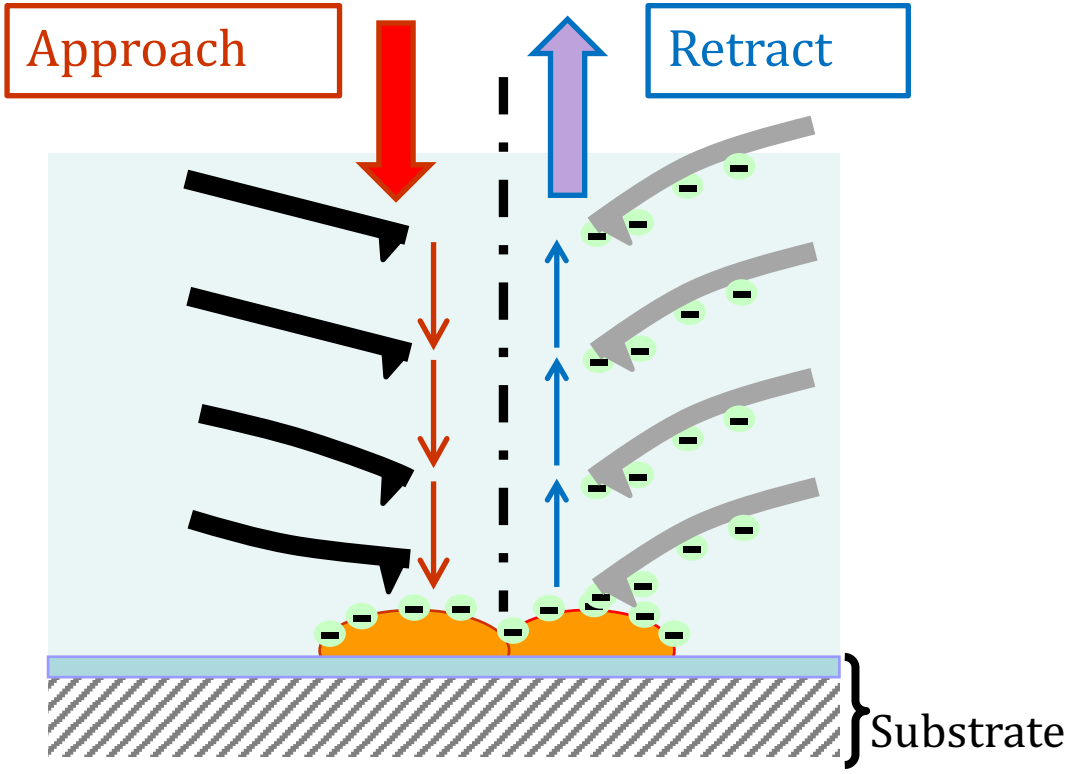
Cite this: *Nanoscale*, 2015, 7, 8843

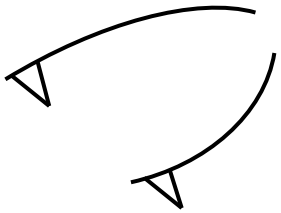
An *in vivo* study of electrical charge distribution on the bacterial cell wall by atomic force microscopy in vibrating force mode†

Christian Marlière*[a] and Samia Dhahri[b]

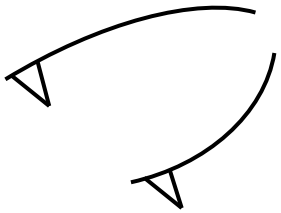


C. Marlière et al., *Nanoscale*, (2015), **7**, 8843

Principle of measurement



Interface Stress: Γ



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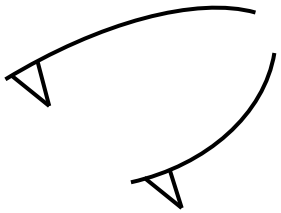


Interface Tension : γ

$$\Gamma_{AB} = \frac{d\gamma_{AB}}{d\varepsilon_{\parallel}} + \gamma_{AB}$$

Shuttleworth's equation

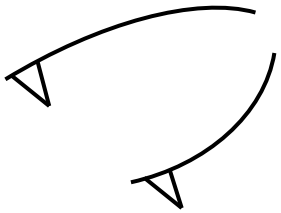
Shuttleworth, R. , Proc. Phys. Soc. Sect. A (1950)

- Under some hypothesis :

$$\Gamma_{AB} \sim \gamma_{AB}$$

Principle of measurement

- Interface Stress: Γ



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- Under some hypothesis :

$$\Gamma_{AB} \sim \gamma_{AB}$$

- $$-d\gamma = \sigma^{M} dE_{-} + \Gamma_{K^+}\, d\mu_{KCl} + \Gamma_{M}\, d\mu_{M}$$

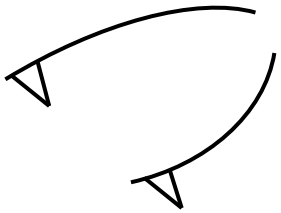
Electro-capillary equation

$\sigma \approx$ excess surface charge density

Bard et Faulkner (2000), Electrochemical Methods

Principle of measurement

- Interface Stress: Γ



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Lippmann's equation

$\sigma \approx$ *excess surface charge density*

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Principle of measurement

- Interface Stress: Γ

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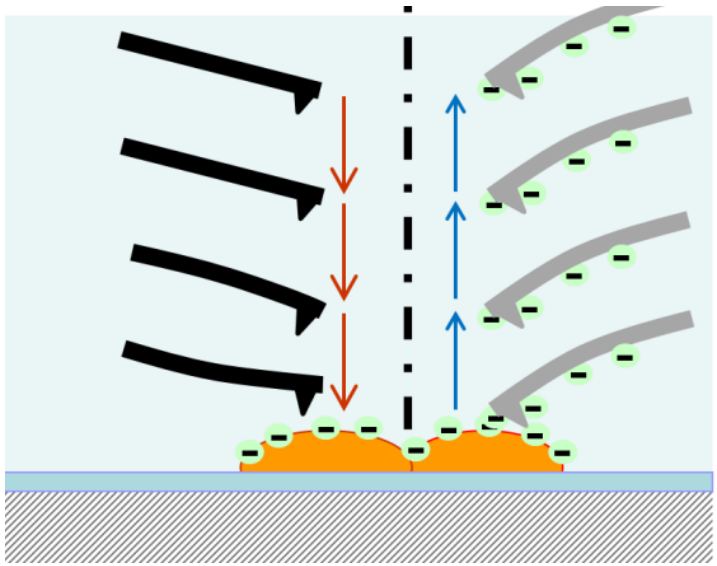
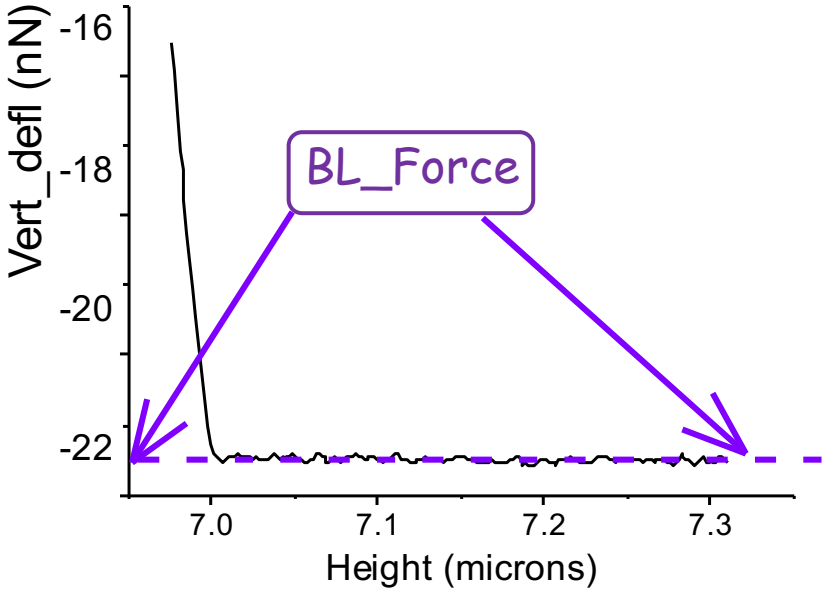
Lippmann's equation

$$\Delta\sigma \rightrightarrows \Delta\gamma_{AB} \rightrightarrows \Delta\Gamma_{AB} \rightrightarrows \Delta\{\text{"}\boldsymbol{BL\_Force}\text{"}\}$$

Principle of measurement



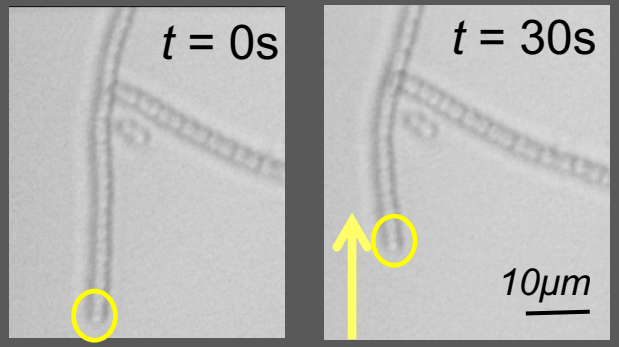


$$\Delta\sigma \rightrightarrows \Delta\gamma_{AB} \rightrightarrows \Delta\Gamma_{AB} \rightrightarrows \boldsymbol{\Delta\{"BL\_Force"\}}$$

Experimental

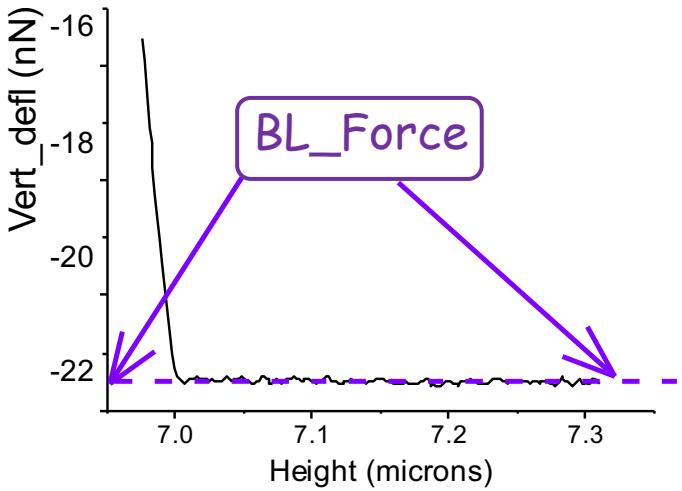
NO ARTIFICIAL IMMOBILIZATION STEP NEEDED:

THEN:



Observation by AFM *without* any immobilisation step

- In physiological medium
- Soft preparation of samples: no deshydratation, etc…
- Mode QI (*Nanowizard 3 JPK*)
  - ✓Approach-Retract Mode
  - ✓Lateral force strictly minimized
  - ✓Minute control of vertical applied force at every pixel
- Cantilevers with metallic layer



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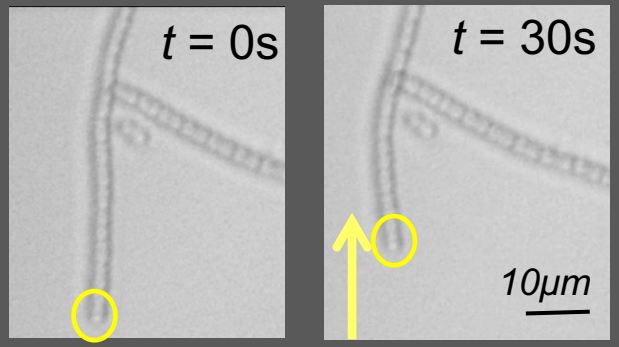
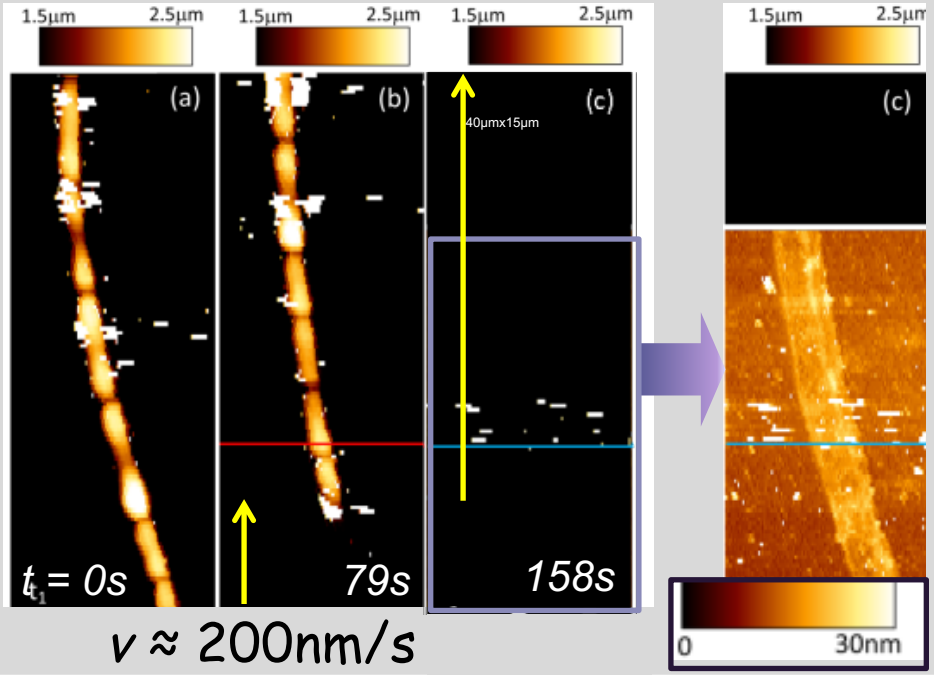
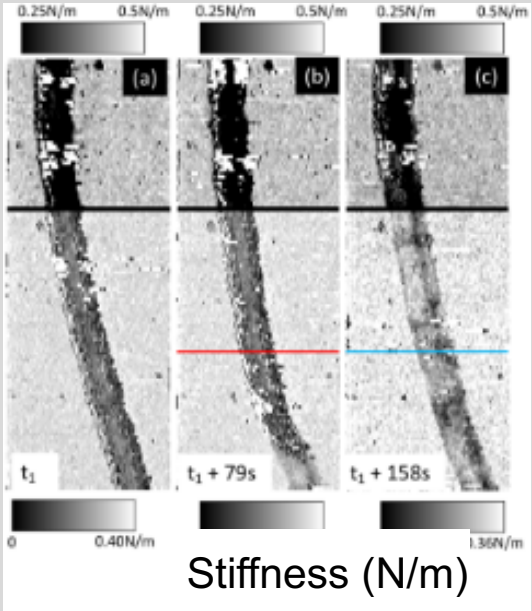


Gliding movement of *Anabaenopsis circularis:* Secretion of **slime**



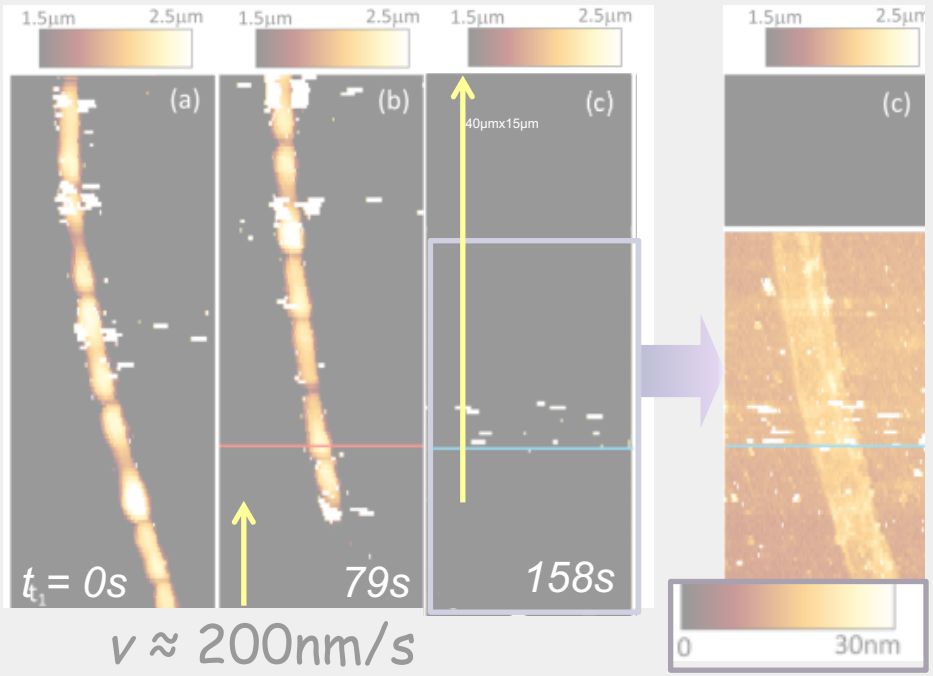
$v \approx 200nm/s$



S. Dhahri et al., PLoS One, (2013)

Experimental

Gliding movement of *Anabaenopsis circularis:* Secretion of **slime**



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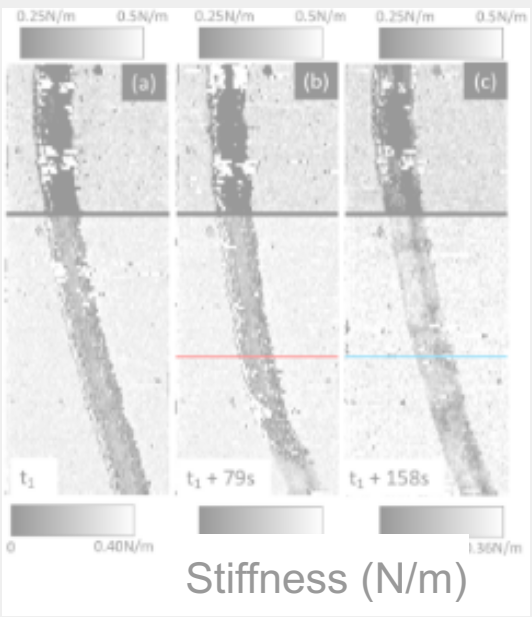
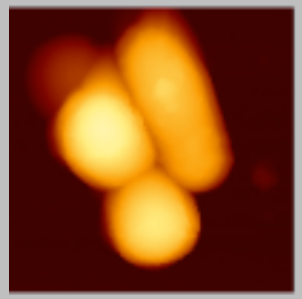


S. Dhahri et al., PLoS One, (2013)

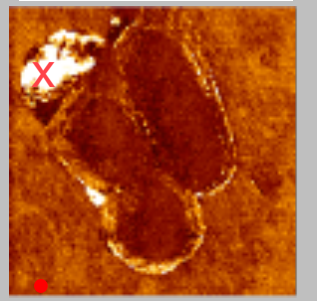
Self-immobilizing bacterium: *Rhodococcus. wratislaviensis*

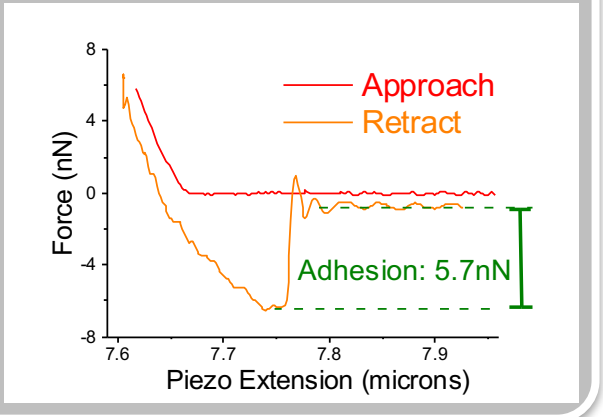
Height imaging



Mechanical imaging

Adhesion imaging





Experimental

Nanoscale

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Cite this: *Nanoscale*, 2015, **7**, 8843

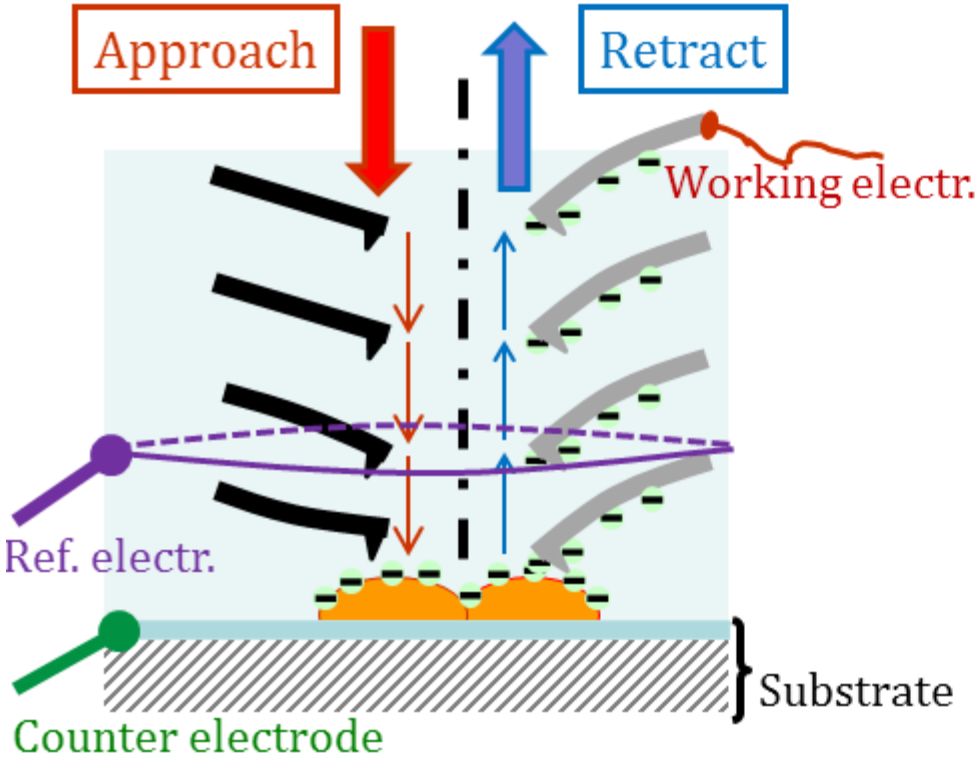
An *in vivo* study of electrical charge distribution on the bacterial cell wall by atomic force microscopy in vibrating force mode†

Christian Marlière*[a] and Samia Dhahri[b]

WE: levier AFM

CE: Substrat (ITO)

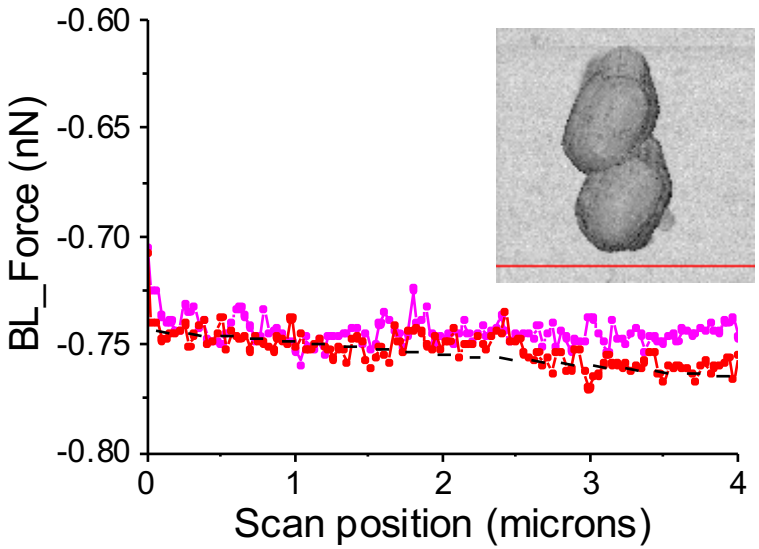
REF: anneau Pt

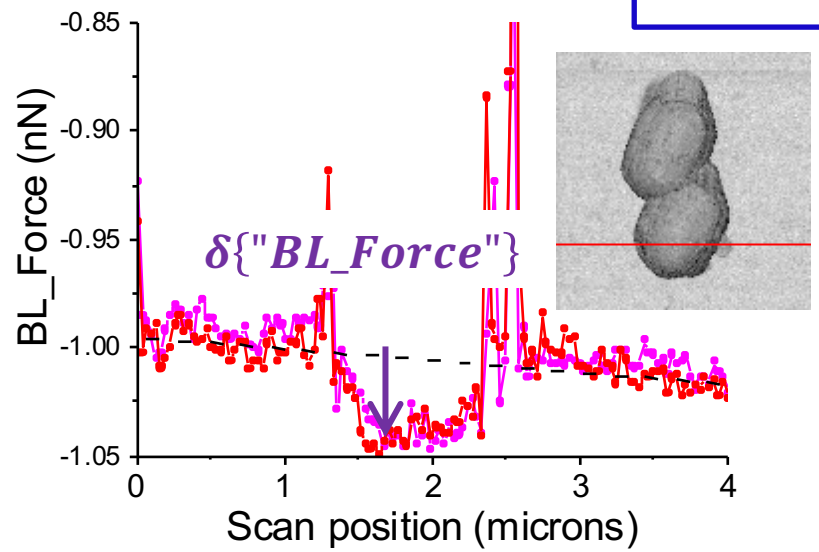


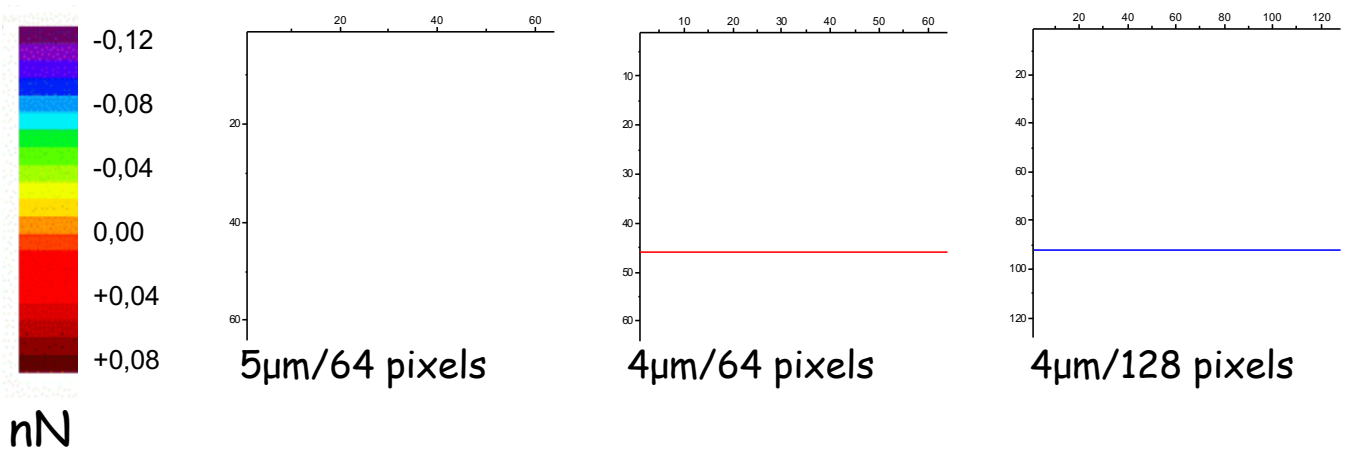
C. Marlière et al., *Nanoscale*, (2015), **7**, 8843

Results: 1. ‘’*fast*’’ regime

0mV

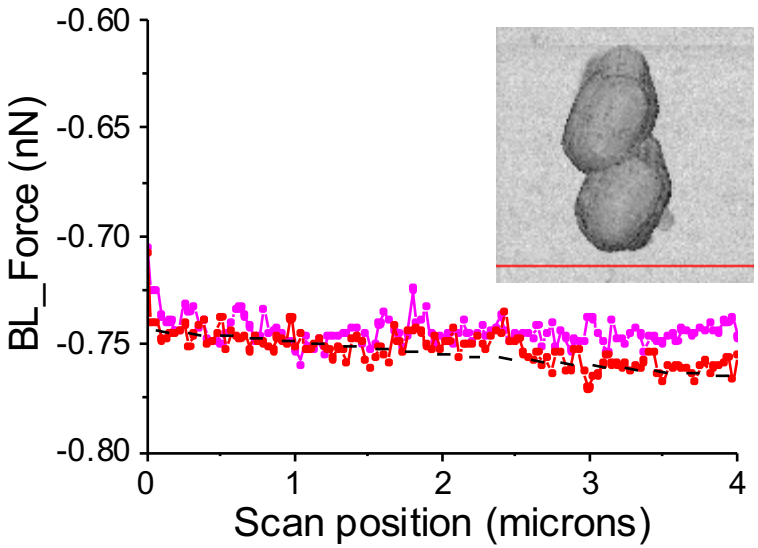


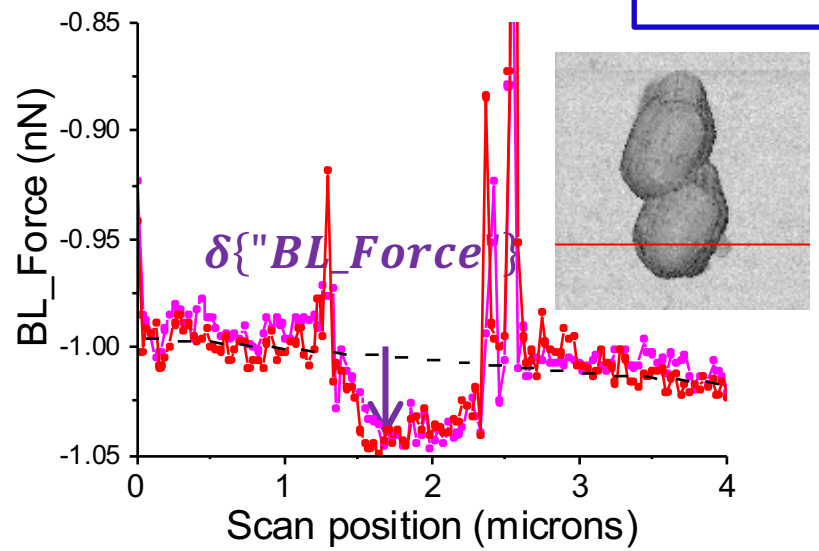




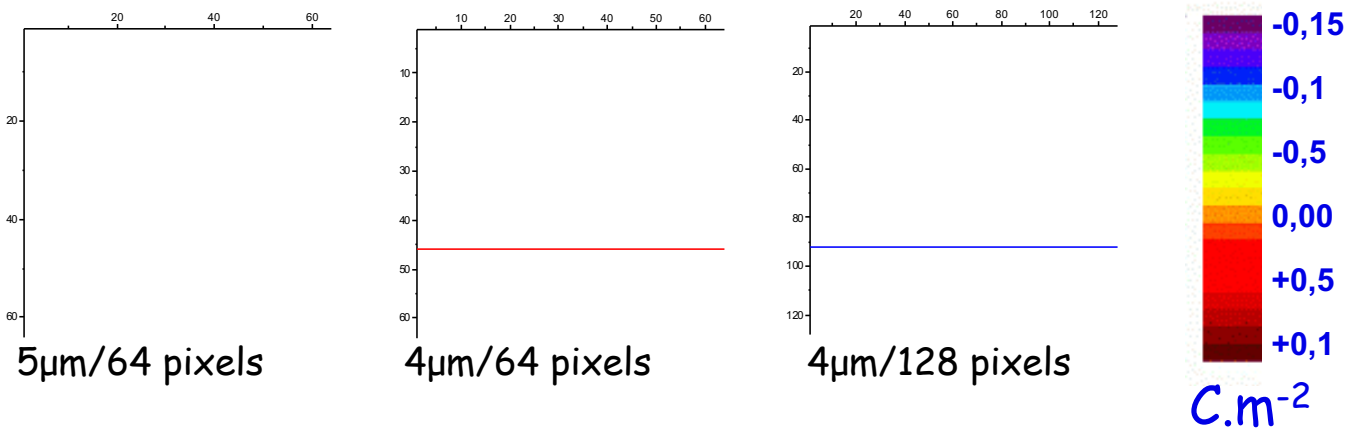
Results: 1. ‘’*fast*’’ regime

0mV

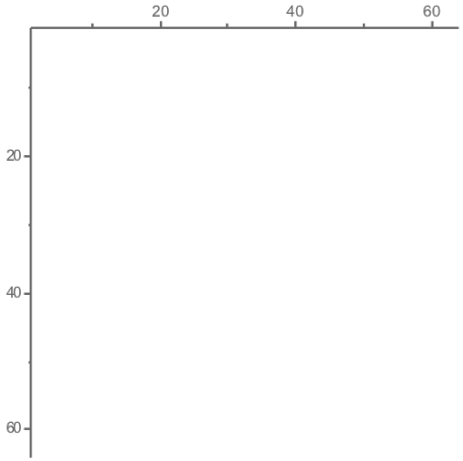




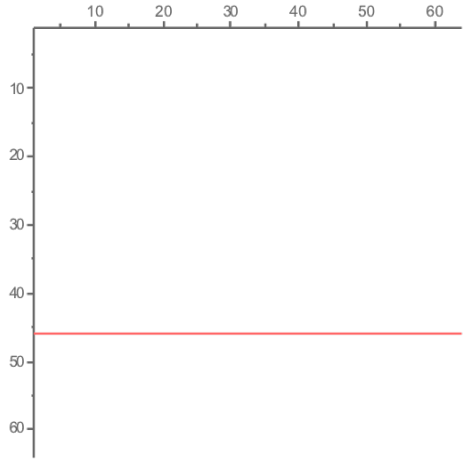
The so-called “fast” regime : quantification of **surface charge density**



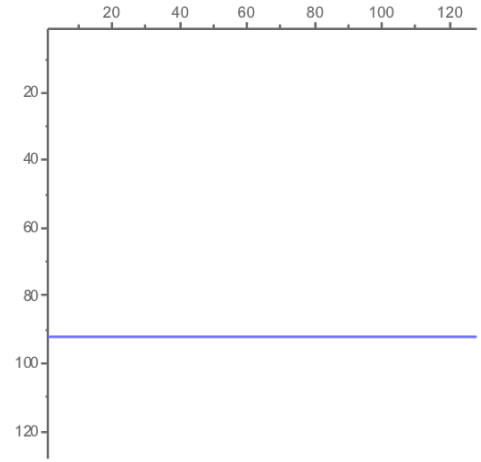
Results: 1. ‘’*fast*’’ regime



5µm/64 pixels

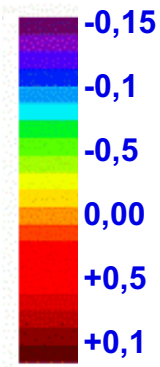


4µm/64 pixels

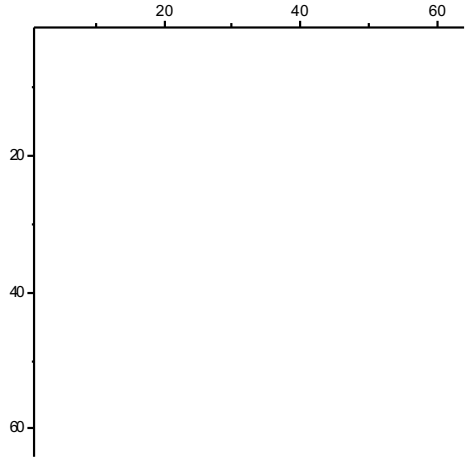


4µm/128 pixels

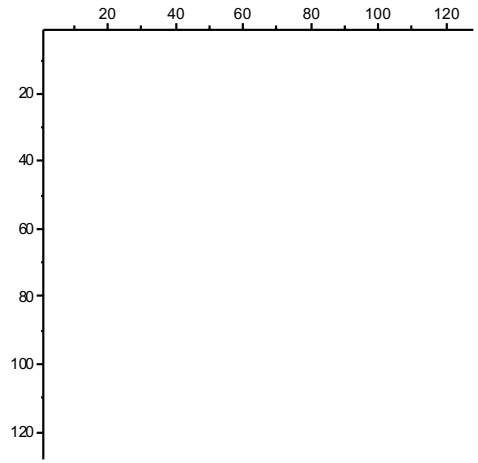
OmV



$C.m^{-2}$



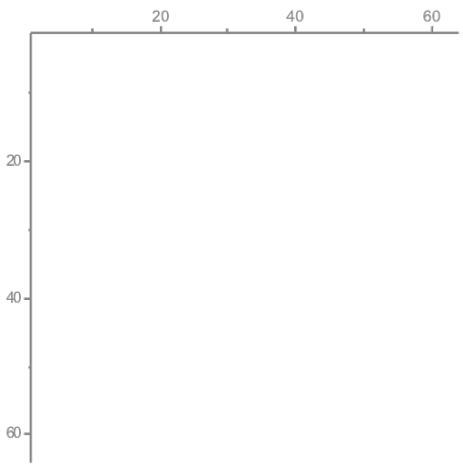
4µm/64 pixels



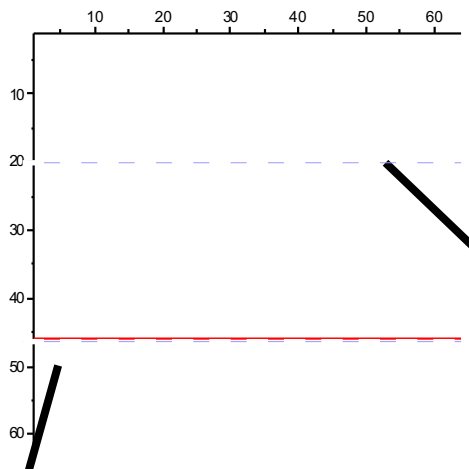
4µm/128 pixels

O.C.

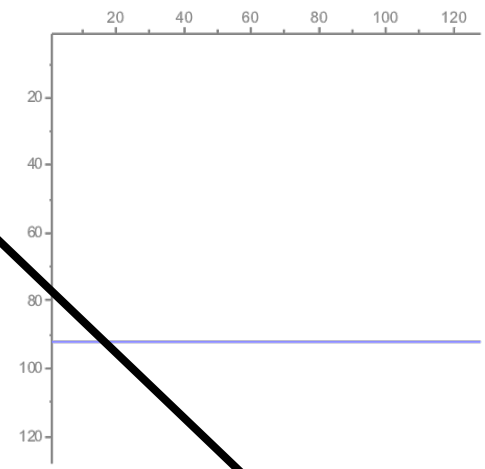
Results: 2. ''*slow*'' regime



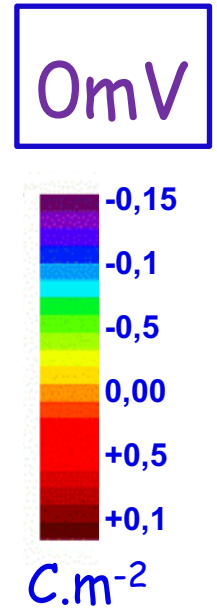
5µm/64 pixels

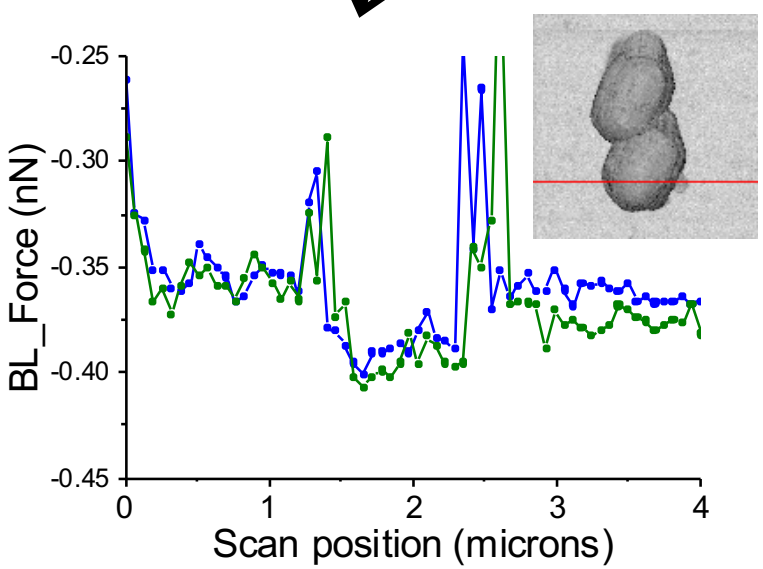


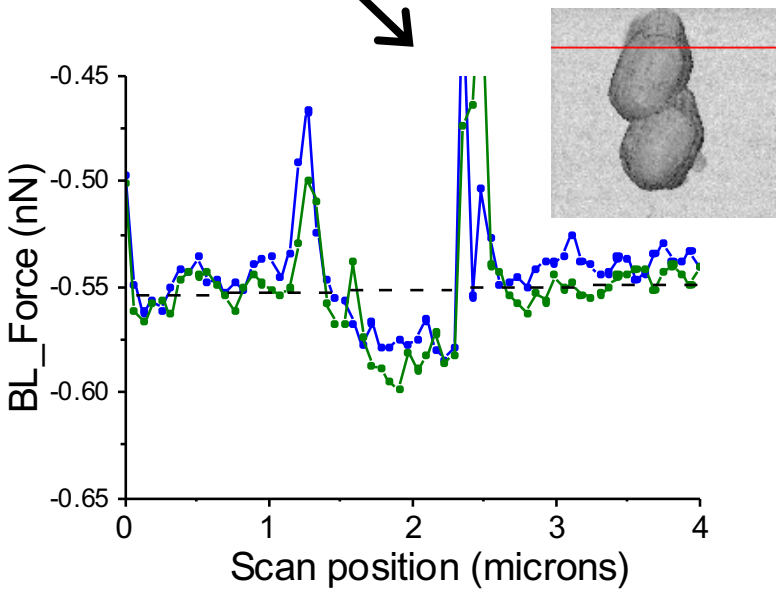
4µm/64 pixels



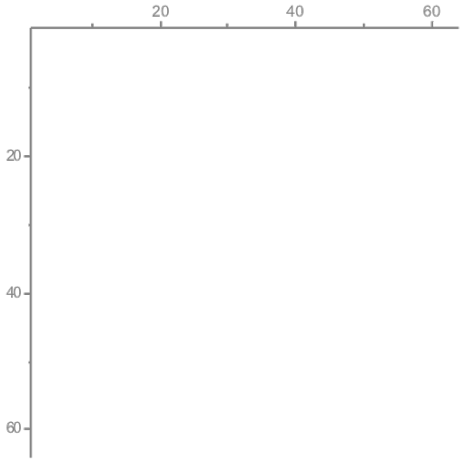
4µm/128 pixels



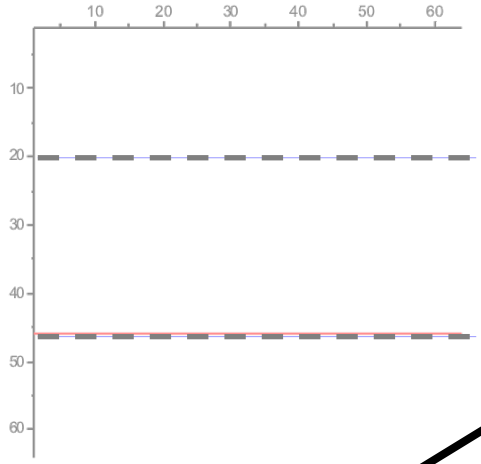




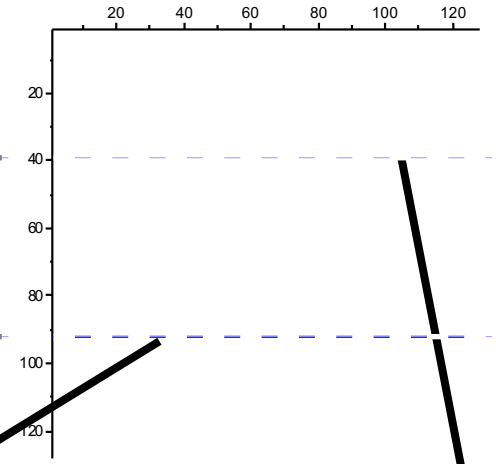
Results: 2. ''*slow*'' regime



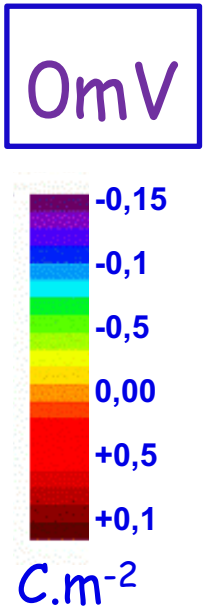
5µm/64 pixels

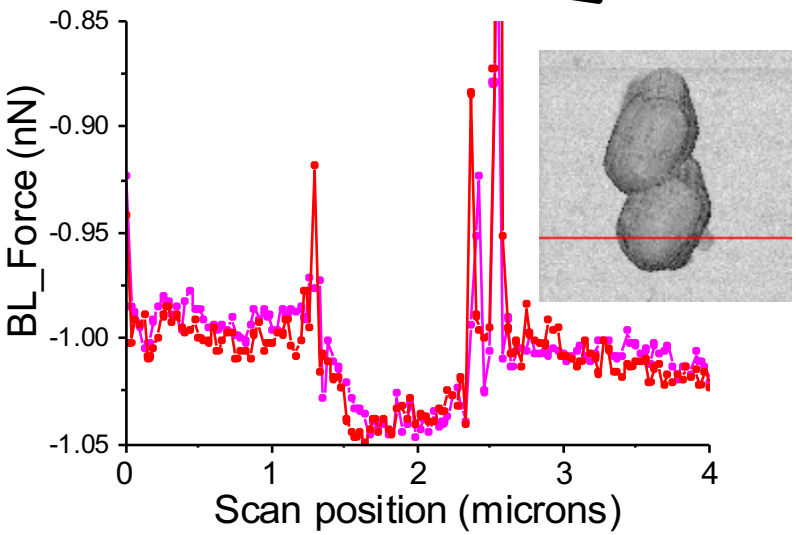


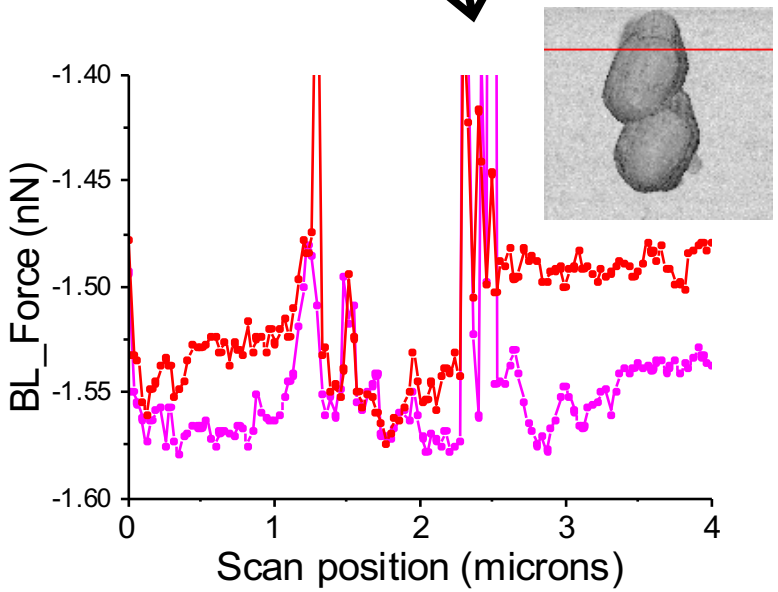
4µm/64 pixels



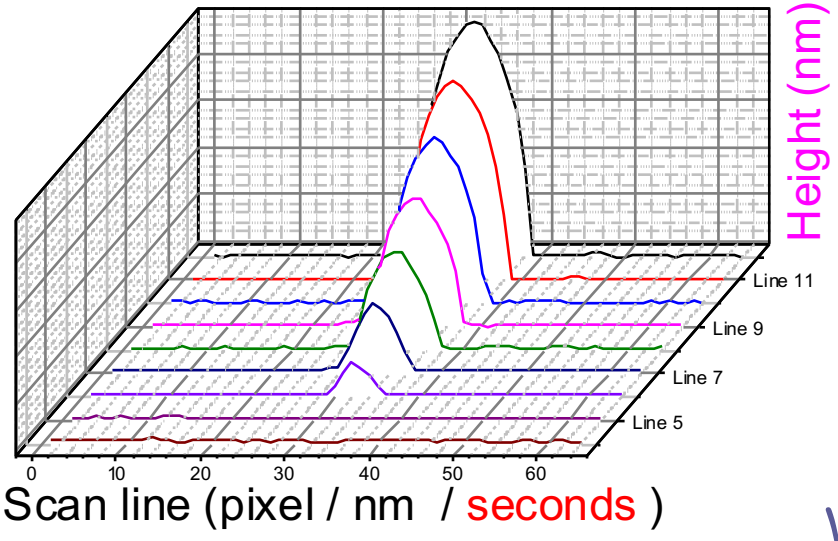
4µm/128 pixels

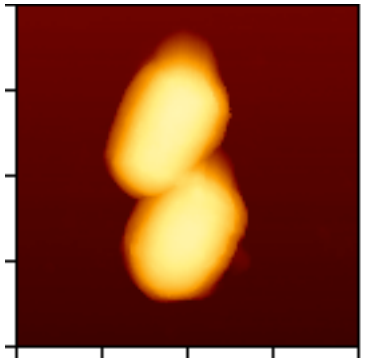






Results: 2. ''*slow*'' regime





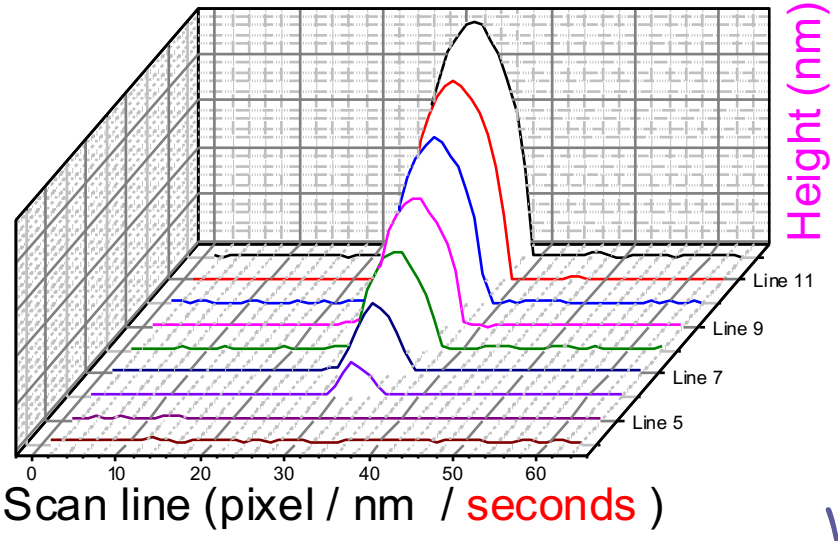
WE: levier AFM

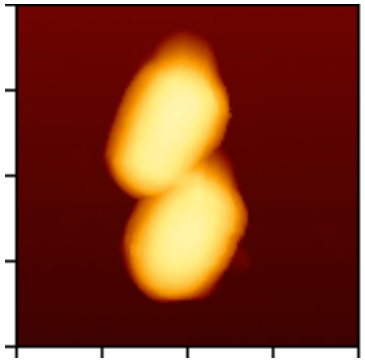
CE: Substrat (ITO)

REF: anneau Pt

4µm/64 pixels

Results: 2. ''*slow*'' regime

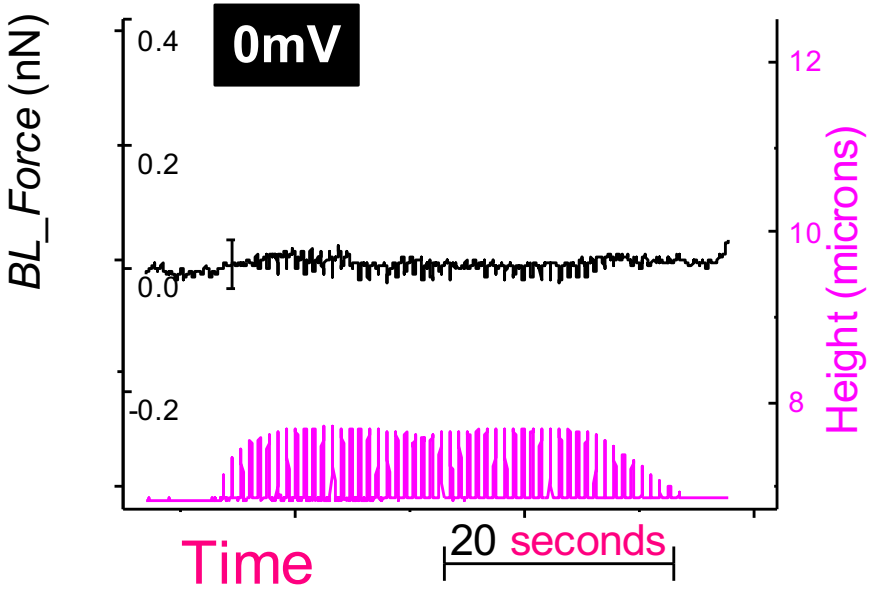




WE: levier AFM

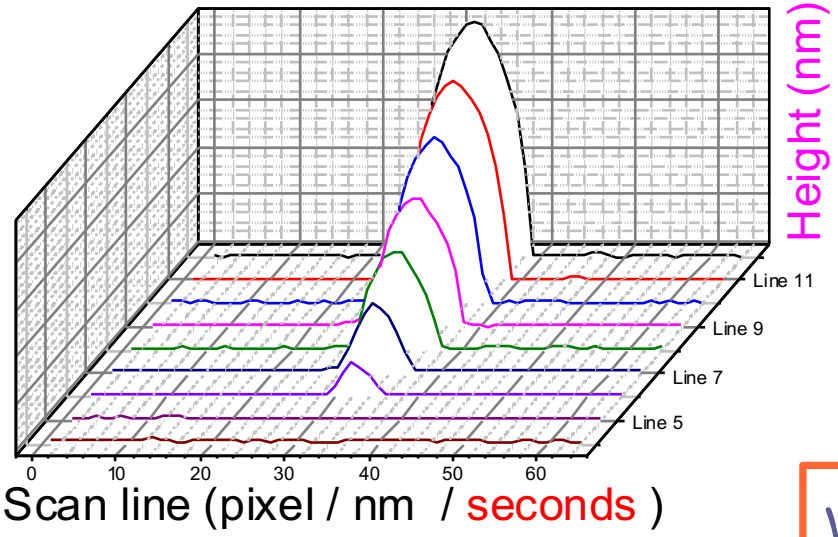
CE: Substrat (ITO)

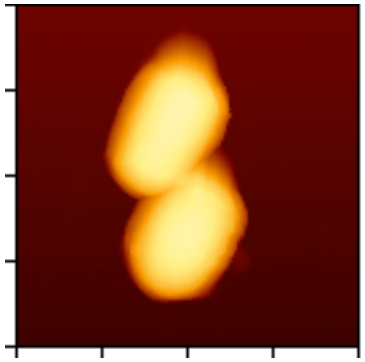
REF: anneau Pt



4µm/64 pixels

Results: 2. ''*slow*'' regime

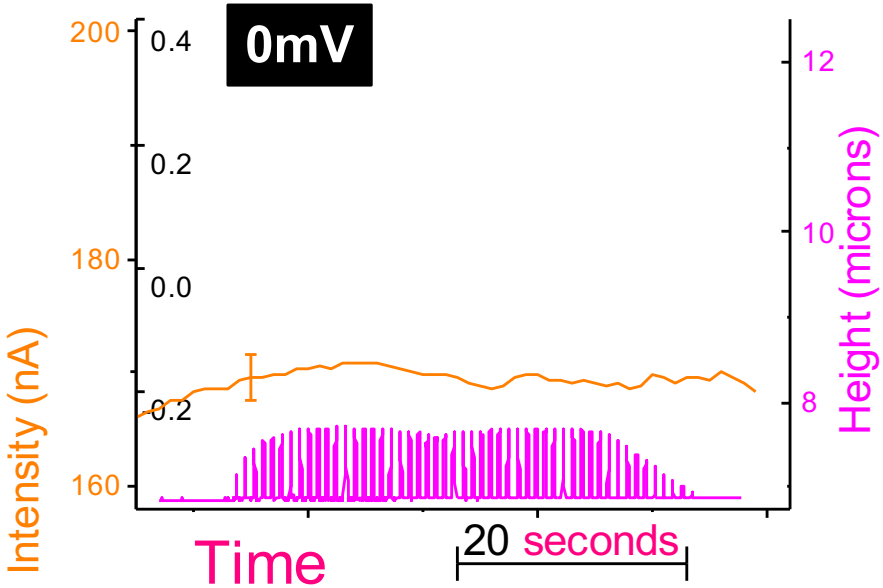




WE: levier AFM

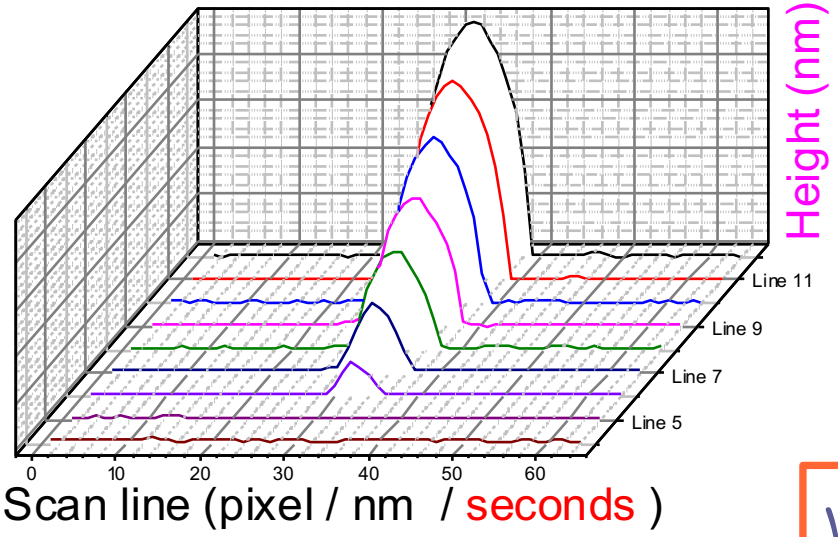
CE: Substrat (ITO)

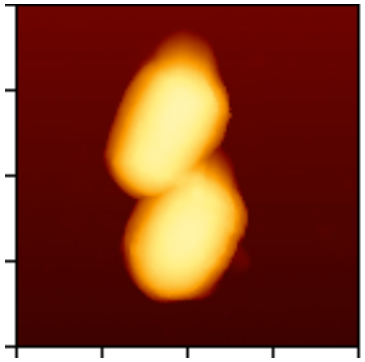
REF: anneau Pt



4µm/64 pixels

Results: 2. ''*slow*'' regime

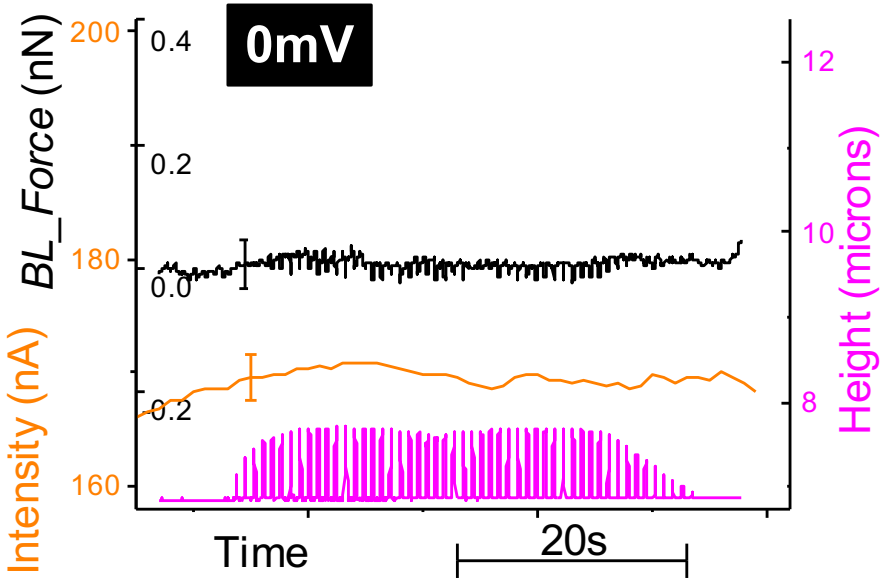




WE: levier AFM

CE: Substrat (ITO)

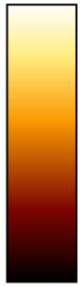
REF: anneau Pt

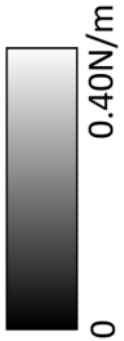


4µm/64 pixels

Results: 2. ''*slow*'' regime

0mV

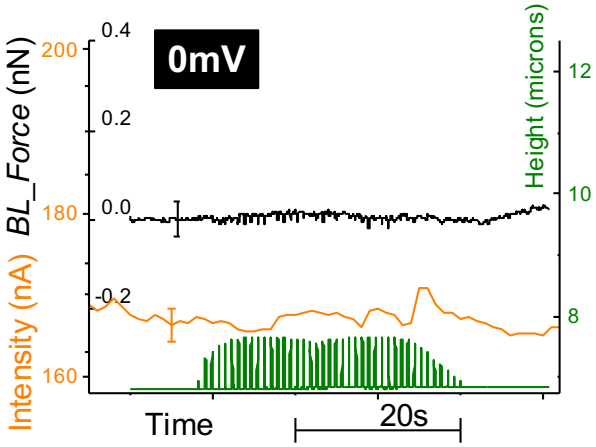




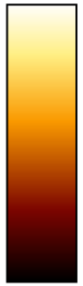
5µm/64 pixels

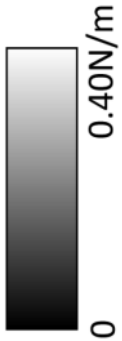
4µm/64 pixels

4µm/128 pixels



Results: 2. ''*slow*'' regime



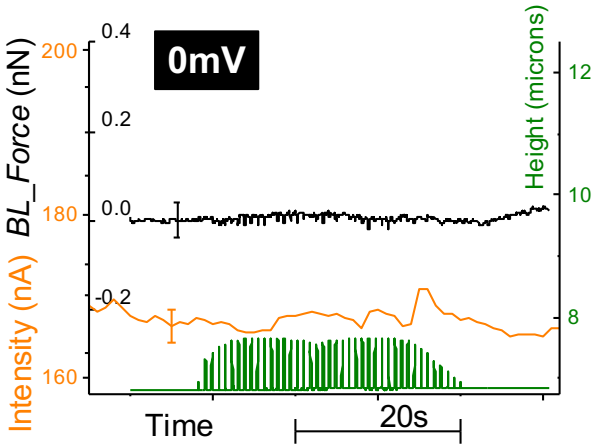


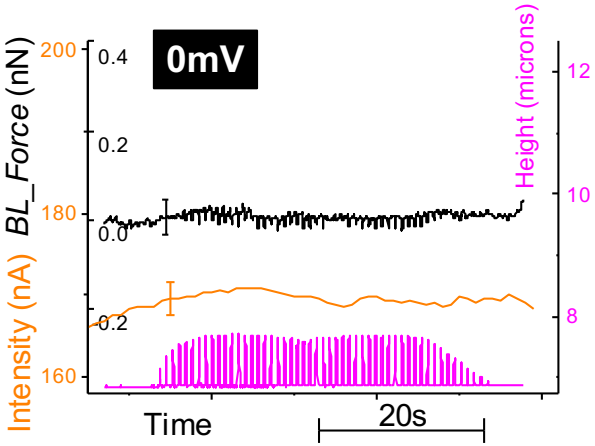
0mV

5µm/64 pixels

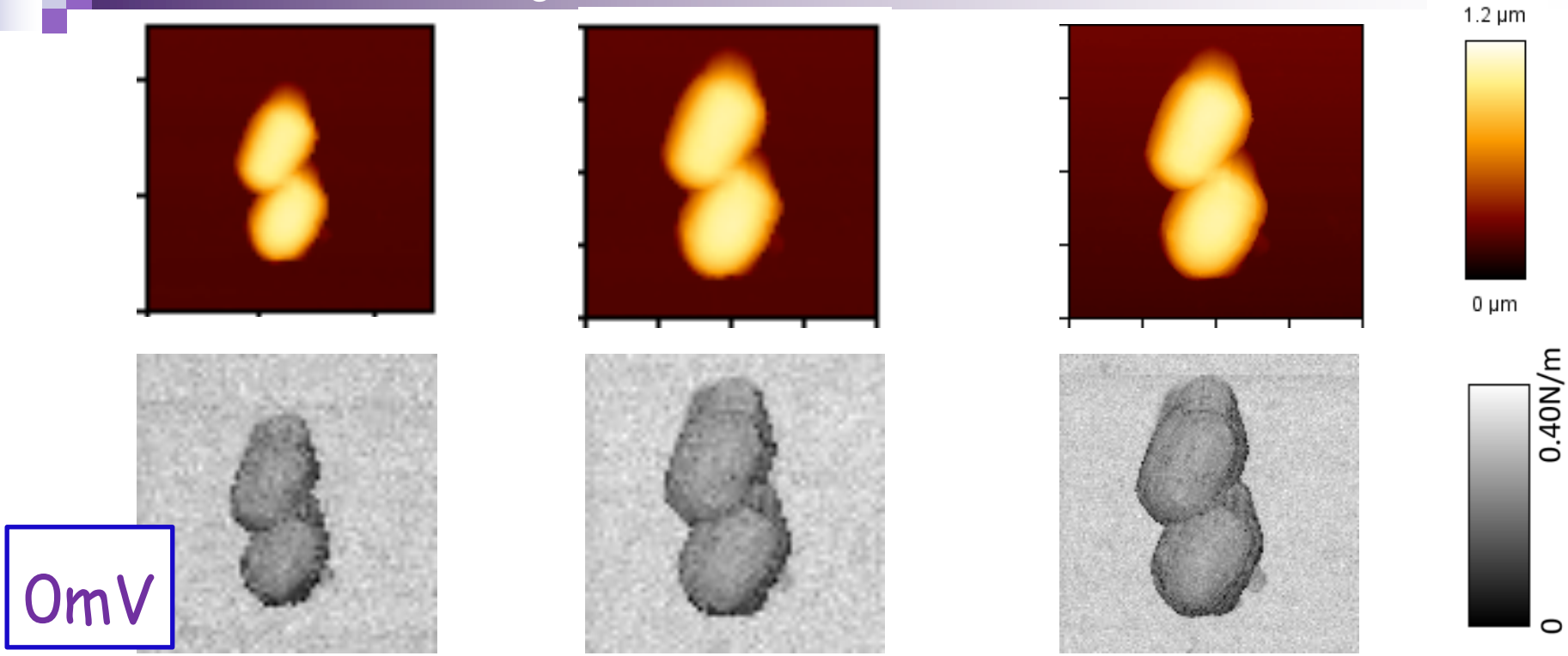
4µm/64 pixels

4µm/128 pixels





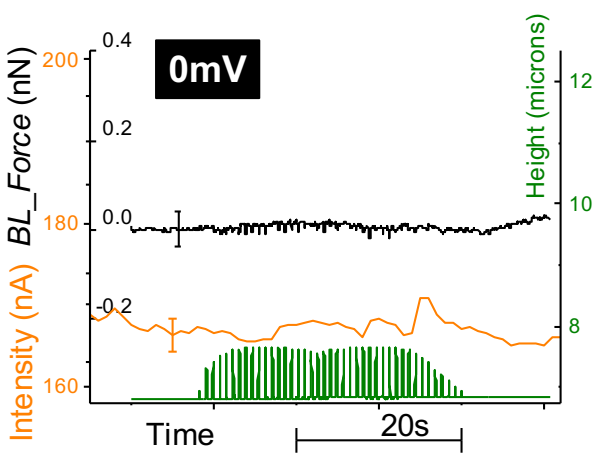
Results: 2. ''*slow*'' regime

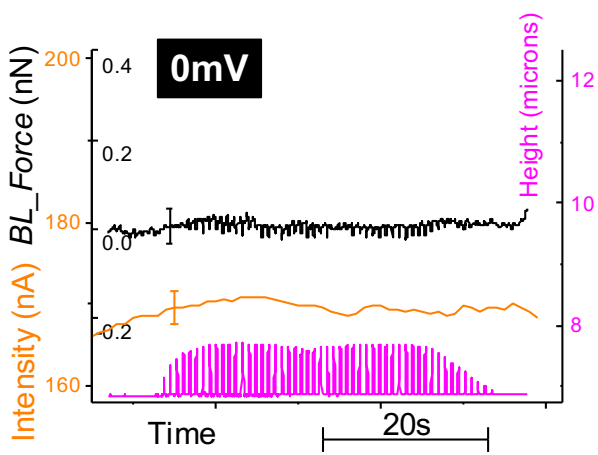


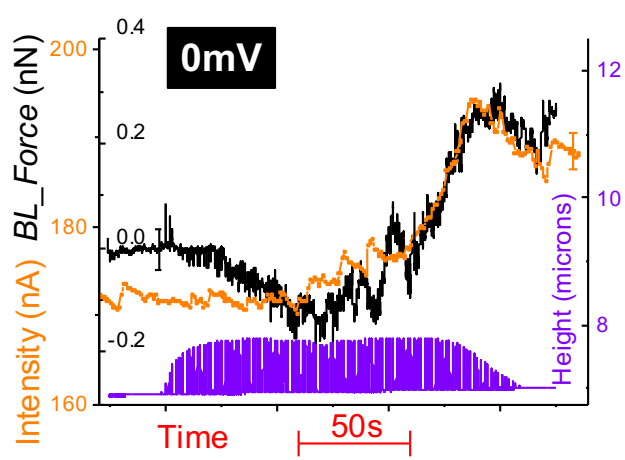
5µm/64 pixels

4µm/64 pixels

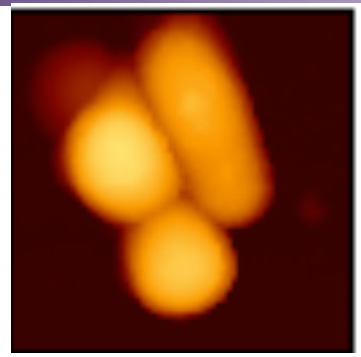
4µm/128 pixels

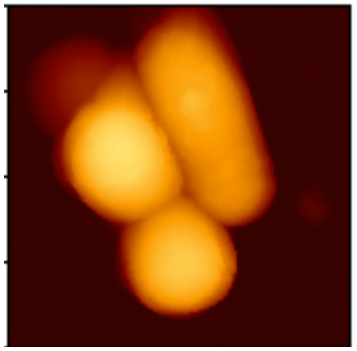


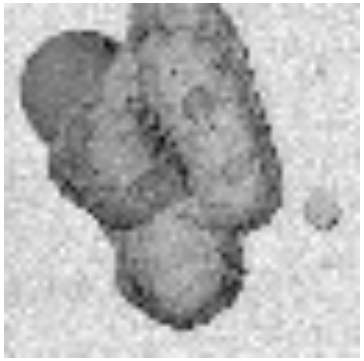




Results: 2. ''*slow*'' regime

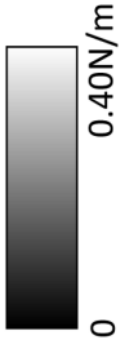




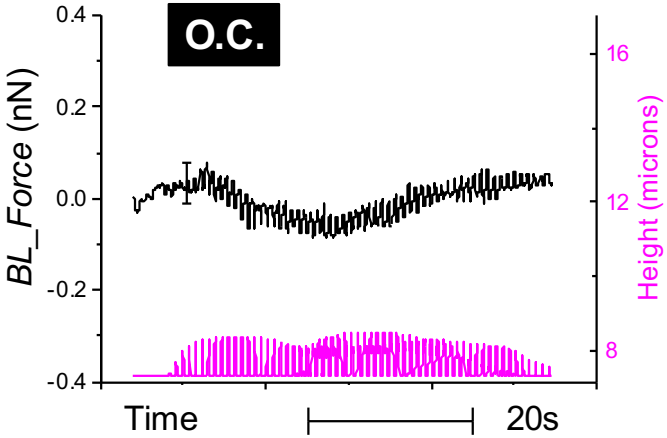


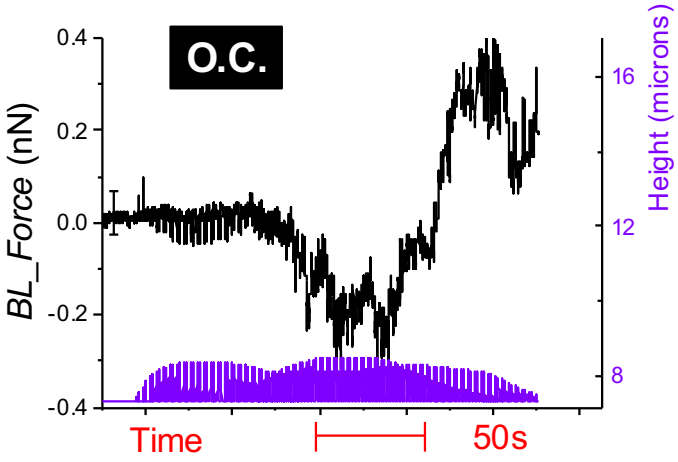
4µm/64pixels

4µm/128 pixels



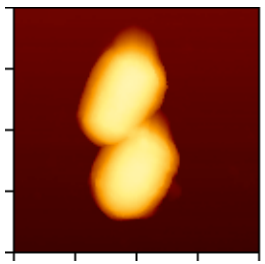
O.C.

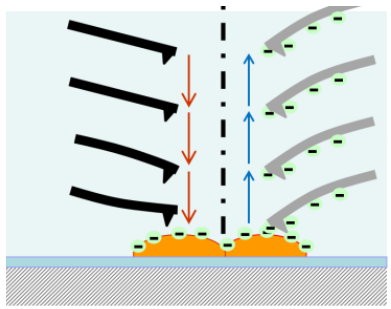


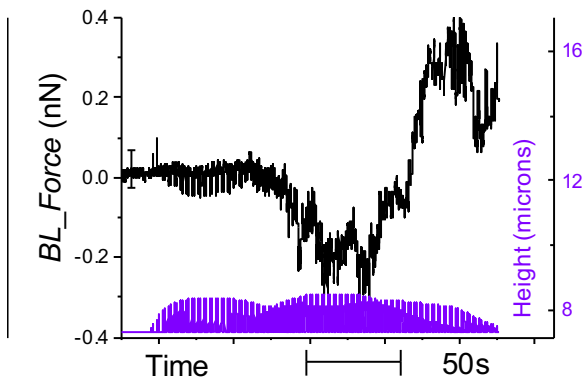


Results: 2. ''*slow*'' regime

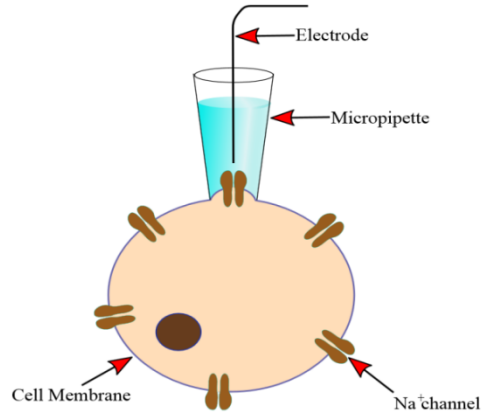
AFM

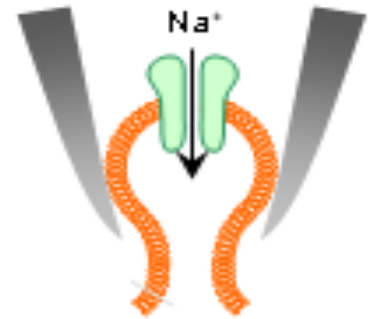


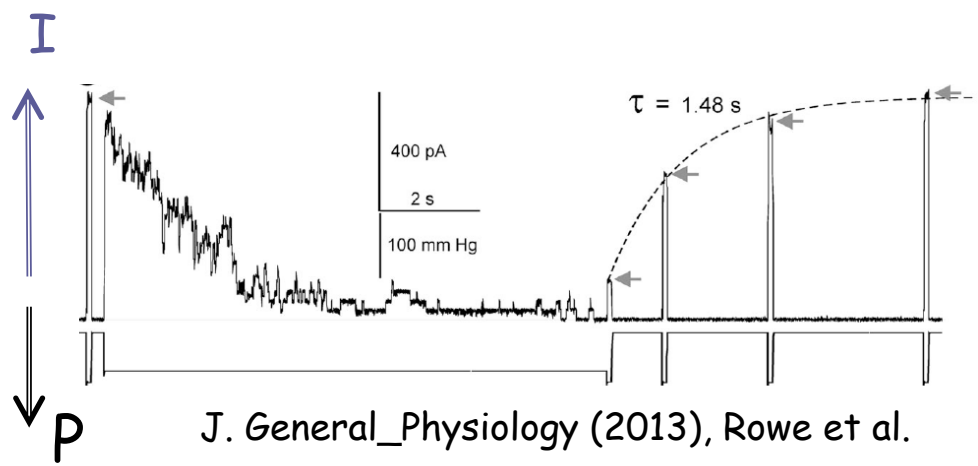




Patch clamp





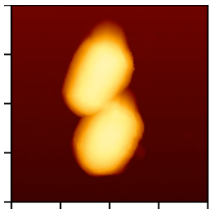


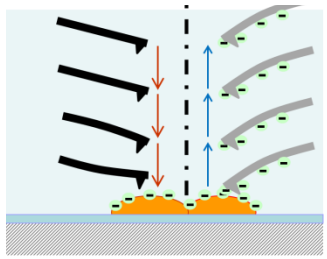
J. General_Physiology (2013), Rowe et al.

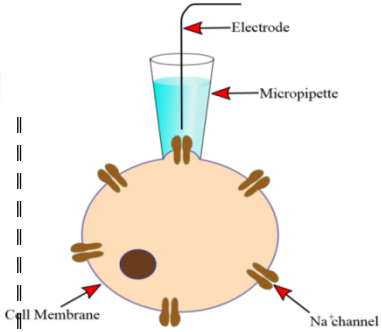
The so-called "slow" regime :
evidence of **ionic currents through mechanosensitive channels**

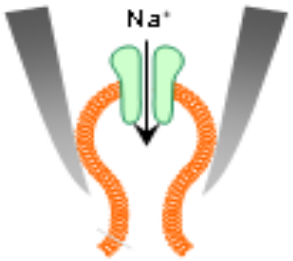
Results: 2. ''*slow*'' regime

AFM ≈ Patch clamp









Contact time between electric double layers at each approach curve:
~ 0.5 ms

For (4µm)² AFM images:

- ✓ Cumulated *contact time for* 0.1µm² area
  - ➢ (64pixels)² ➔ ~ 15 ms
  - ➢ (128pixels)² ➔ ~ **50 ms**

Typically:

- ✓ opening time of ionic channels: ~10–100 ms
- ✓ 10 ionic channels / µm²

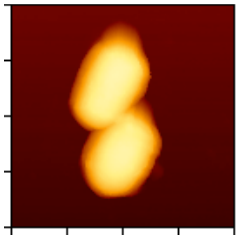
Statistically:

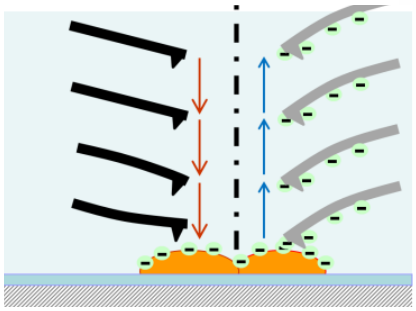
Prerequesite for ejection of ions:

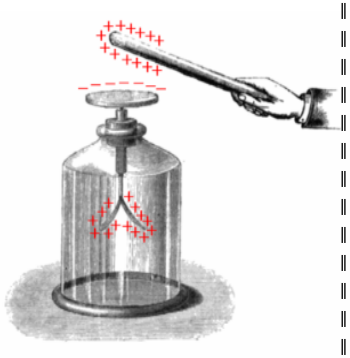
- ➢ Have a look at area of 0.1µm²
- ➢ during at least ~ **10–100 ms**

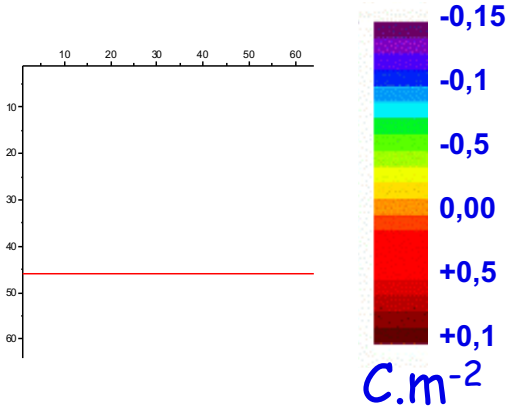
The so-called "slow" regime :
evidence of **ionic currents through mechanosensitive channels**

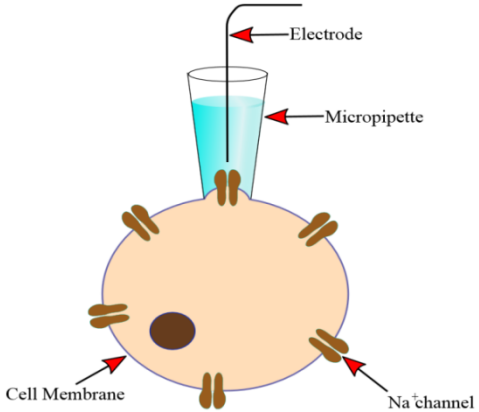
To resume:

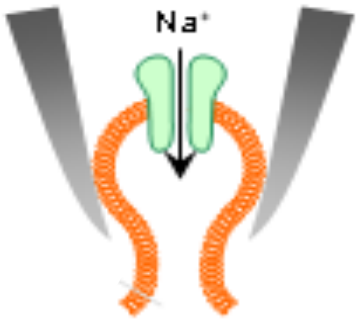


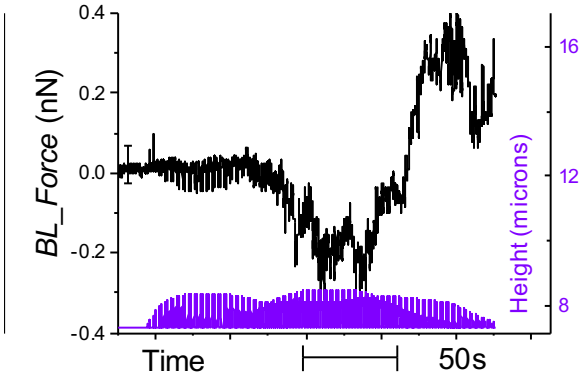












The "*fast*" regime :

❖ quantification of

surface charge density

The "*slow*" regime :

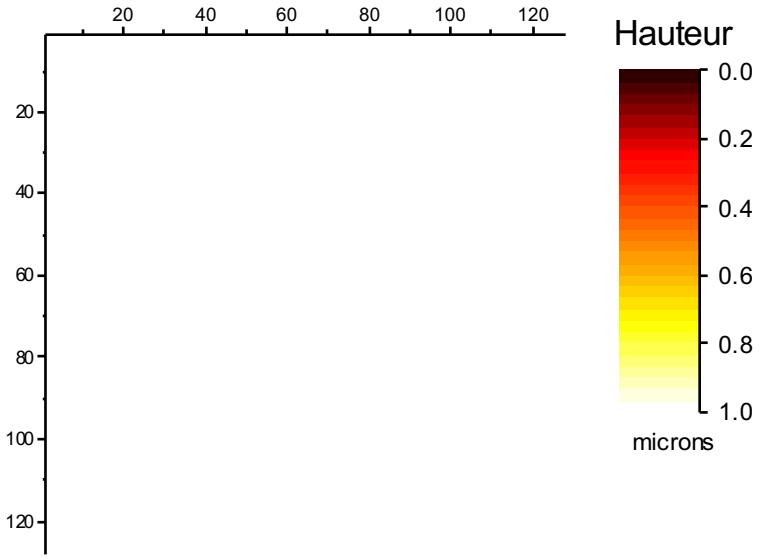
❖ evidence of

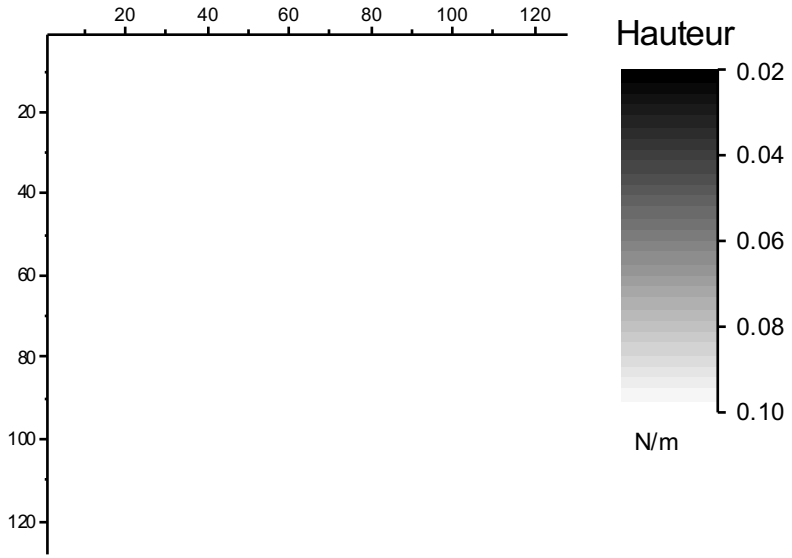
ionic currents through mechanosensitive channels

What next?

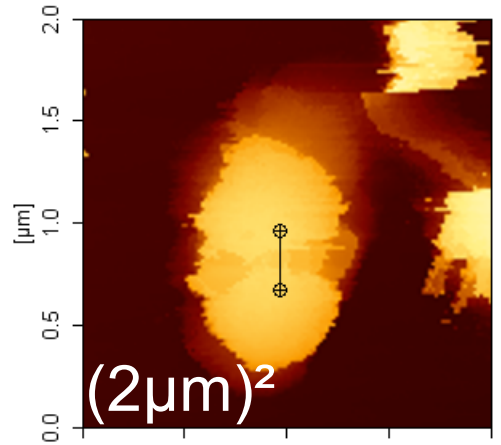
Staphylococcus aureus

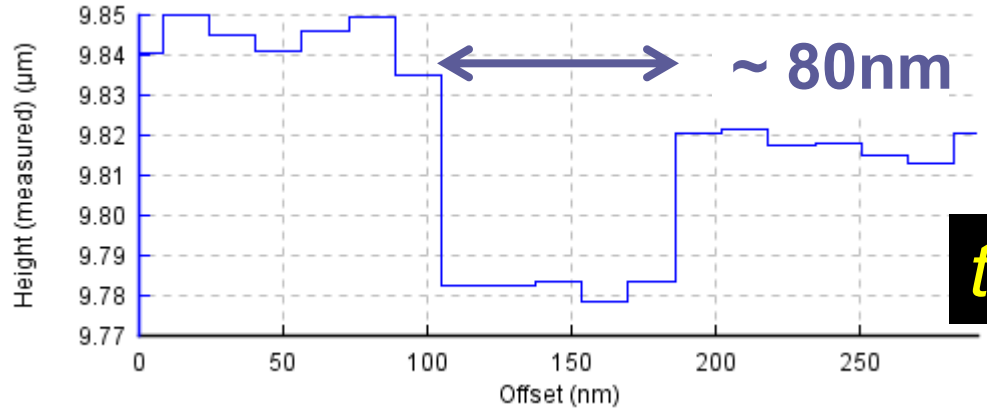
Sans antibiotique



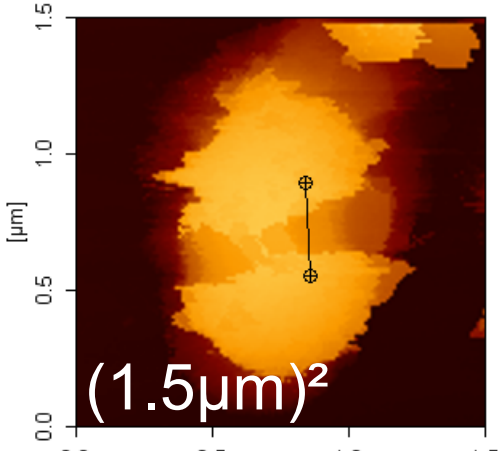


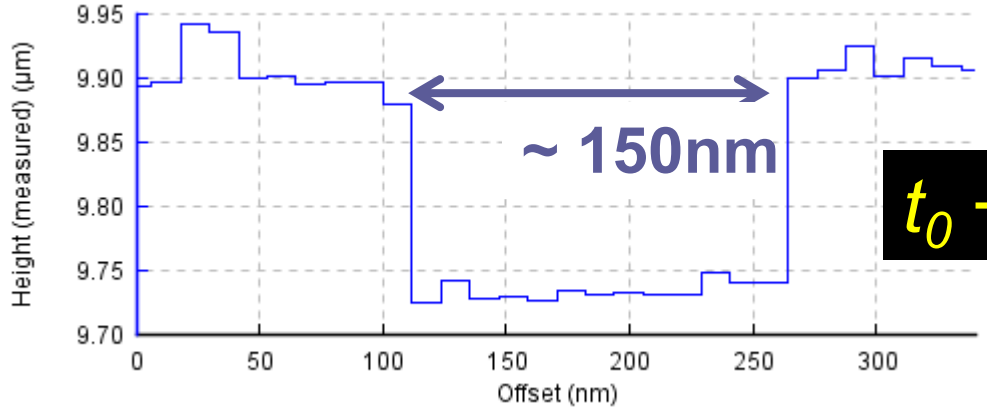
$(3\mu m)^2$



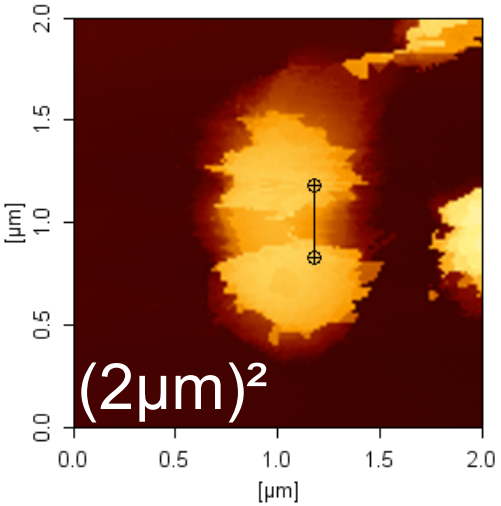


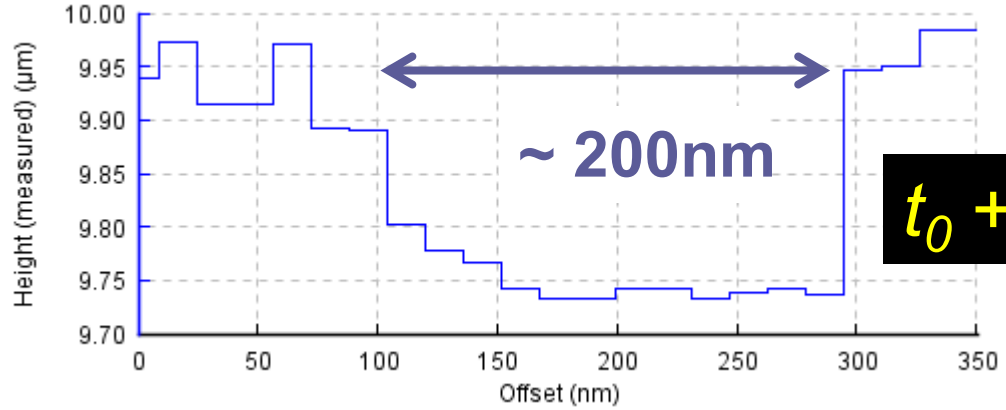
t_0 mn





t_0 + 20mn

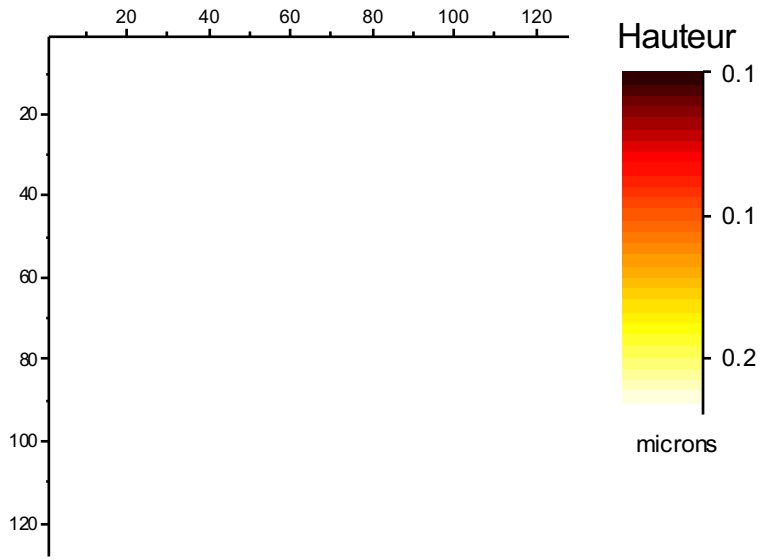


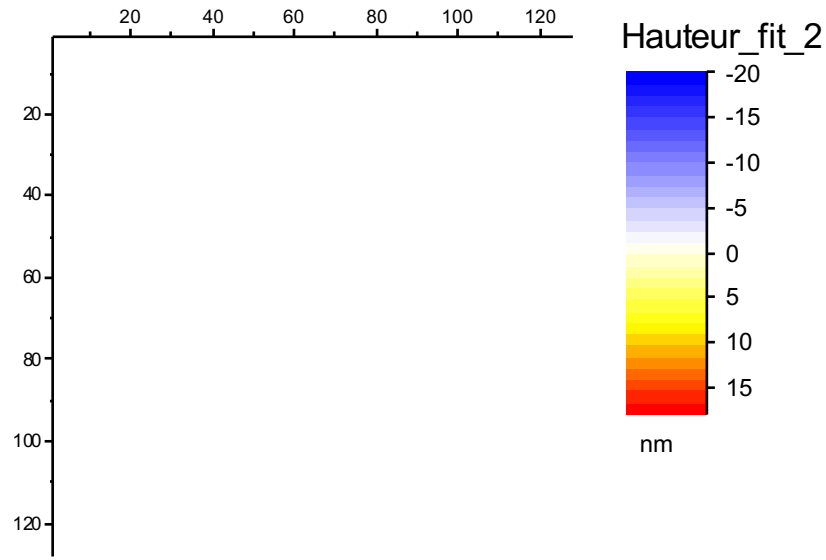


t_0 + 40mn

Staphylococcus aureus

Avec antibiotique (Daptomycine) : $t = 0$

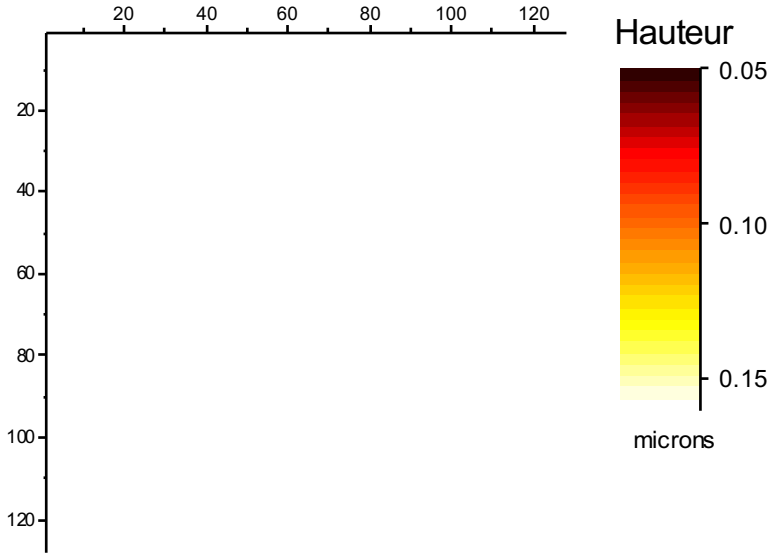


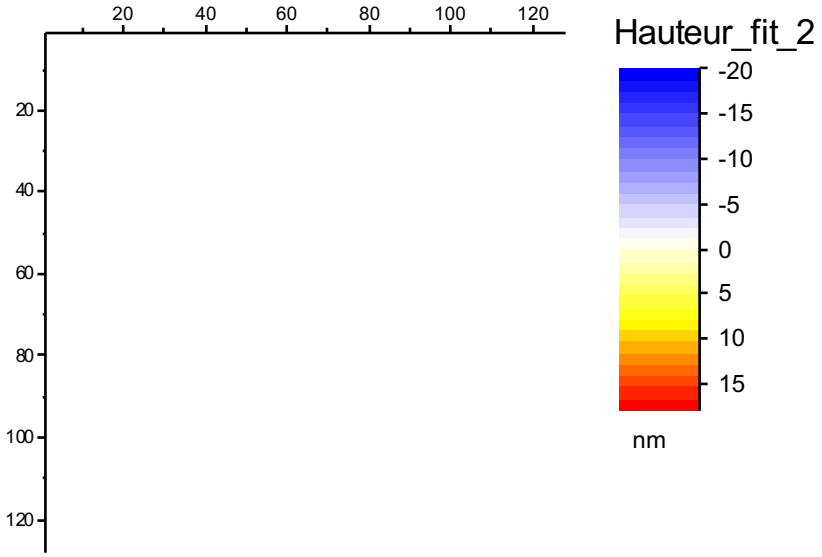


(400nm)²

Staphylococcus aureus

Avec antibiotique (Daptomycine) : t = 25mn

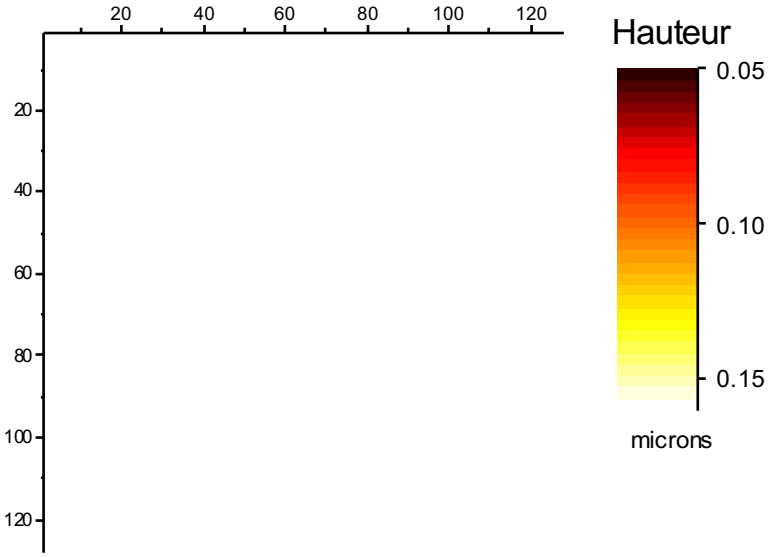


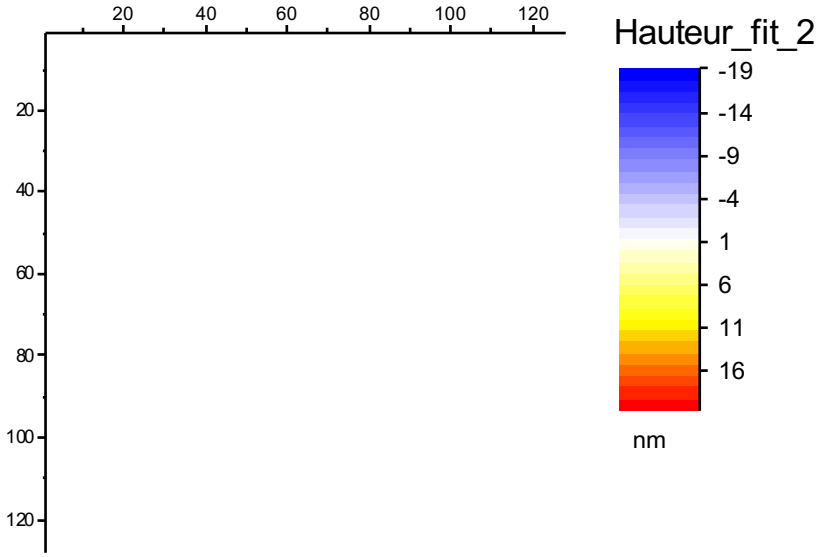


(400nm)²

Staphylococcus aureus

Avec antibiotique (Daptomycine) : t = 50mn

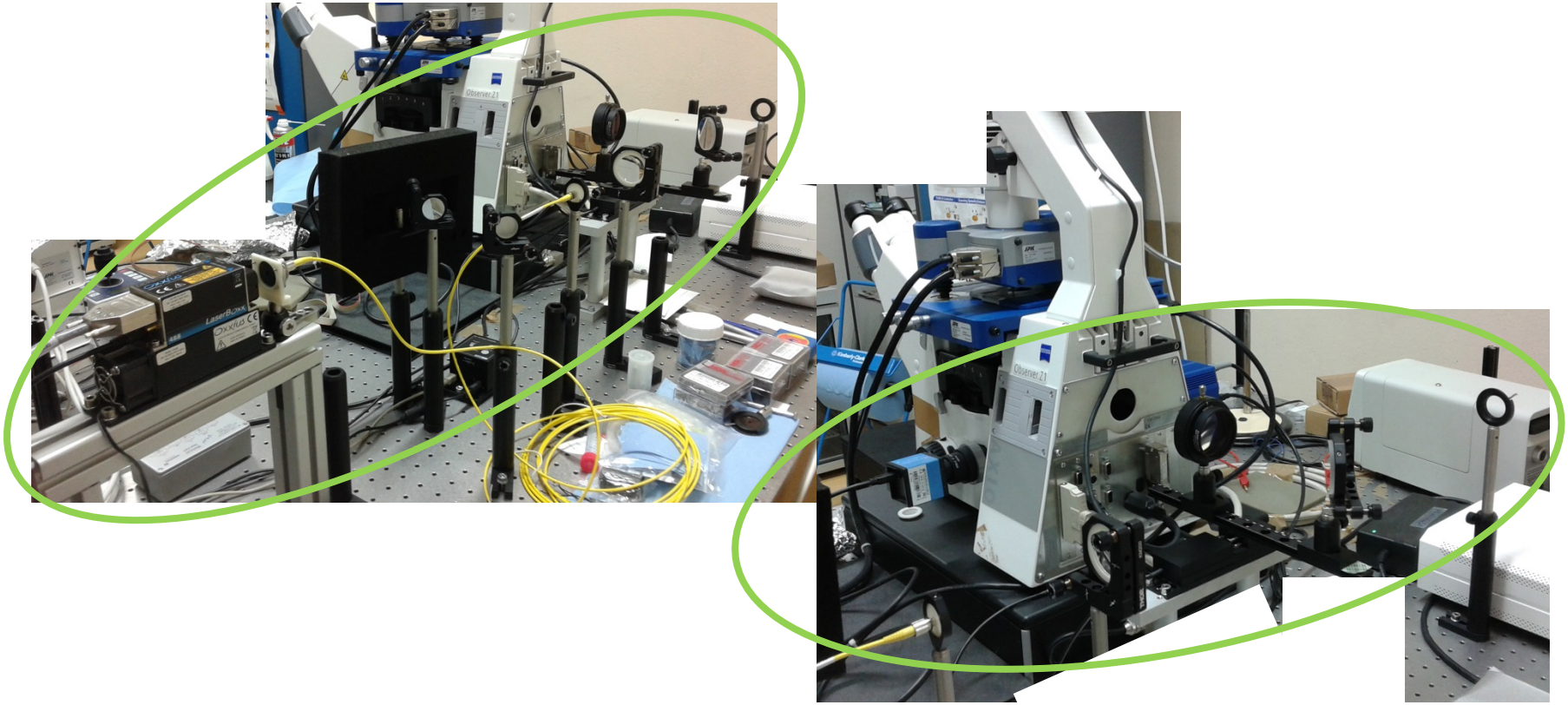




(400nm)²

Home-made TIRF

Catherine Le Bris / Sandrine Lévêque-Fort (ISMO)



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A direct and at nanometer scale study of electrical charge distribution on membranes of alive cells.

Thankyou for your attention

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