

DE LA RECHERCHE À L'INDUSTRIE



*Nouvelles approches  
pour la nano-caractérisation,  
et la micro- et nano- manipulation*

UPMC - Jussieu



CEA Saclay | **Jérôme Polesel**

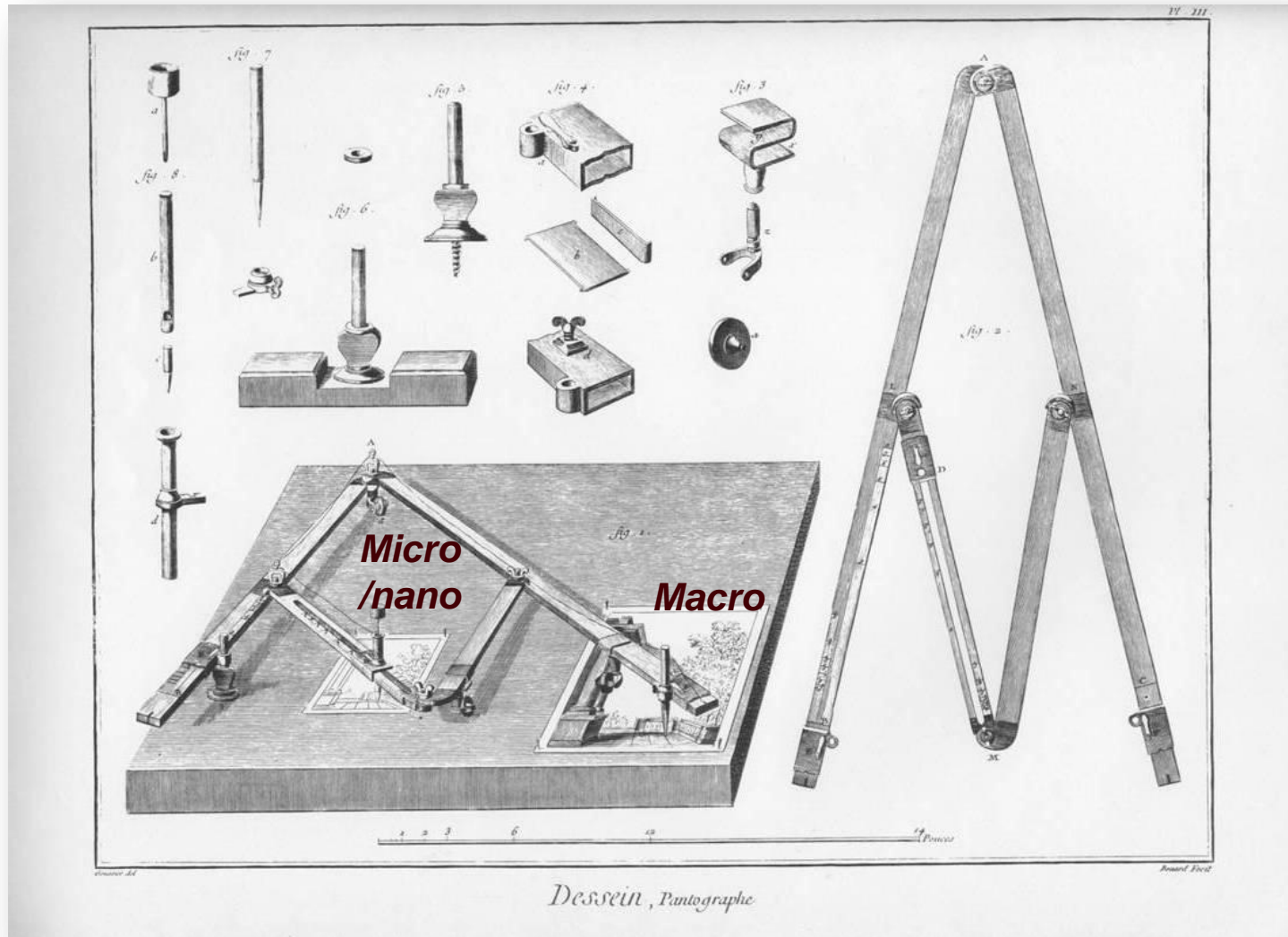
[www.cea.fr](http://www.cea.fr)

21 septembre 2012

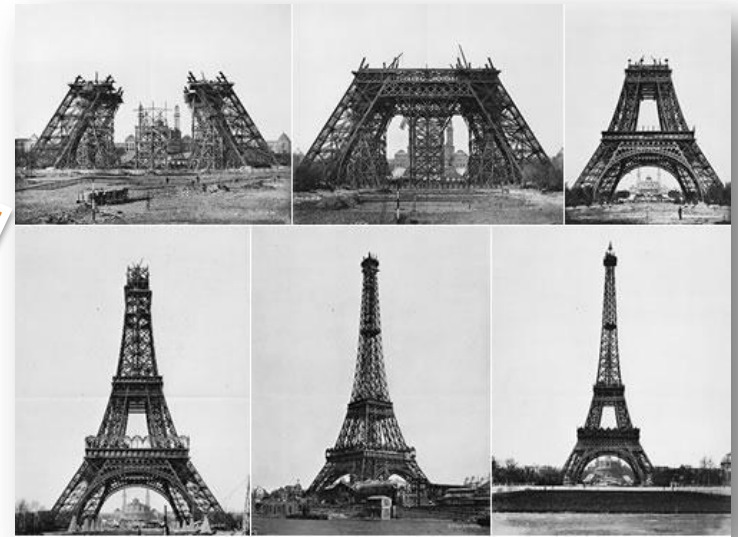
- I. Contexte: Les Nanotechnologies
- II. Avancés scientifiques et microscopies
- III. Les sonde locales
  - I. La microscopie à effet tunnel (STM)
  - II. La microscopie à force atomique (AFM)
  - III. Vers de nouveaux dispositifs à sonde locale ...
- IV. Vers de nouveaux dispositifs à sonde locale ...

# I. Les Nanotechnologies

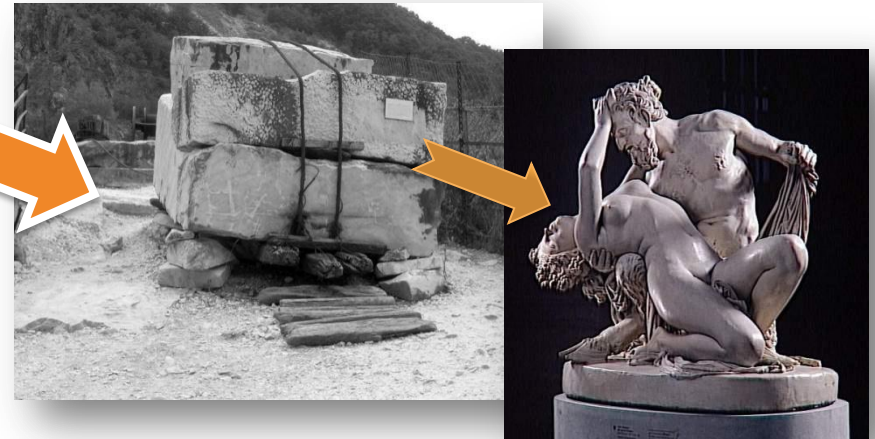
# Agir à l'échelle nanométrique



# Deux approches de fabrication

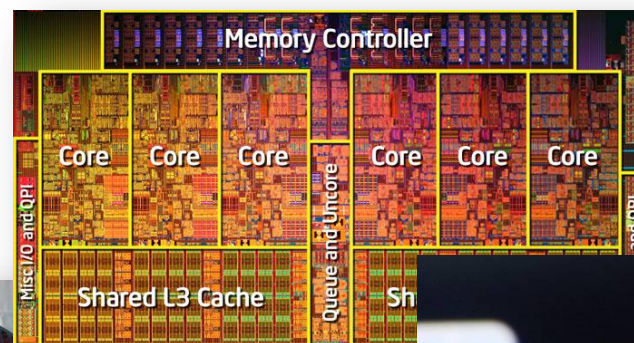
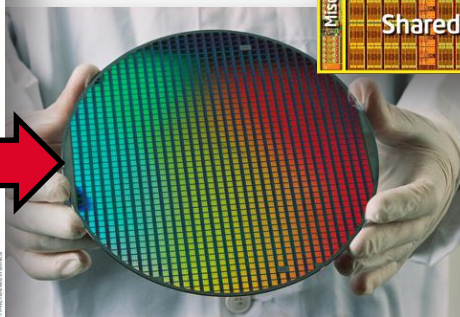
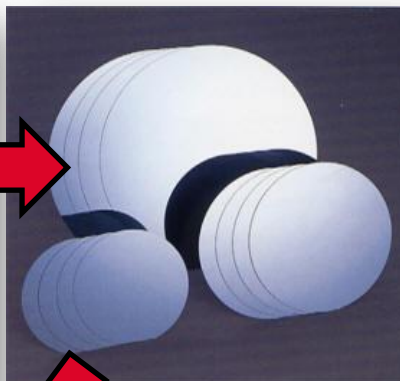
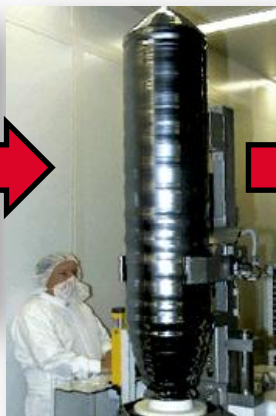


Bottom-Up

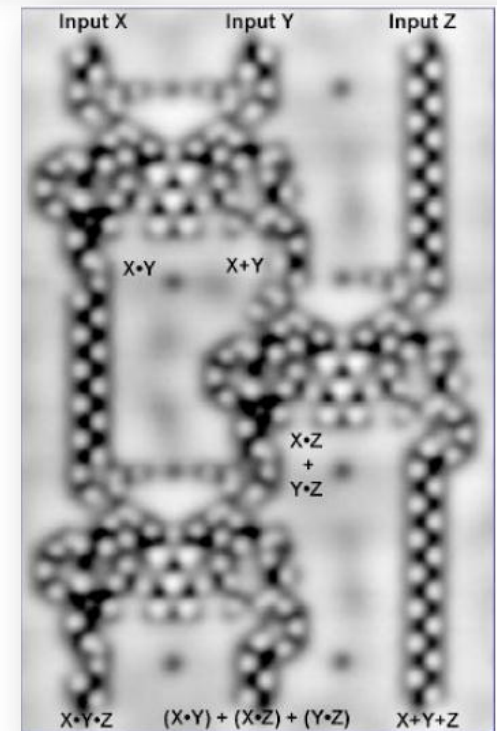
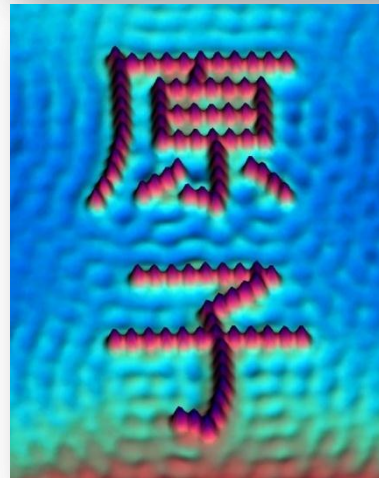
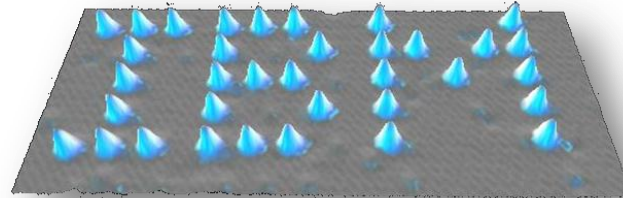
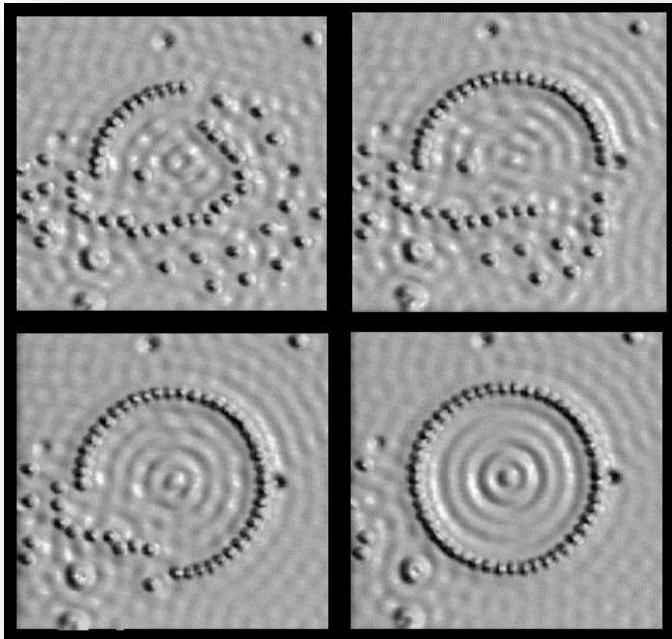


Top-down

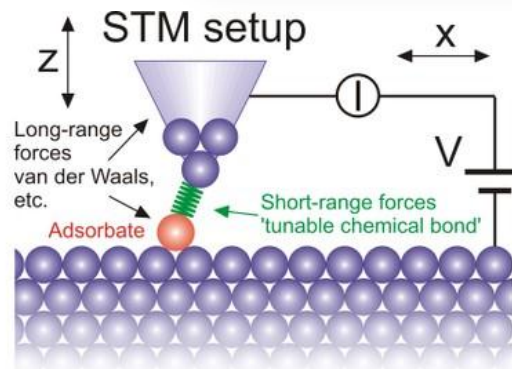
# La microfabrication, la lithographie: top-down



# Manipuler la matière atome par atome: bottom-up

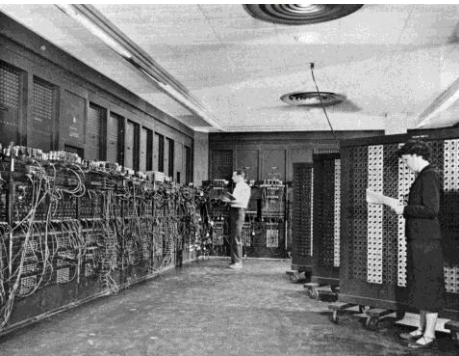


Don Eigler,  
Kauli prize  
en 2010



# La miniaturisation

1943-  
1945



Colossus, 1943

1954



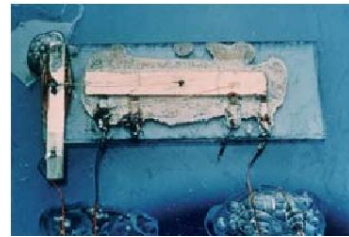
Bell Labs, 1947



William Shockley, John Bardeen et Walter Brattain  
Prix Nobel de Physique 1972

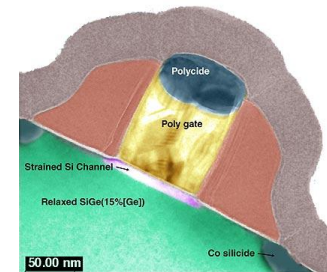


1958

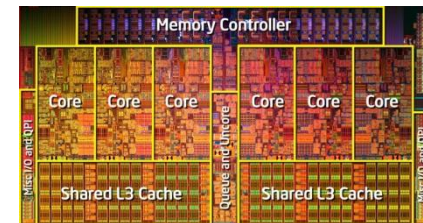
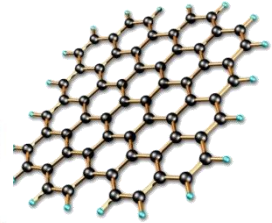


TI, Jack Kilby

2010

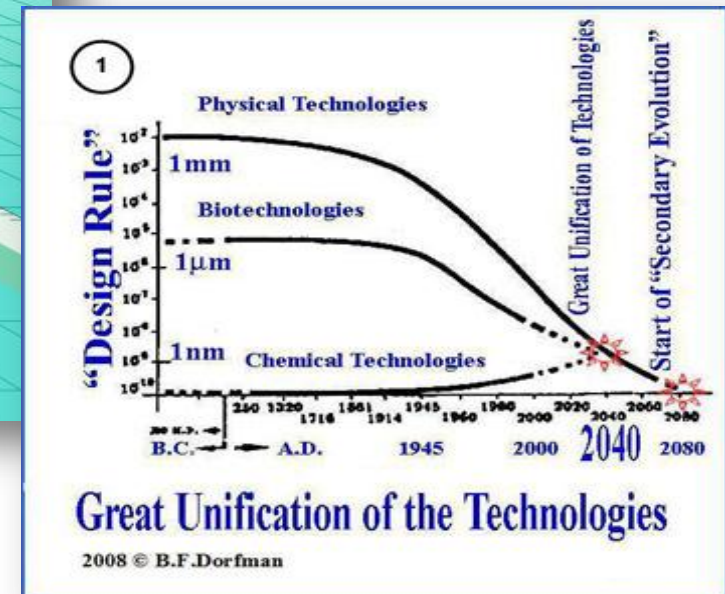
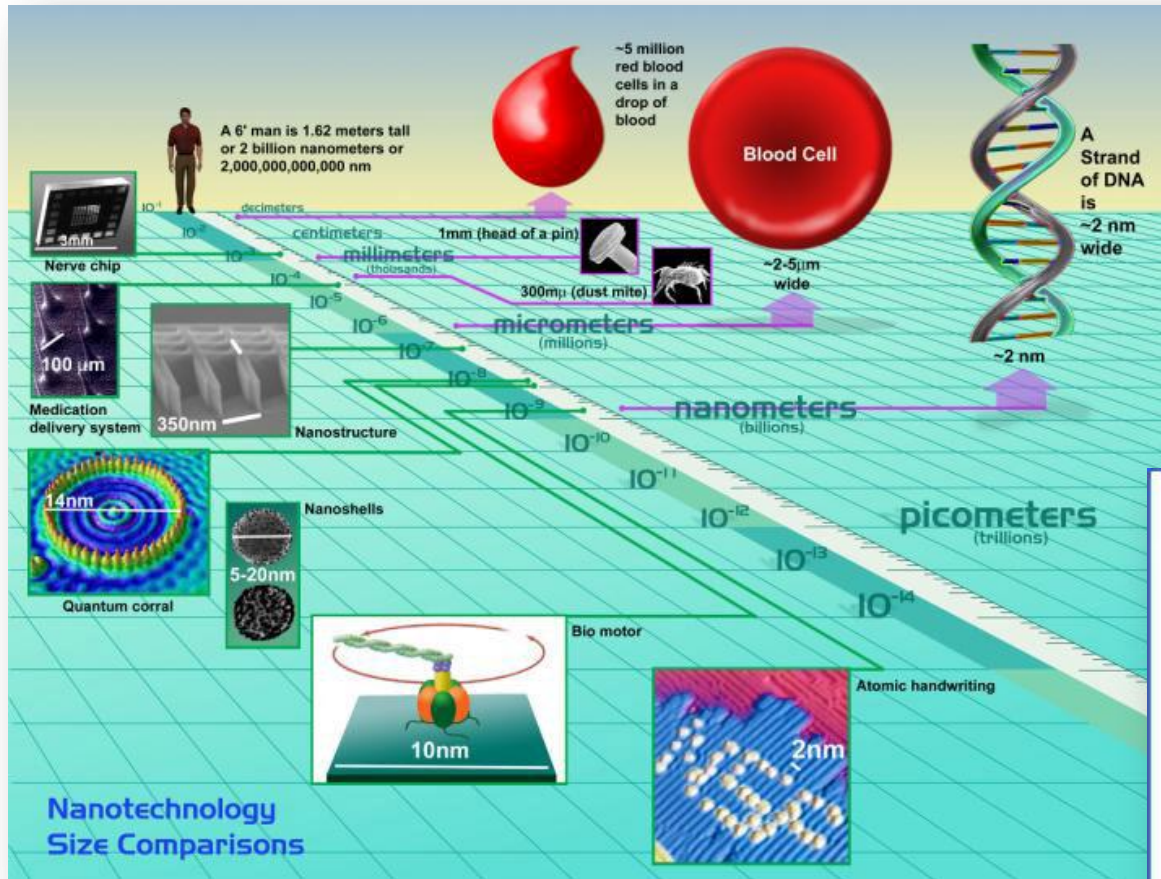


20??



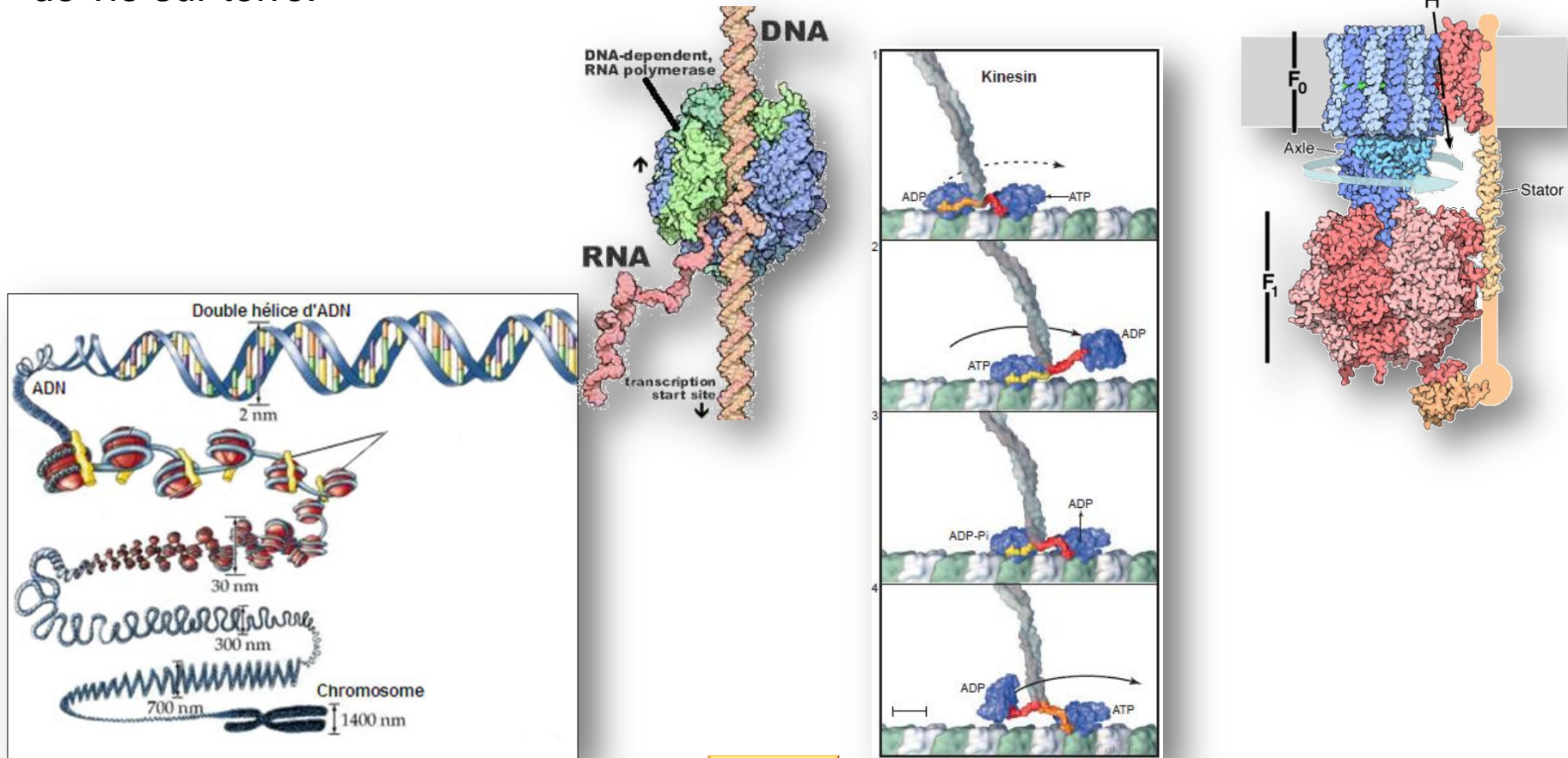
Core 2012

# Unification des domaines par la réduction en taille



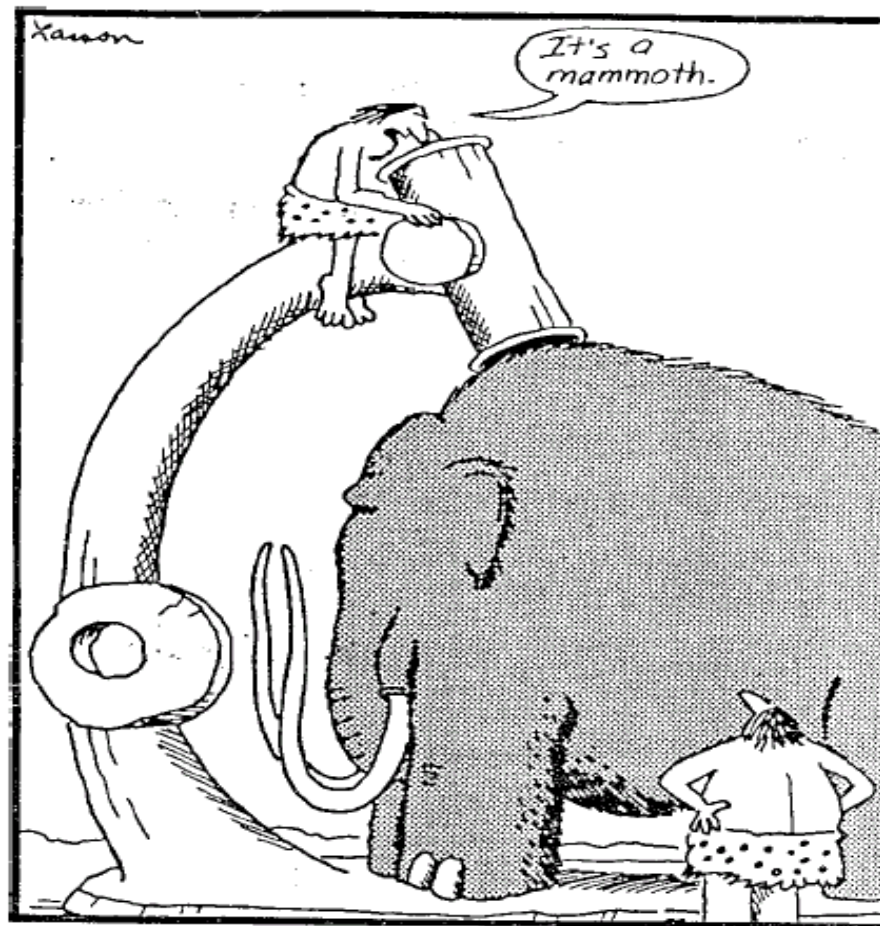
# Nanotechnologies dans la nature

La nature fabrique des nanomachines depuis l'apparition des premières formes de vie sur terre:



Nécessité de caractériser la matière à des échelles de plus en plus petites !

## II. Avancés scientifiques et microscopies

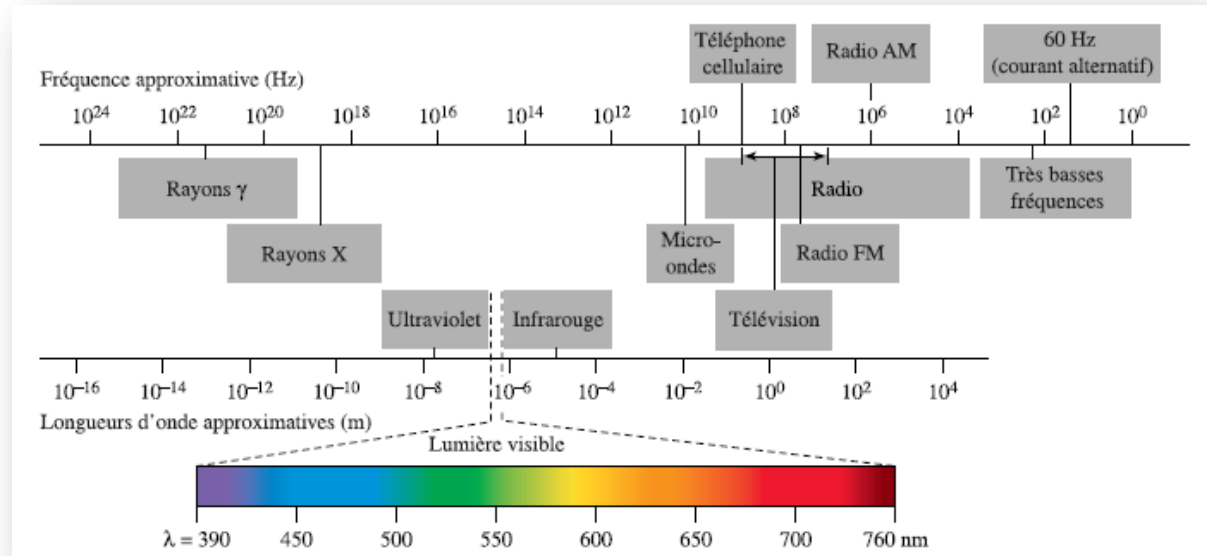


Early microscope

# Comment caractériser la matière ?

## – Sonde électro-magnétique, “champ”:

photon

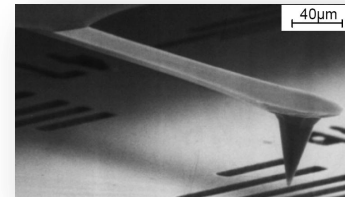


## – Sonde “particulaire”:

électron, neutron, ions/atomes (ex: He), ...

## – Sonde mécanique:

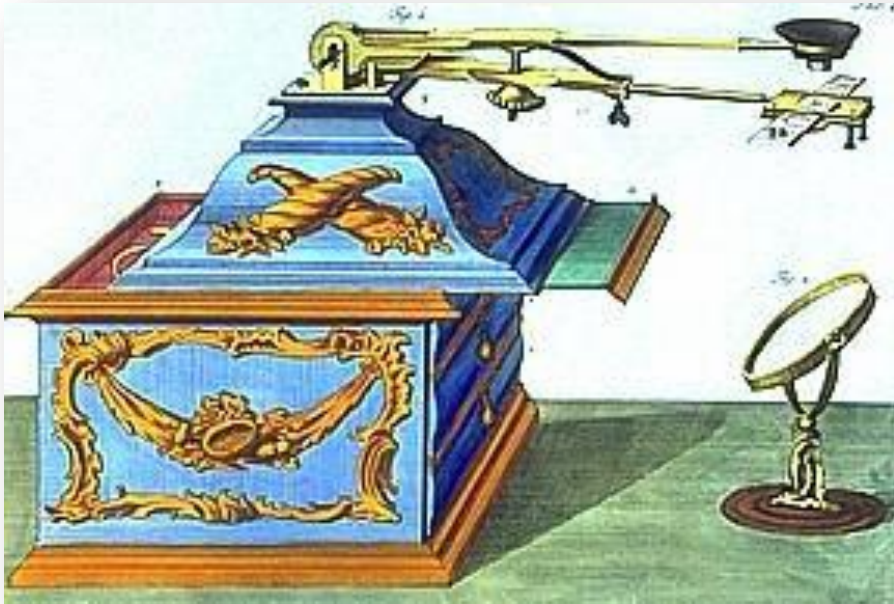
cantilever AFM = nano-palpeur



# Antonie van Leeuwenhoek (1630 - 1723)



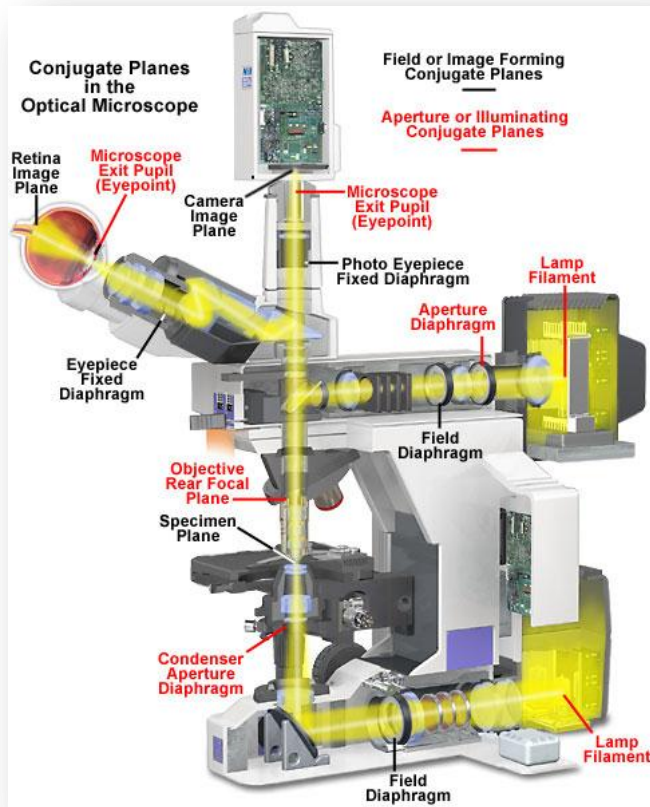
# Antonie van Leeuwenhoek (1630 - 1723)



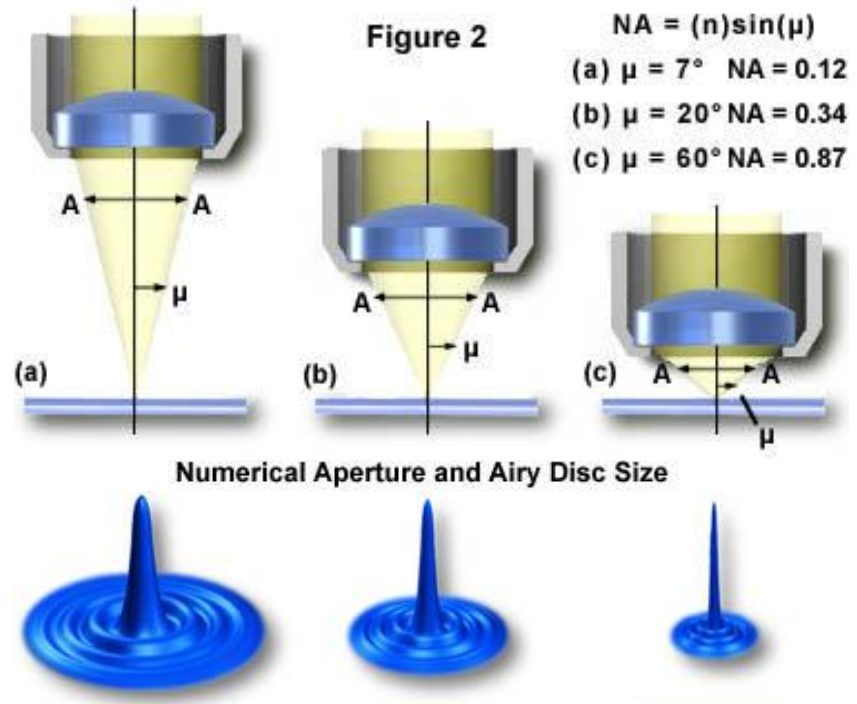
- Grossissements jusqu'à 300 fois !
- Suffisant pour observer les cellules



# La microscopie optique moderne



Des limites fixés par la nature ondulatoire de la lumière :



Limite de résolution latérale:

$$\Delta x \approx 1.22 \frac{\lambda}{2.ON} \quad (\sim 200 \text{ nm})$$

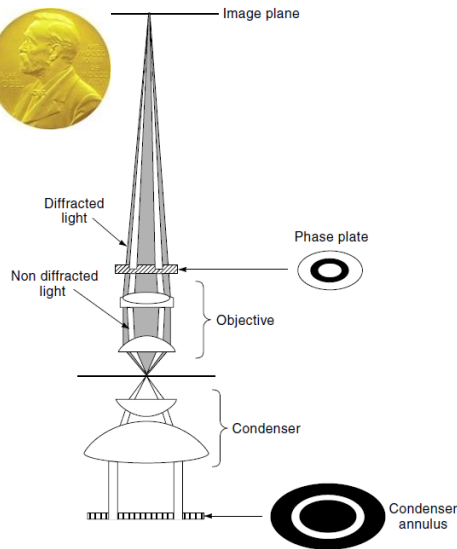
Profondeur de champ:

$$DOF \approx 4 \frac{\lambda}{ON^2} \quad (\sim 1 \mu\text{m})$$

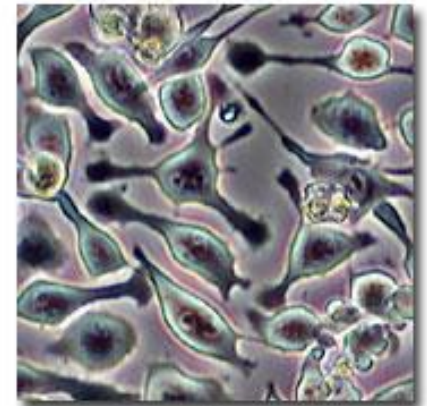
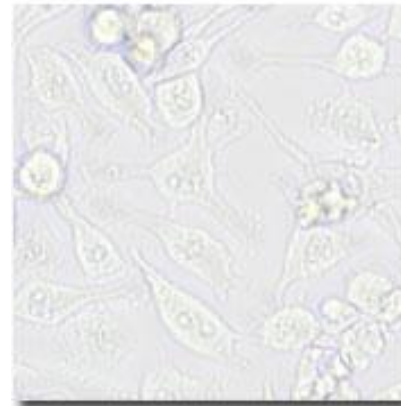
# Voir les objets transparents



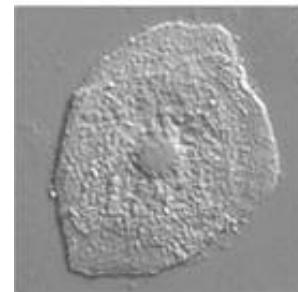
Frederik Zernike  
prix Nobel de  
physique 1953



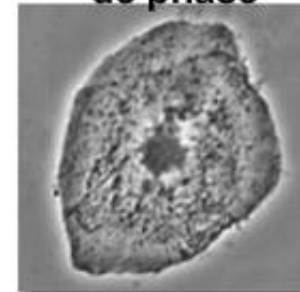
Living Cells in Brightfield and Phase Contrast



DIC



Contraste  
de phase

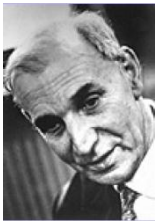


- Microscope exploitant le déphasage et non l'absorption lumineuse

## LASER



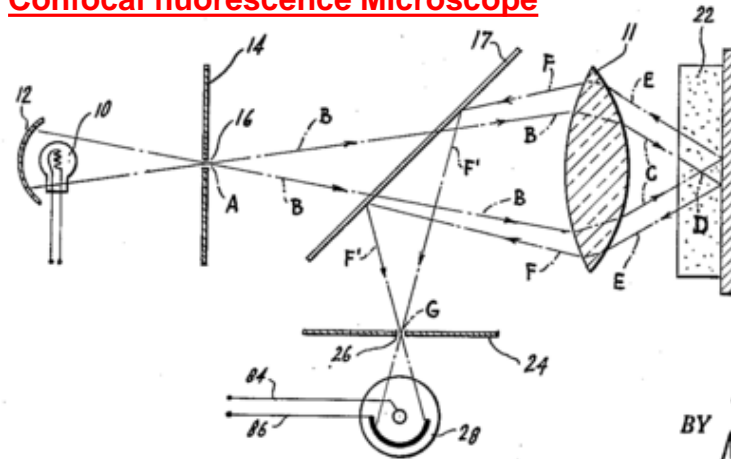
Nobel Physics, 1964  
Townes, Basov, Prokhorov  
La théorie du LASER



Alfred Kastler  
Nobel Physics, 1966  
Pompage optique



## Confocal fluorescence Microscope



1957 INVENTOR.  
MARVIN MINSKY  
BY Ameter & Levy  
ATTORNEYS

## Green Fluorescence Protein (GFP)



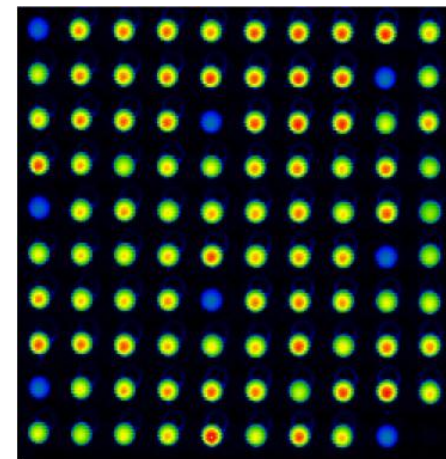
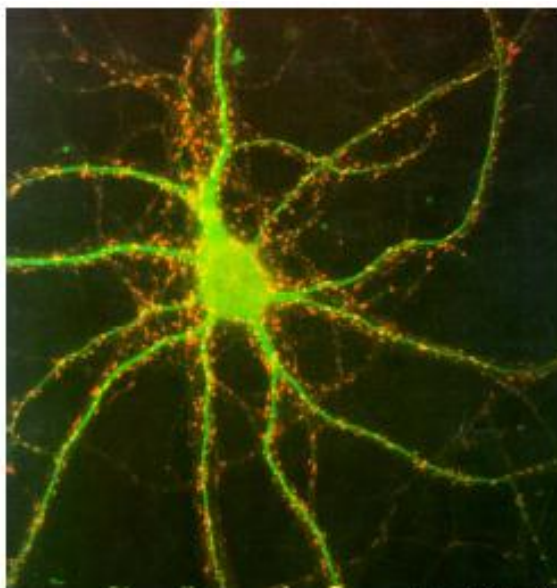
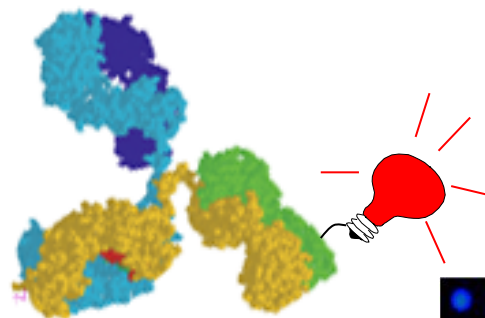
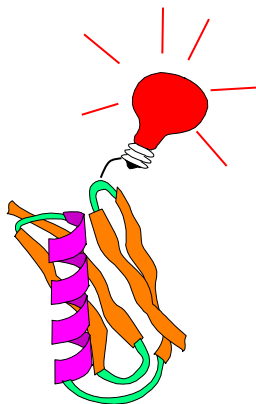
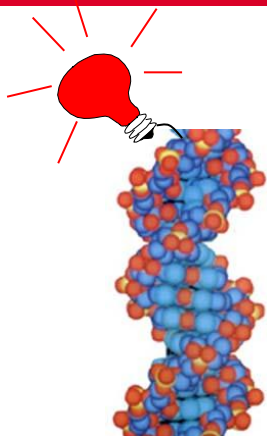
Nobel Chemistry 2008:  
R. Tsien, M. Chalfie, O.  
Shimomura



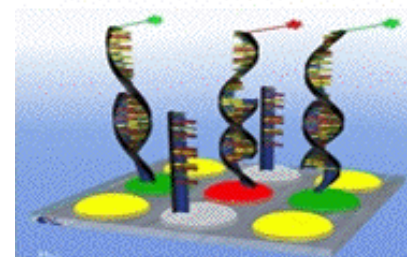
1974



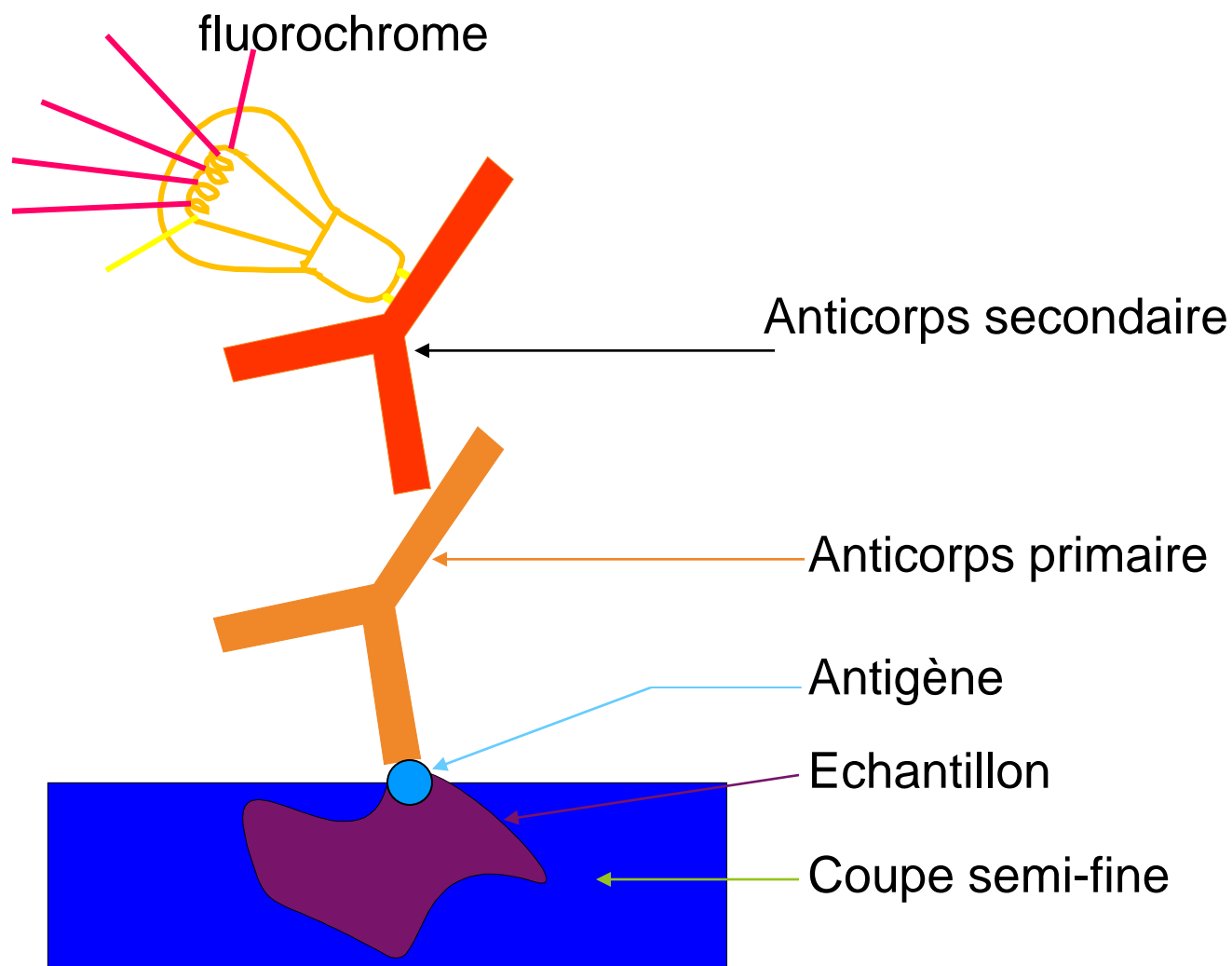
# Marquage à l'échelle moléculaire



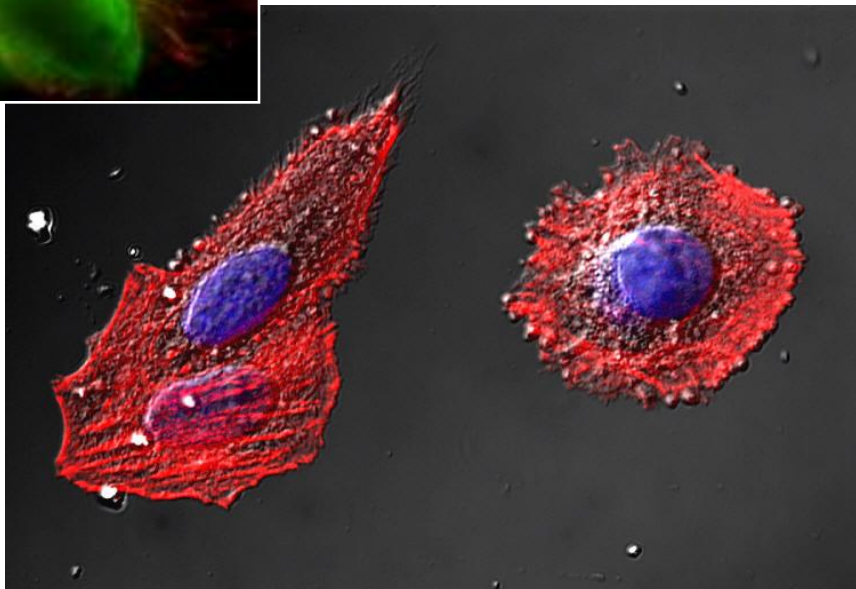
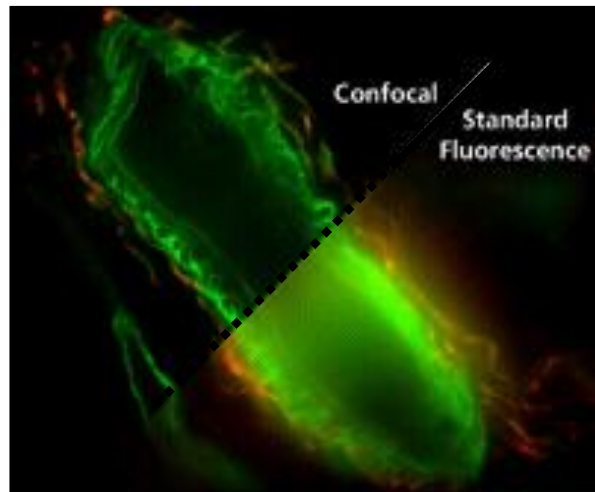
● Sample A > B  
● Sample B > A  
● Sample A = B



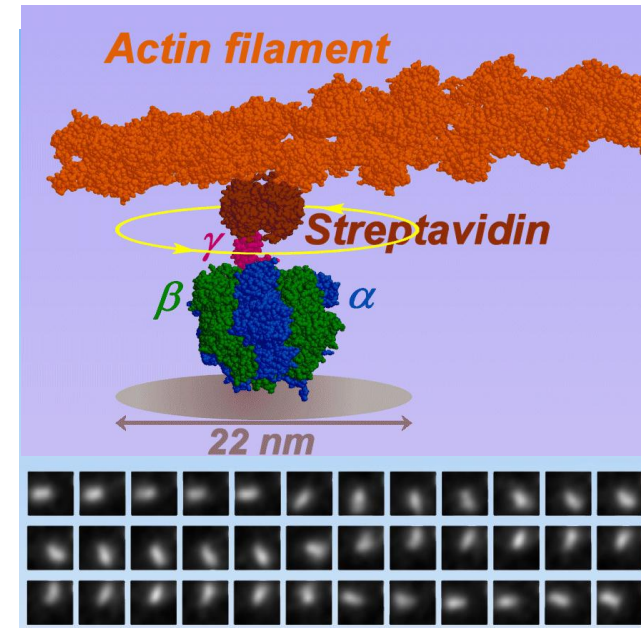
# Les marquages en immunofluorescence



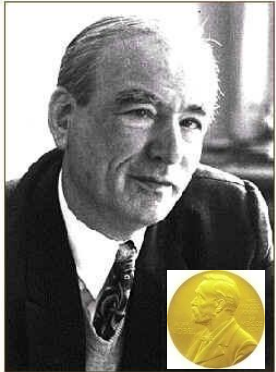
# Suivi de protéines



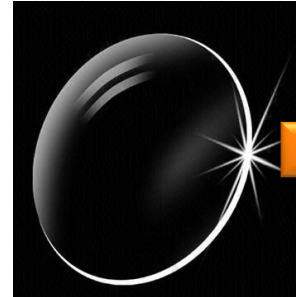
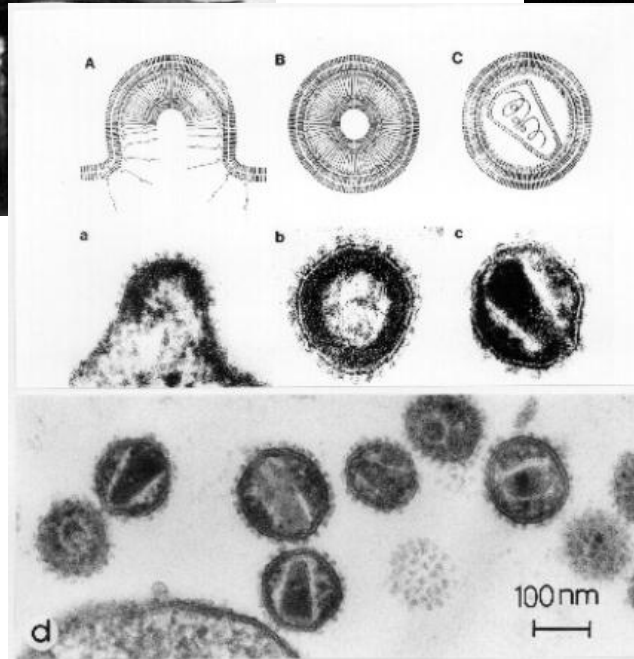
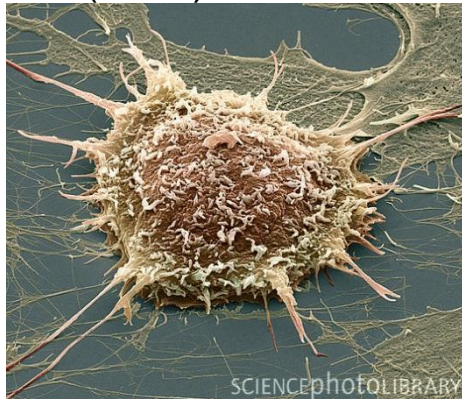
F1ATPase: the smallest motor !



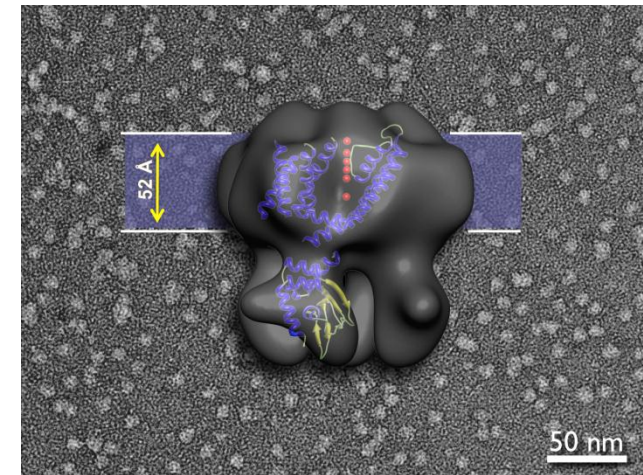
# La microscopie électronique



Ernst Ruska,  
Nobel  
Physique  
(1986)



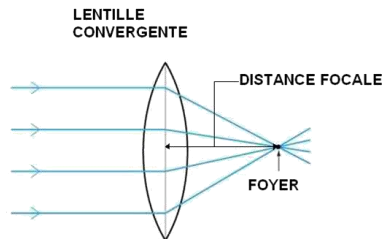
$$\text{Spatial resolution} = 1.22 \frac{\lambda}{2 \cdot \text{NA}}$$



cyclic nucleotide gated potassium  
channel MloK1

# Analogie Microscopie Electronique/Microscopie optique

photons

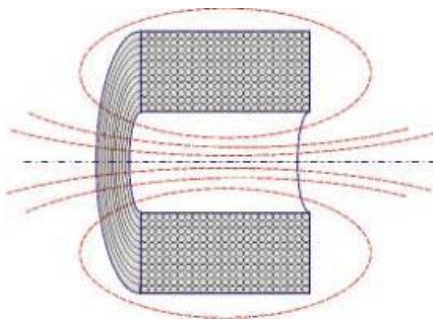


**Résolution spatiale =  $1.22 \lambda / 2.NA$**

$$\lambda_{electron} = \frac{h}{\sqrt{2.m_0.q.U.\left(1 + \frac{q.U}{2.m_0.c^2}\right)}}$$

$$\lambda_{electron} = 12.2 \text{ pm à } U=10\text{kV}$$

électrons

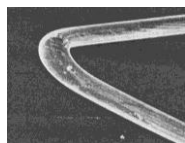


$$\vec{F}_{em} = q.\vec{v} \wedge \vec{B}$$

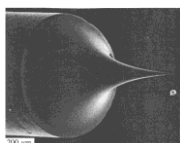


Lentille  
électromagnétique

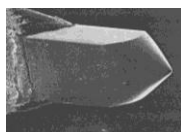
# Structure d'un microscope électronique à balayage



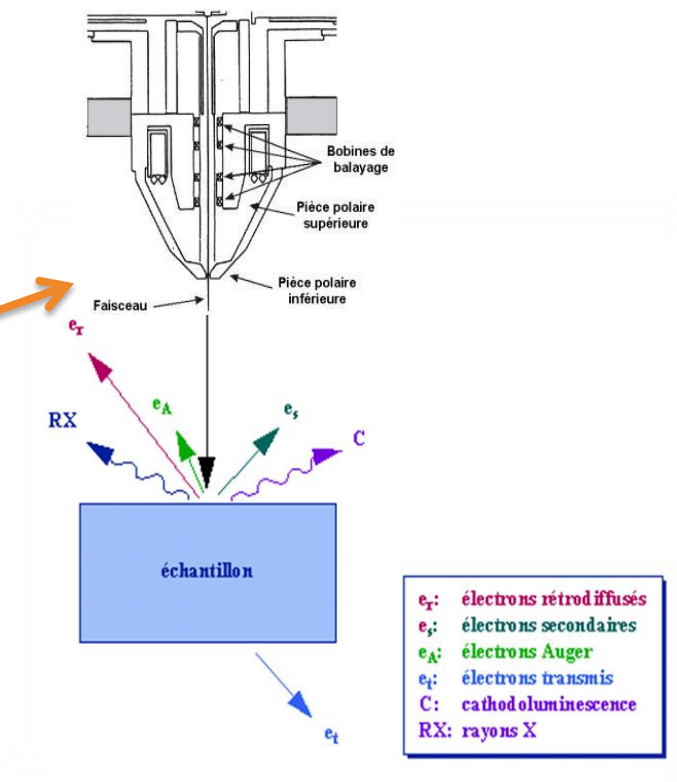
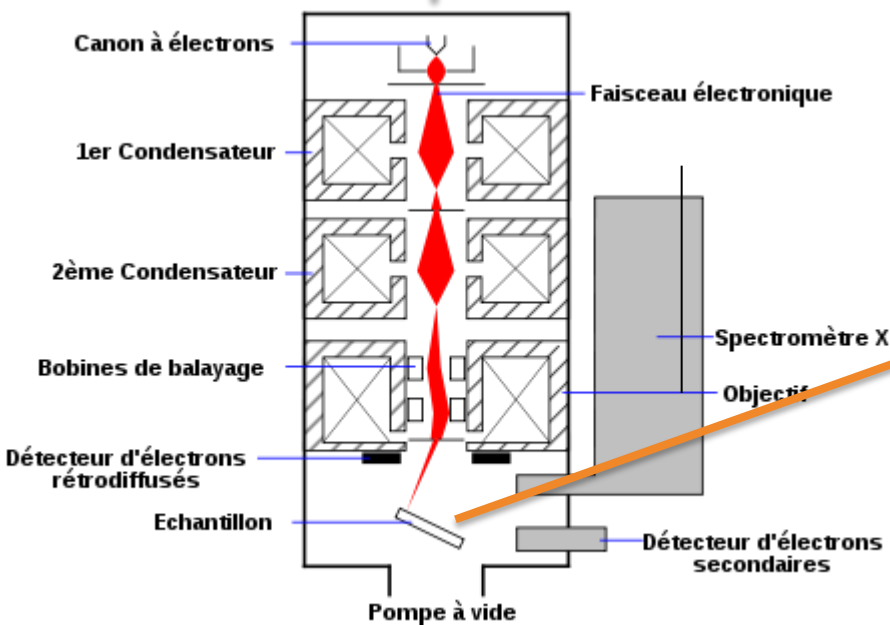
Filament de tungstène



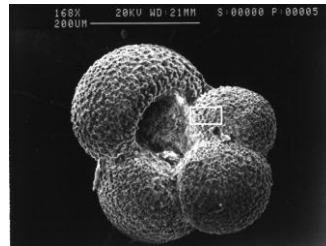
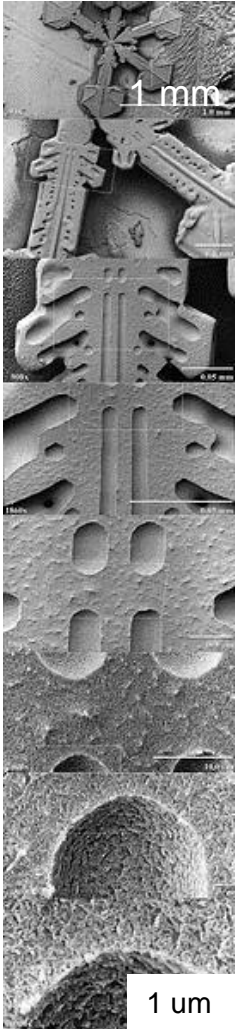
FEG



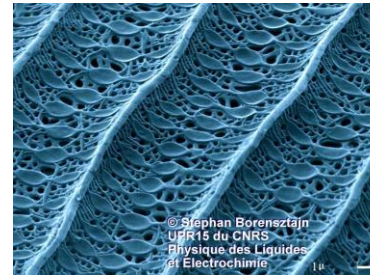
Cristal de  $\text{LaB}_6$



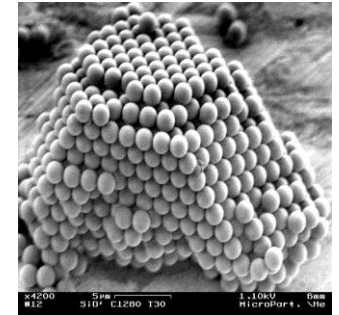
# Microscopie électronique à balayage (MEB ou SEM)



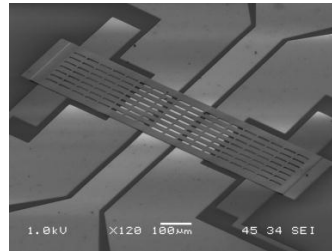
Foraminifère  
planctonique



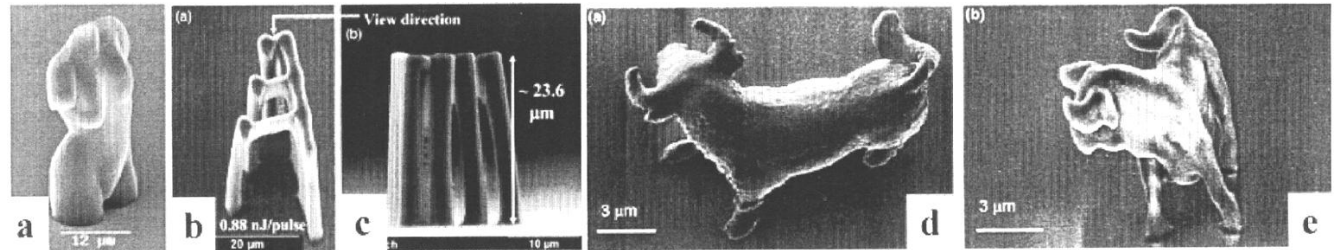
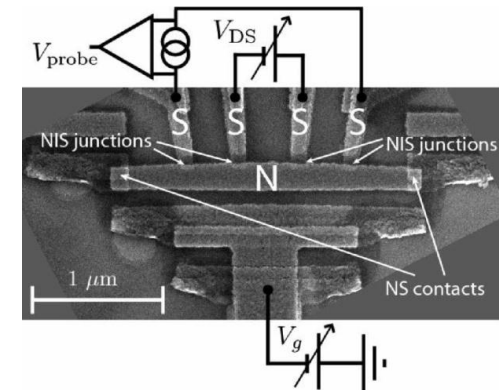
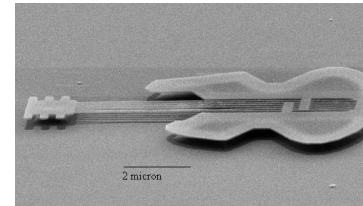
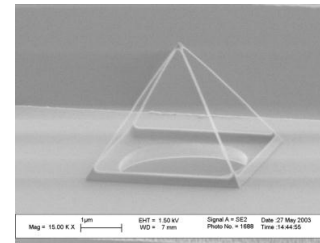
Détail aile de papillon



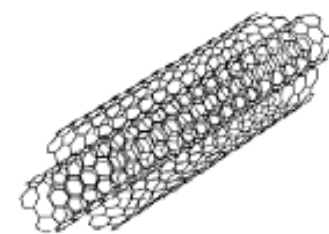
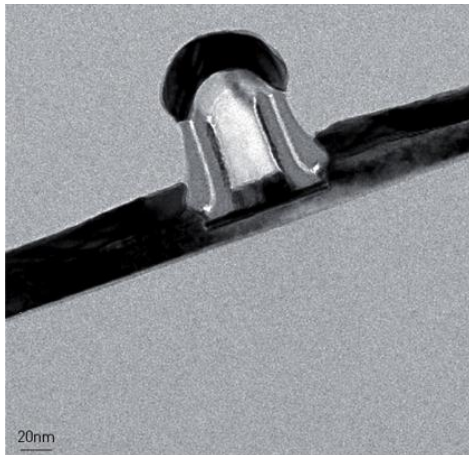
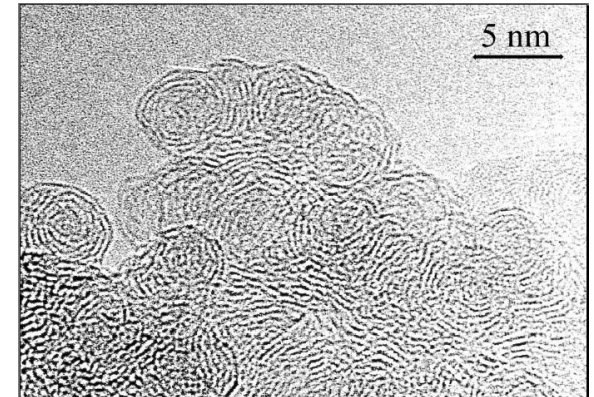
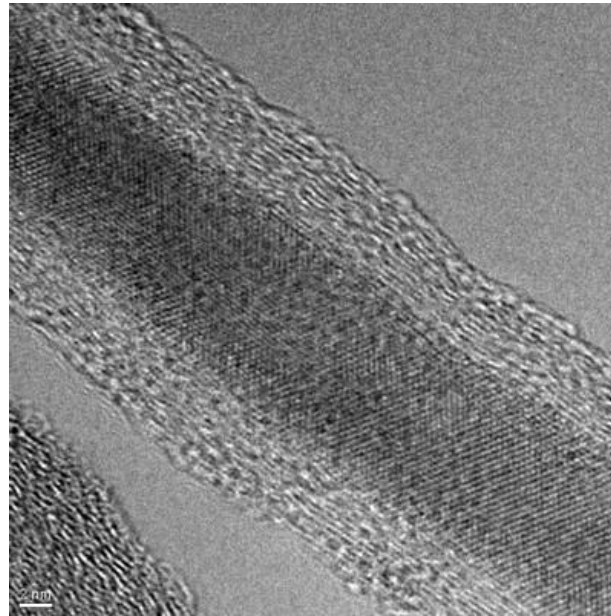
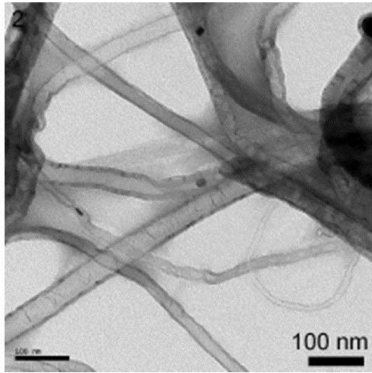
Assemblage 3D de  
billes de silice



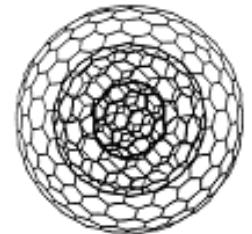
Résonateur RF 4GHz



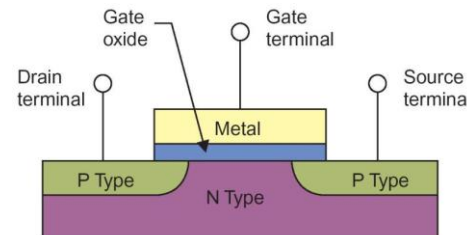
# Microscopie électronique à transmission (MET ou TEM)



Multi-Wall NT



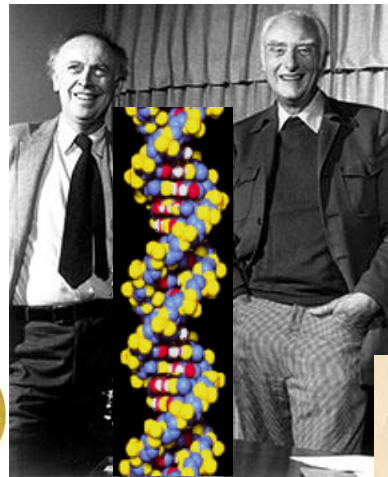
Nano-onions



# Rayons X et structures des protéines



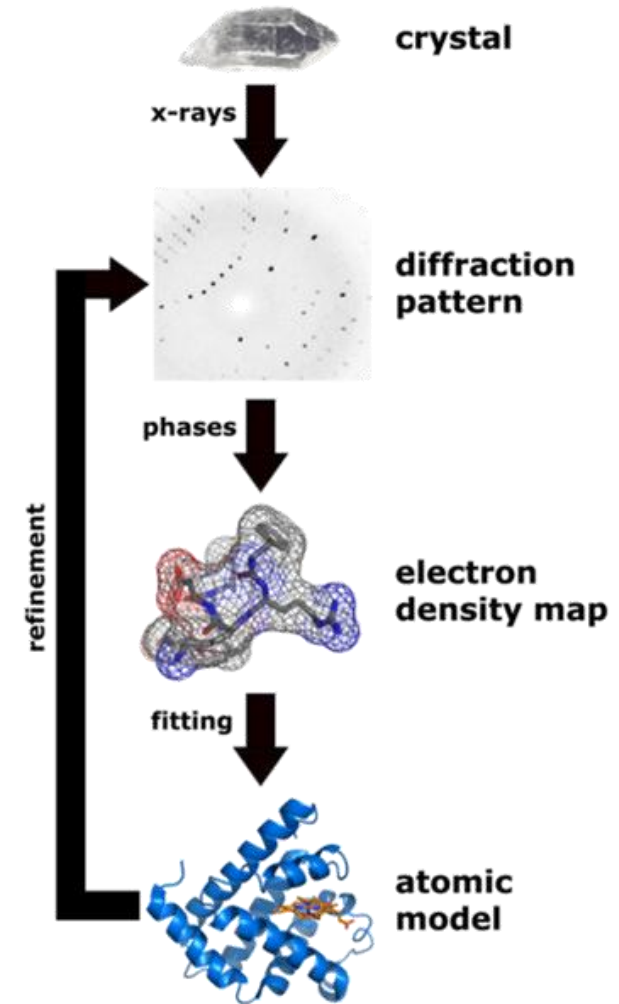
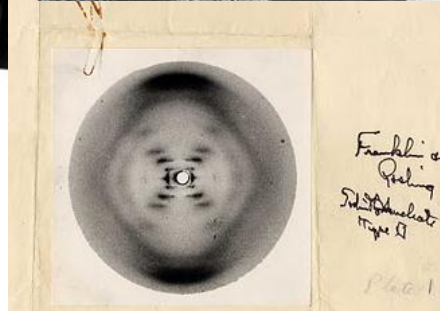
Wilhelm Röntgen  
prix Nobel de  
physique 1901



J. Watson, F.  
Crick, M. Wilkins  
, Nobel de  
physiologie et de  
médecine, 1962

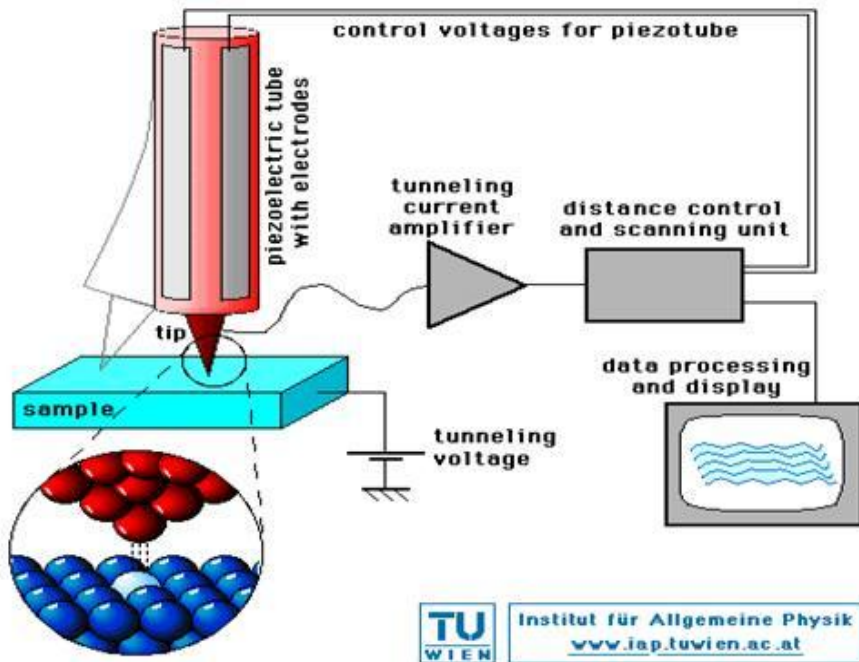


Rosalind Franklin



# III. Les sonde locales

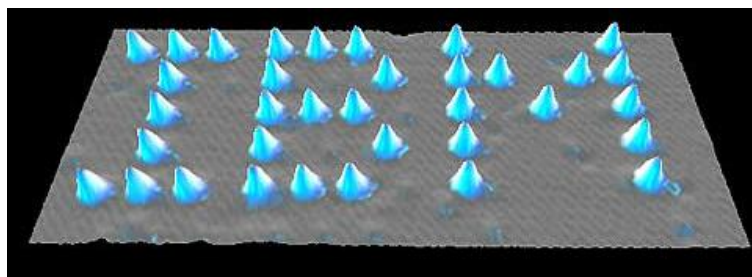
# III.1 La microscopie à effet tunnel (STM)



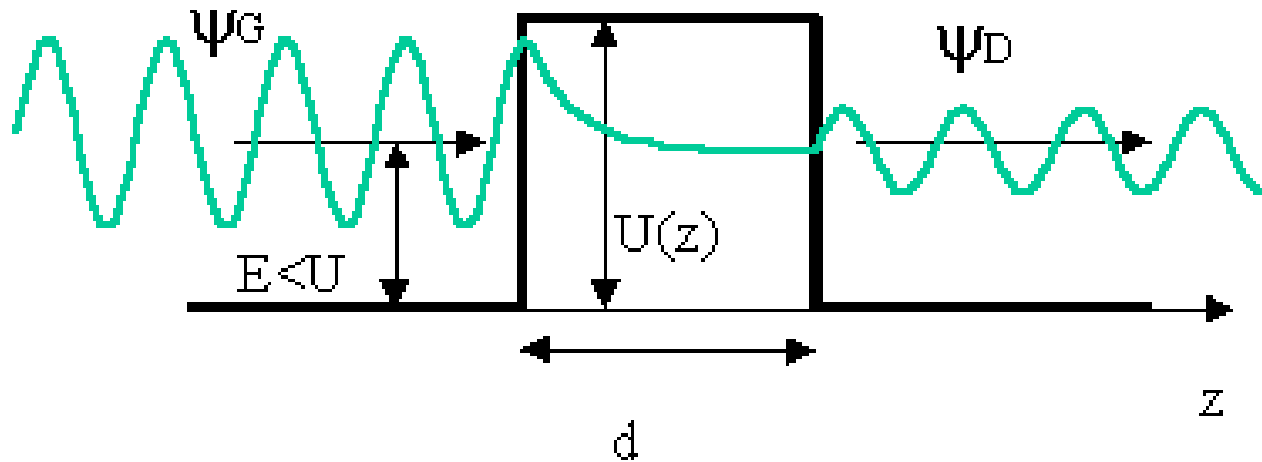
**Schéma de principe  
du microscope à effet tunnel (STM)**



**Prix Nobel (1986)**

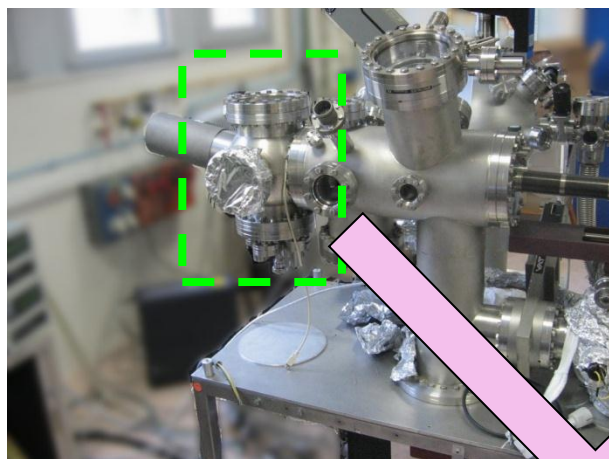


# L'effet tunnel électronique

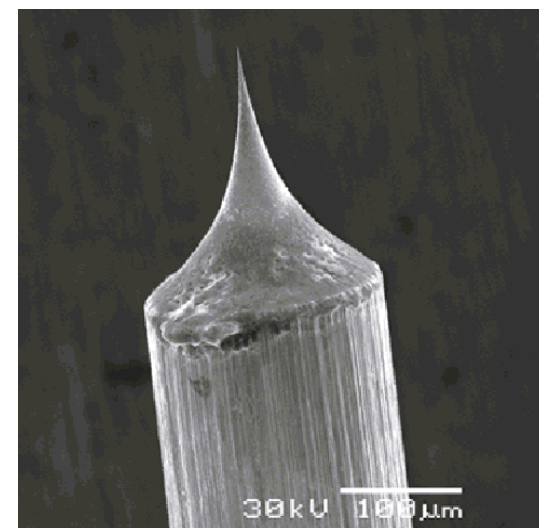
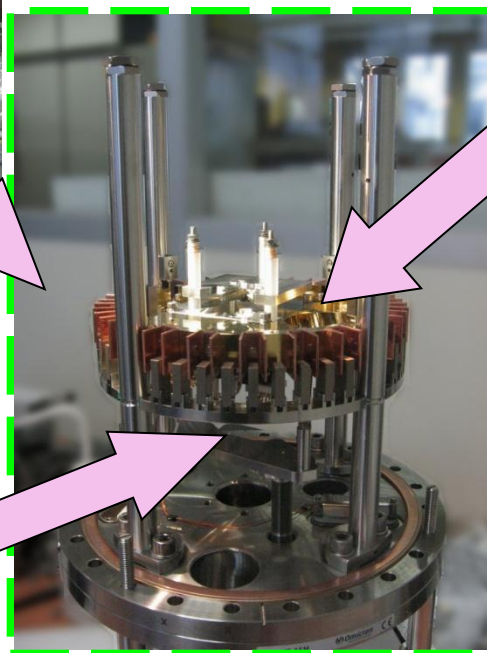
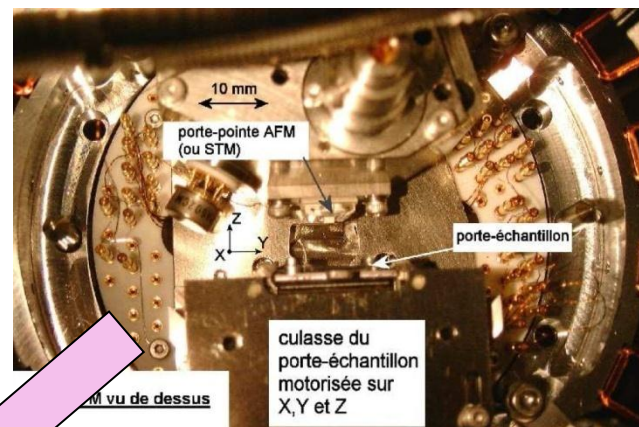


$$I_{\text{tunnel}} = I_0 \cdot \exp(-2 \cdot \kappa \cdot d) \quad \text{avec} \quad \kappa = \frac{\sqrt{2 \cdot m \cdot \phi}}{\hbar} \sim 10 \text{ Angströms}$$

# Un microscope STM sous Ultra-Vide

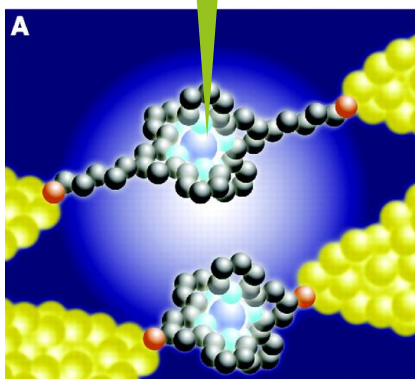


Chambre Ultra-Vide du STM

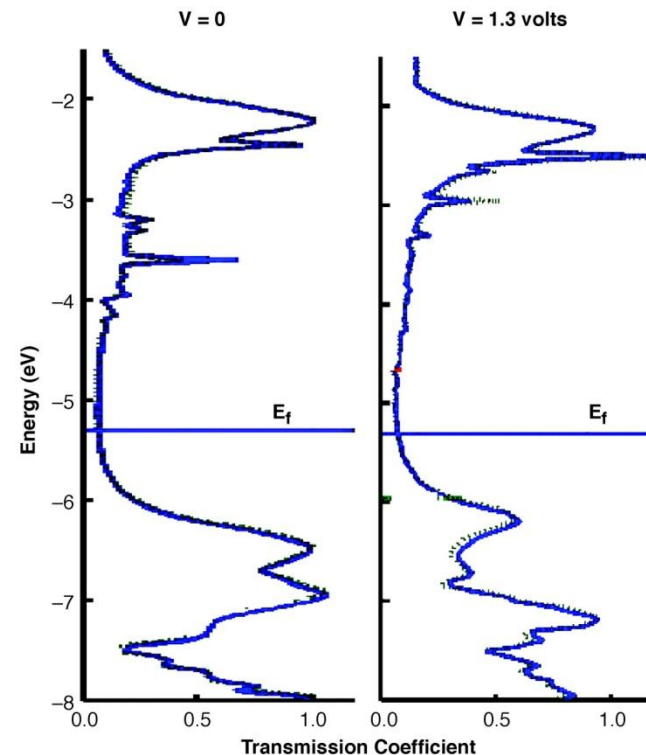
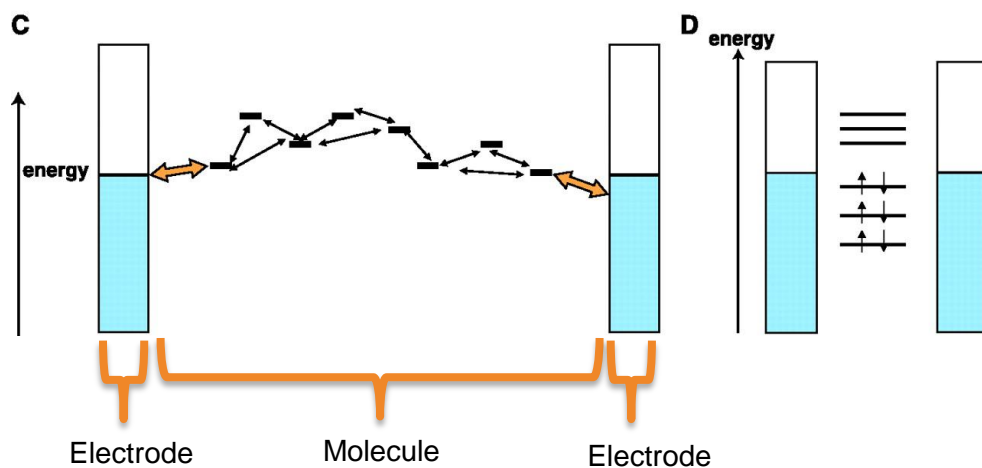


# Densité locale d'état électronique

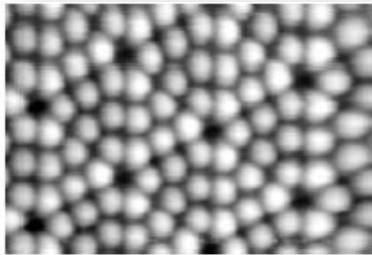
Pointe STM



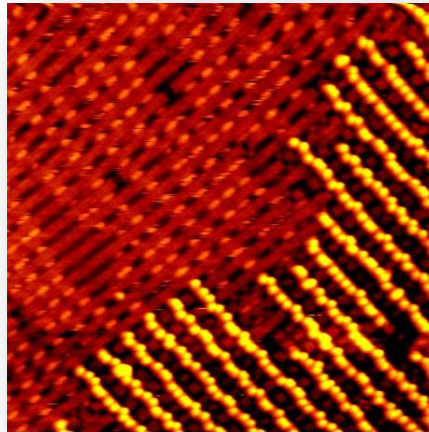
A. Nitzan et al., Science 300, 1384 (2003)



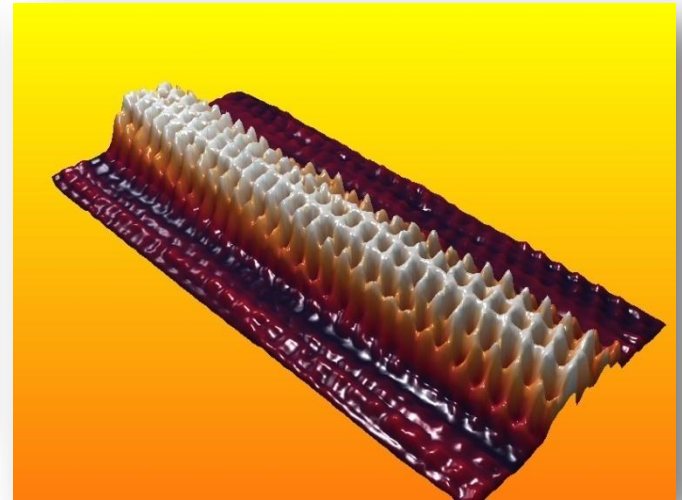
# Résolution atomique



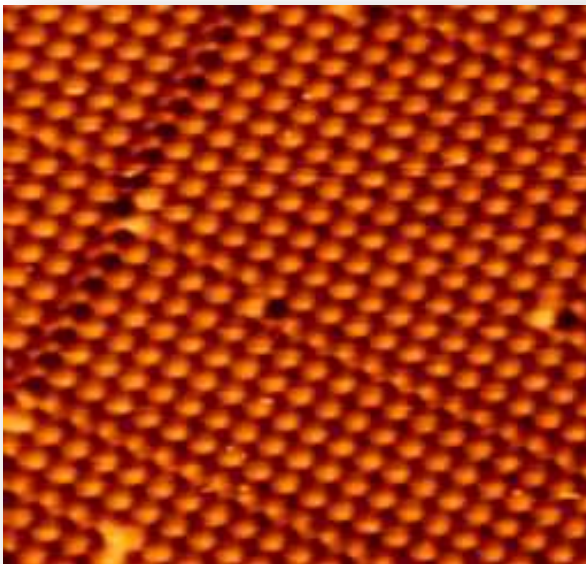
Si(111)(7x7)



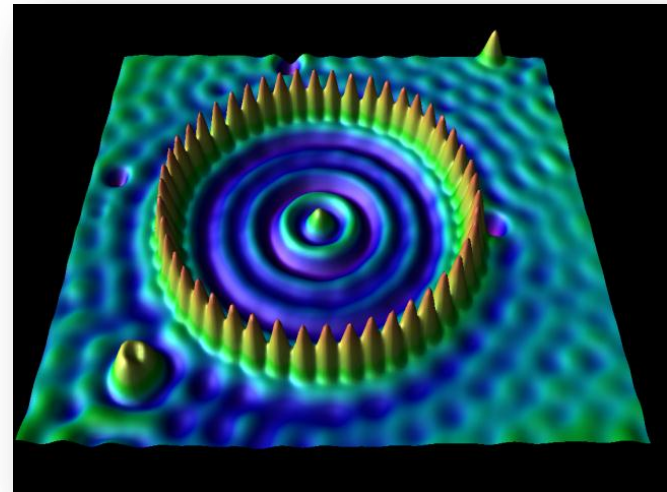
GaAs(001)



Nanotube de carbone

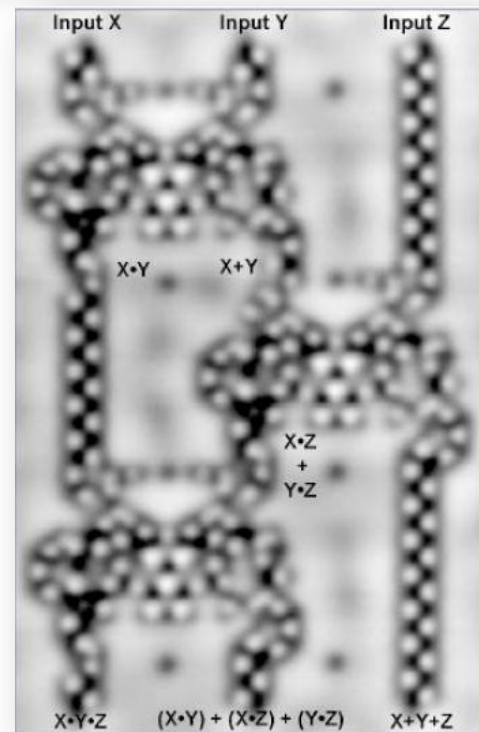
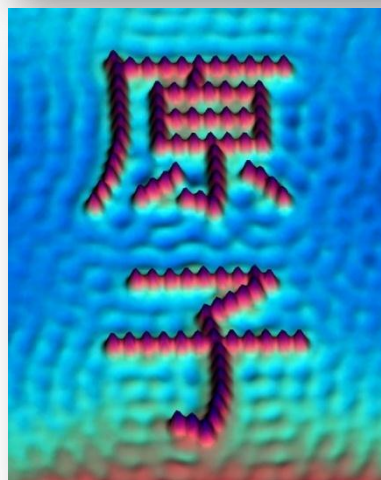
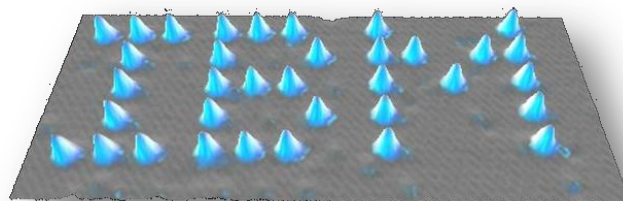
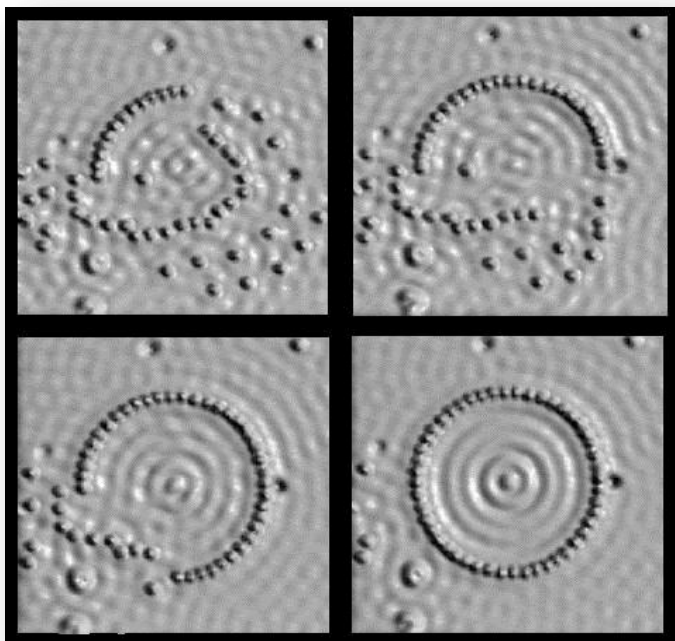


Surface de cuivre (001)

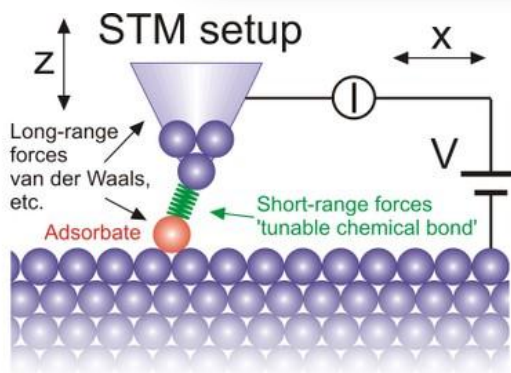


Fe adatoms sur cuivre (111)

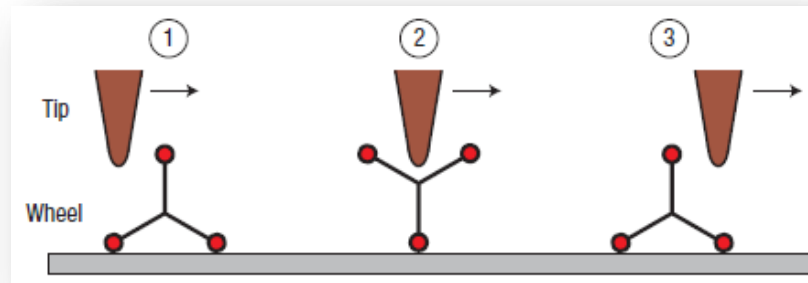
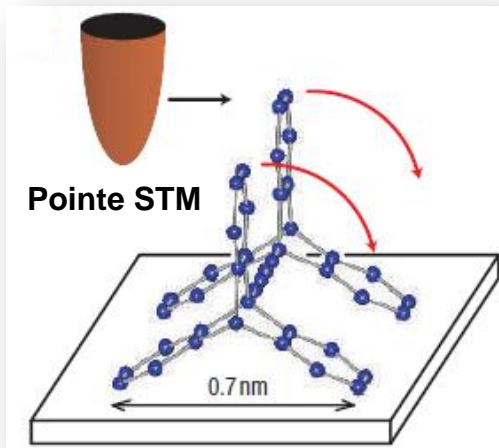
# Manipuler la matière atome par atome



Don Eigler,  
Kauli prize  
en 2010



# Nanomanipulation et la Nanofabrication



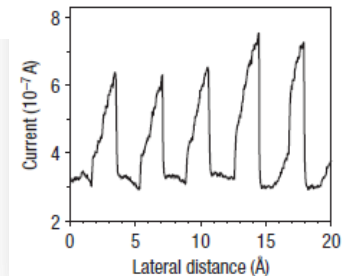
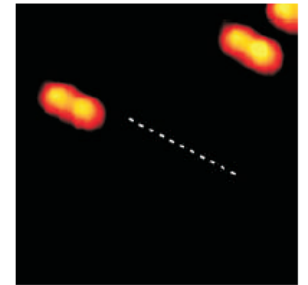
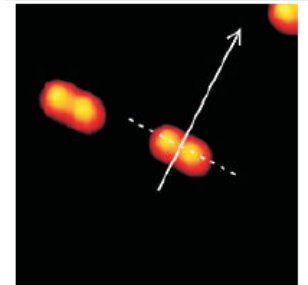
L. Grill et al., 2007

$$\mathbf{U} = \mathbf{R} \cdot \mathbf{I}$$

$$\sum \vec{F} = k \cdot \vec{x}$$

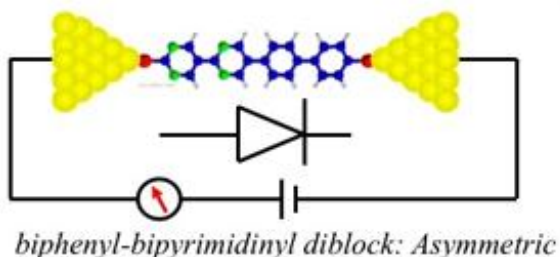
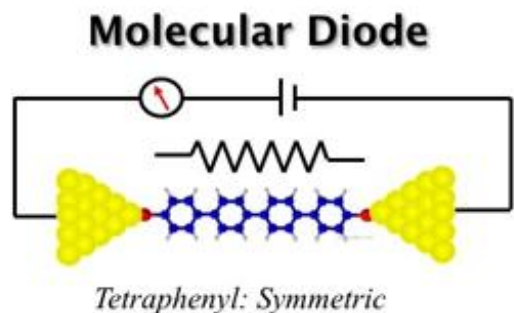
$$\Delta E$$

- Signature du courant tunnel du déplacement (« Hopping »)
- Mais pas de mesures directes de la force nécessaire au déplacement et à l'énergie dissipée ...

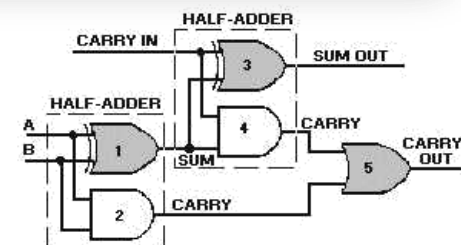
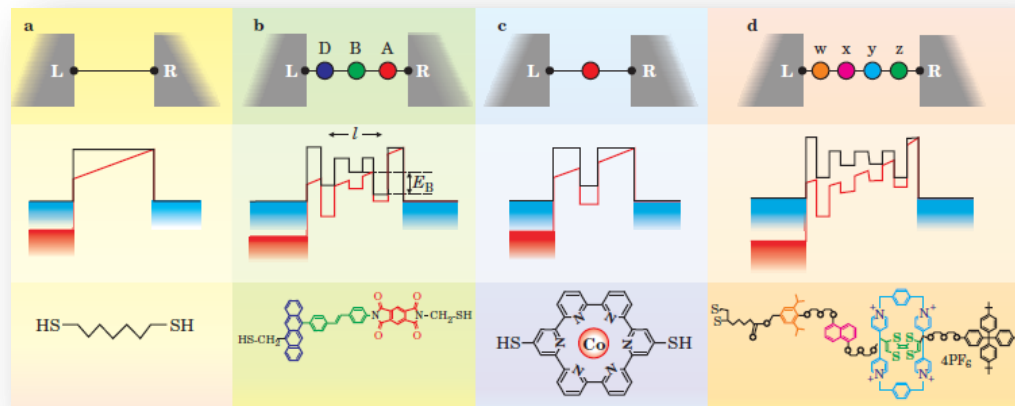


# L'électronique à une molécule !

Ari Aviram : "**Les molécules remplaceront le silicium !**"



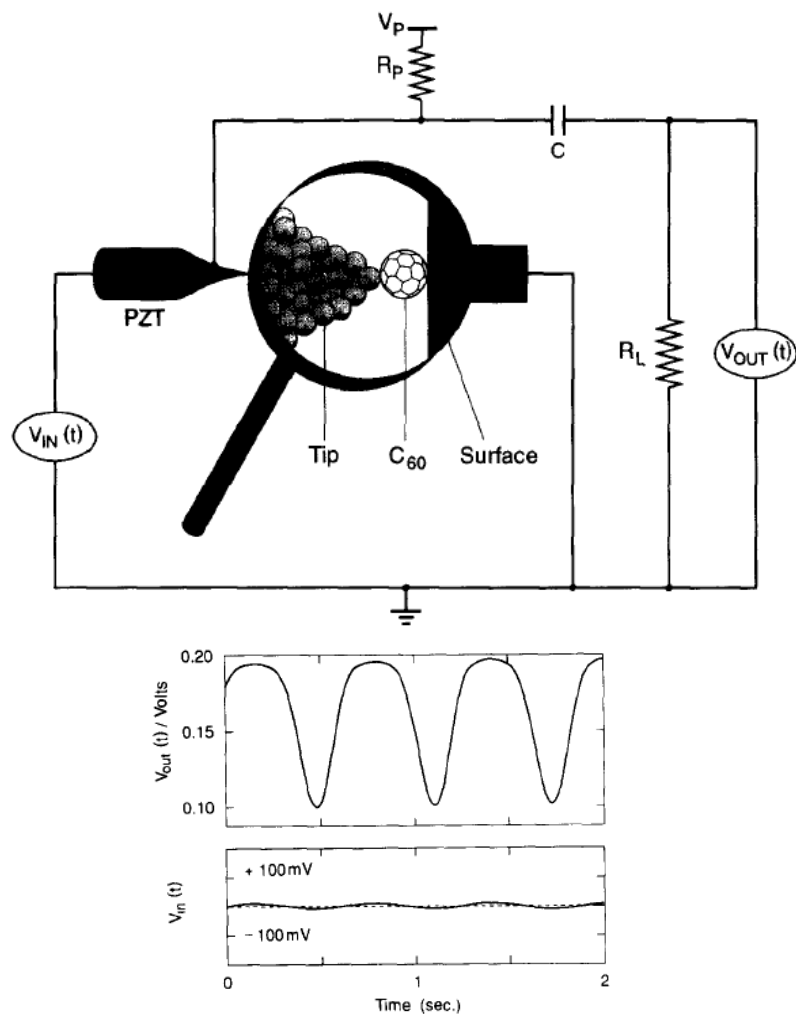
● Sulfur  
● Carbon  
● Nitrogen



A	B	CARRY IN	SUM OUT	CARRY OUT
0	0	0	0	0
0	1	0	1	0
1	0	0	1	0
1	1	0	0	1
0	0	1	1	0
0	1	1	0	1
1	0	1	0	1
1	1	1	1	1

Calcul moléculaire: la molécule intègre toute les « portes logiques » nécessaire pour être une entité calculatrice

# Amplificateur moléculaire



C. Joachim et al., Chem. Phys. Lett. 265, 353 (1997)

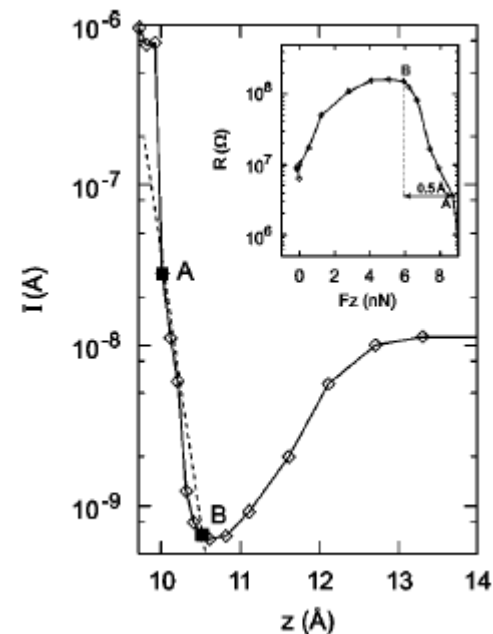
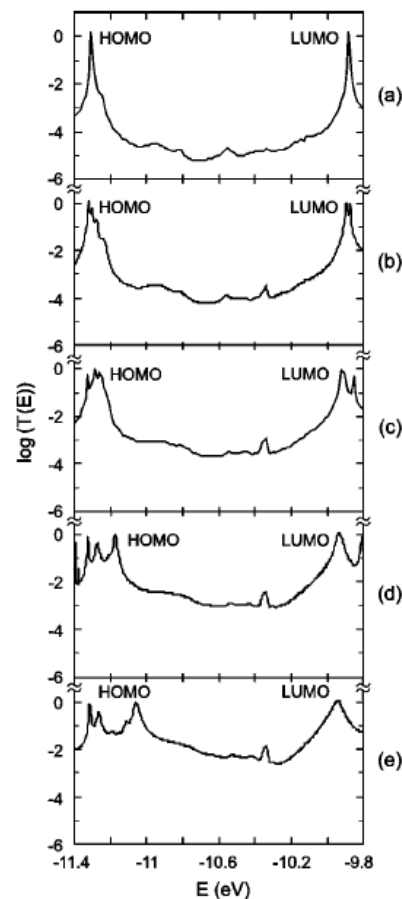
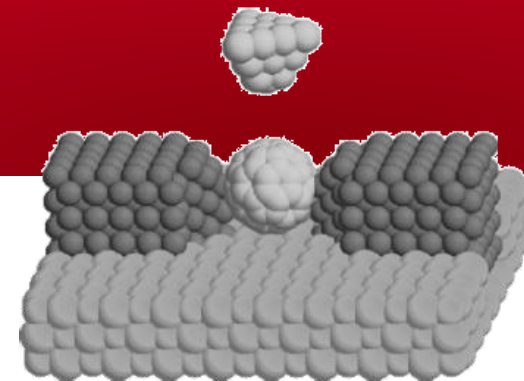
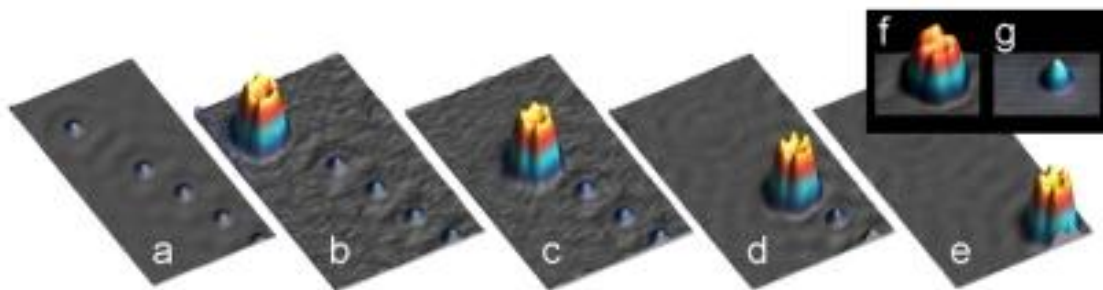
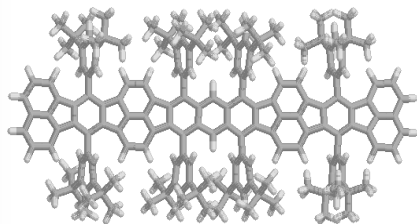
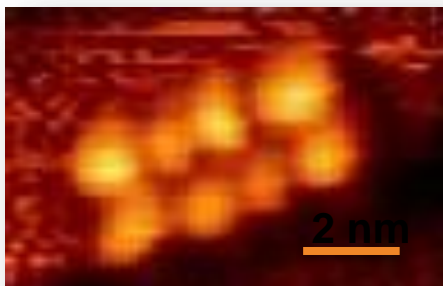
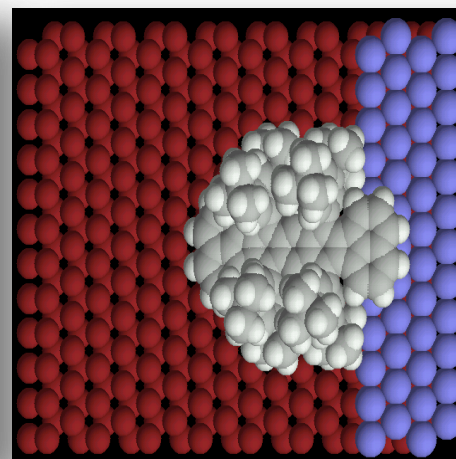
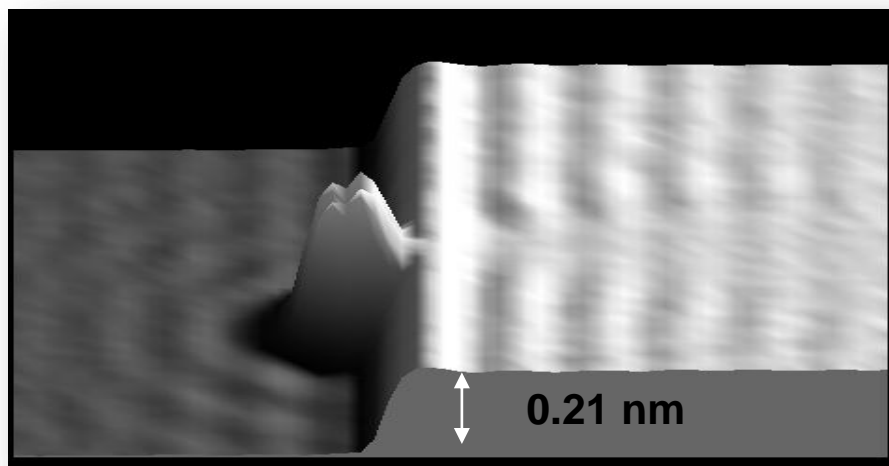
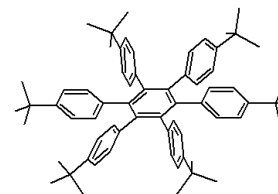


FIG. 11.  $T(E)$  spectra for a single  $C_{60}$  molecule compressed by the STM tip apex. The tip apex-surface distance is (a) 13.85, (b) 13.35, (c) 12.85, (d) 11.85, and (e) 11.35 Å. Only the HOMO-LUMO gap region is represented.

# Mécanique moléculaire

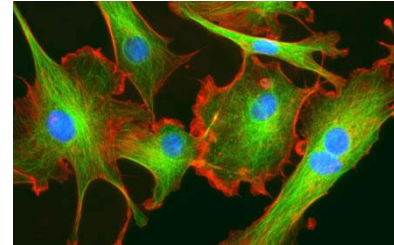
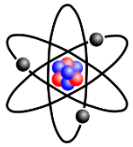


L'aspirateur à atomes !



les effets  
électroniques  
(ondes de  
surface) !

# Techniques de caractérisations complémentaires

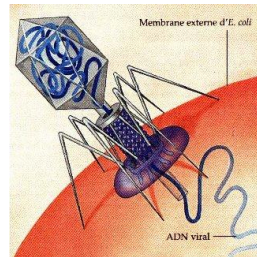


pm

nm

um

mm



Oeil

Microscope optique

Microscope électronique

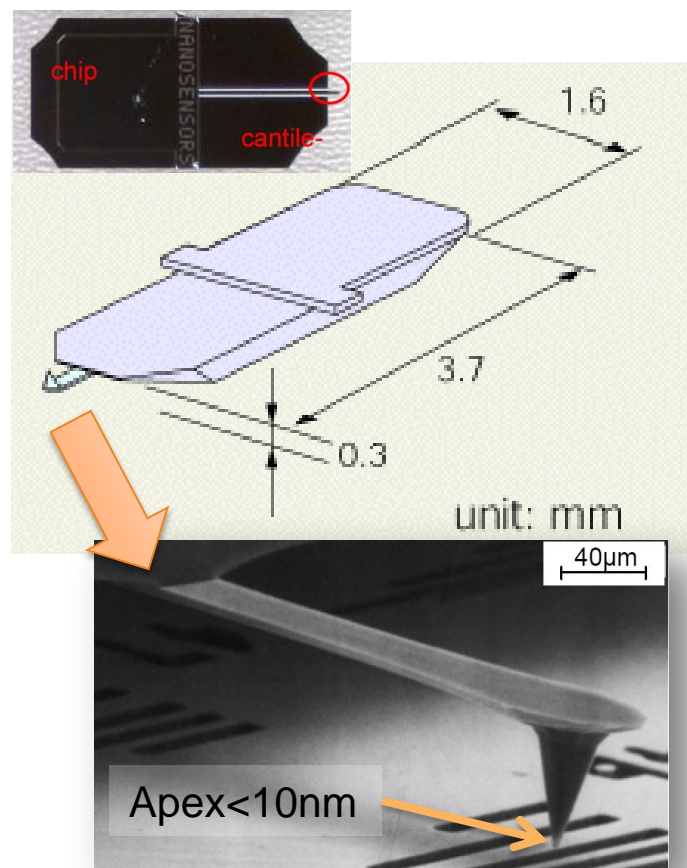
Diffraction Rayons X

STM

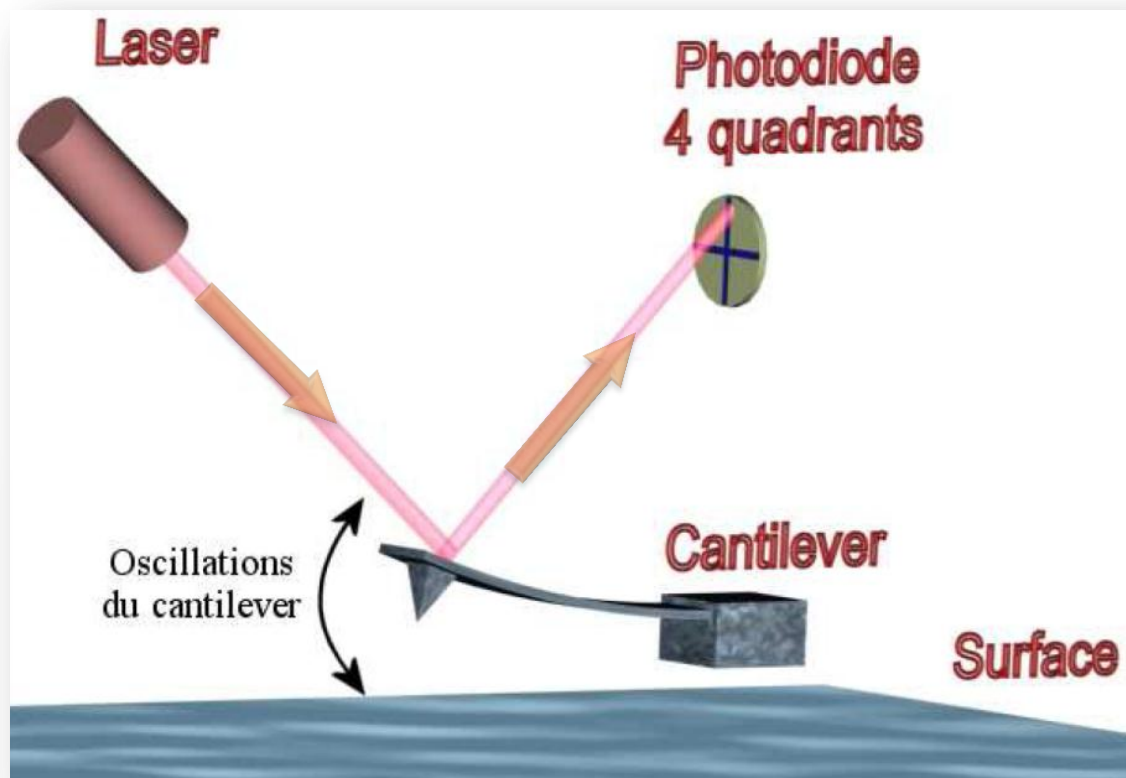
AFM: Microscopie à force atomique

## III.2 La microscopie à force atomique (AFM)

# La microscopie à force atomique (AFM)

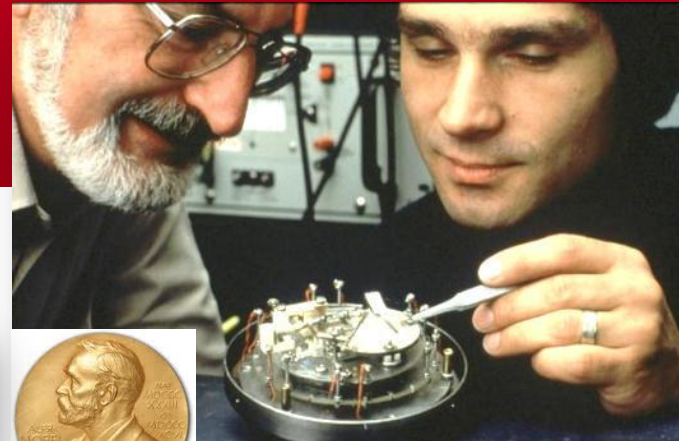


Cantilever



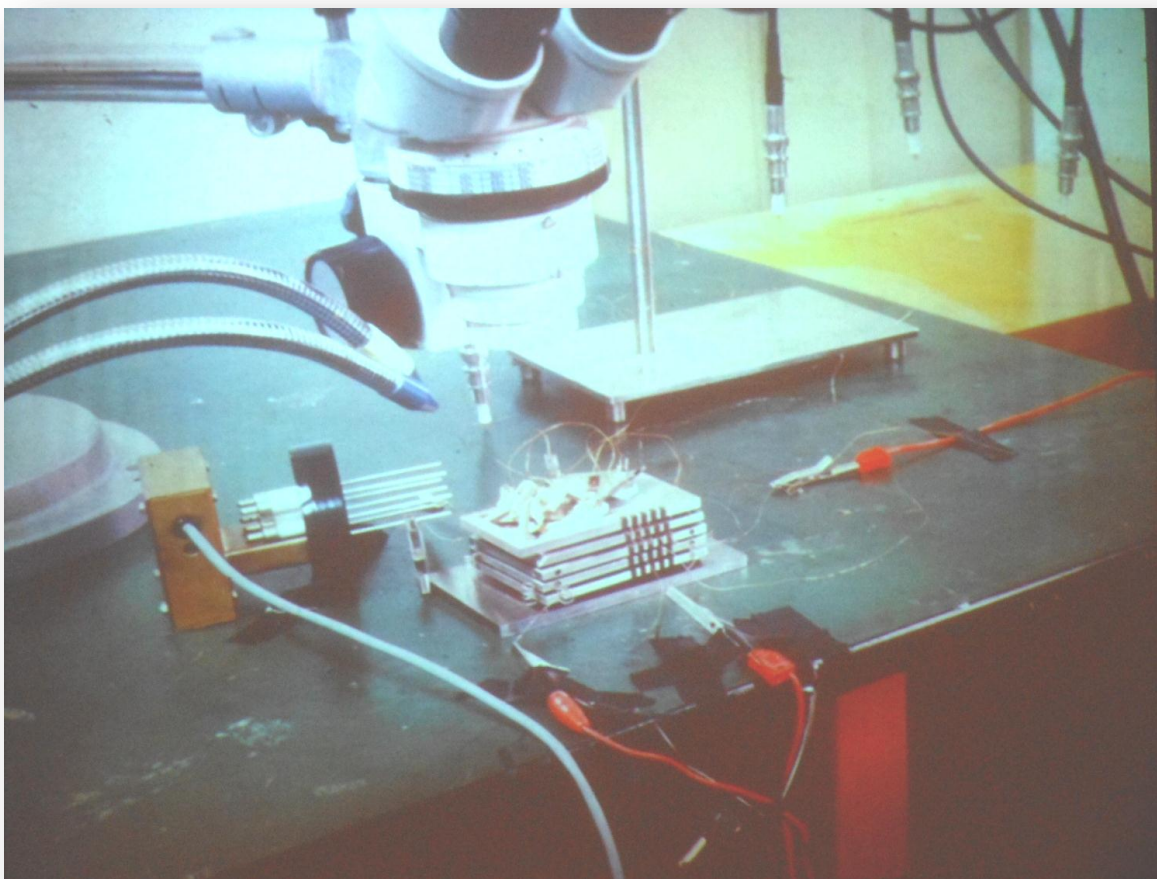
# Les pères de l'AFM

Heinrich  
Rohrer



STM Nobel Prize,  
1986

**Gerd Binnig**

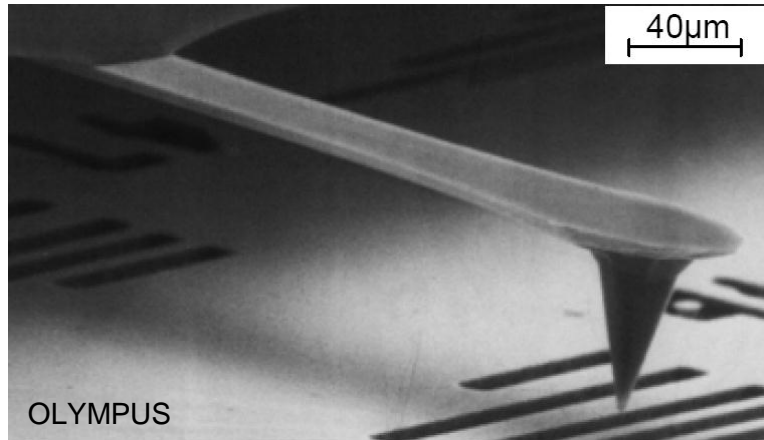


**Christoph Gerber**

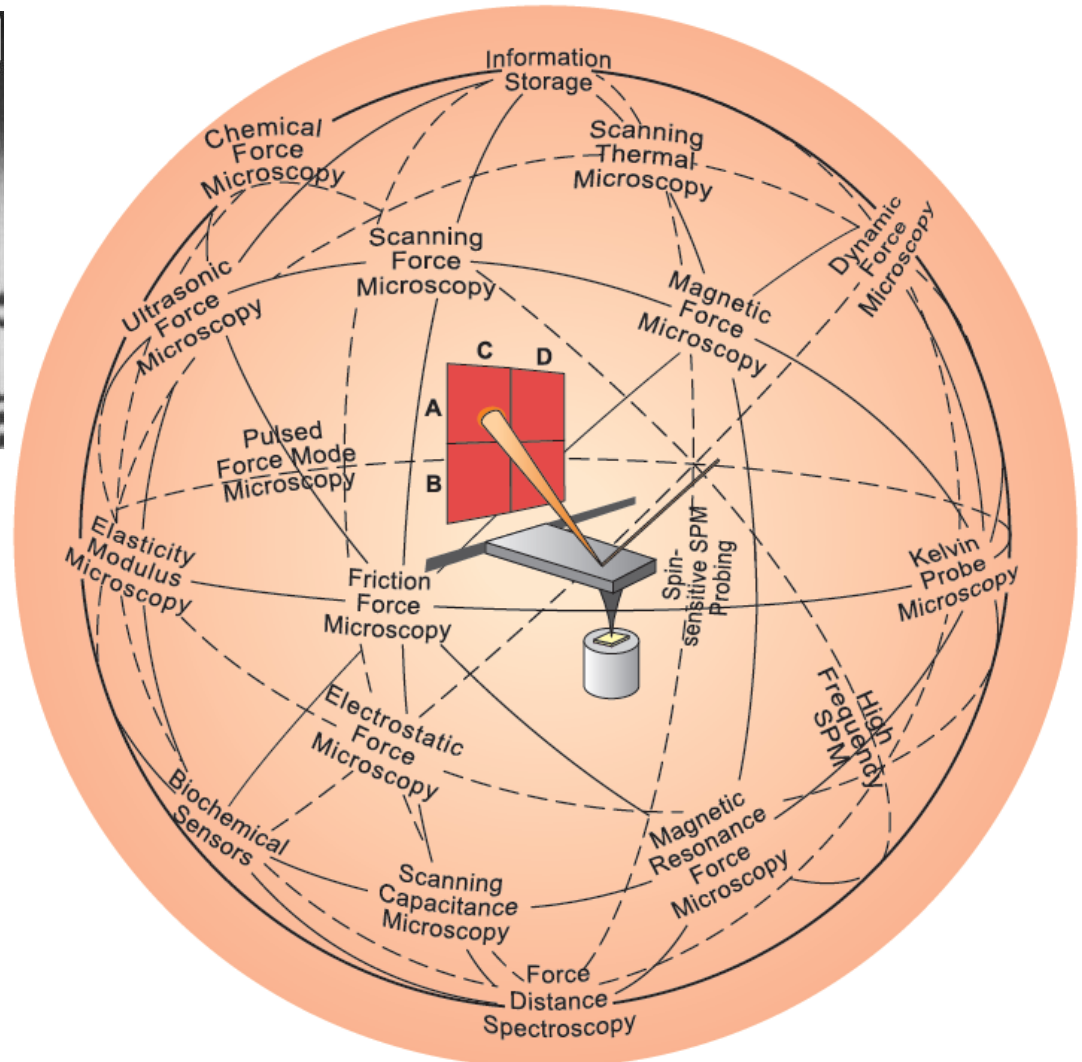


**Carl Quate**

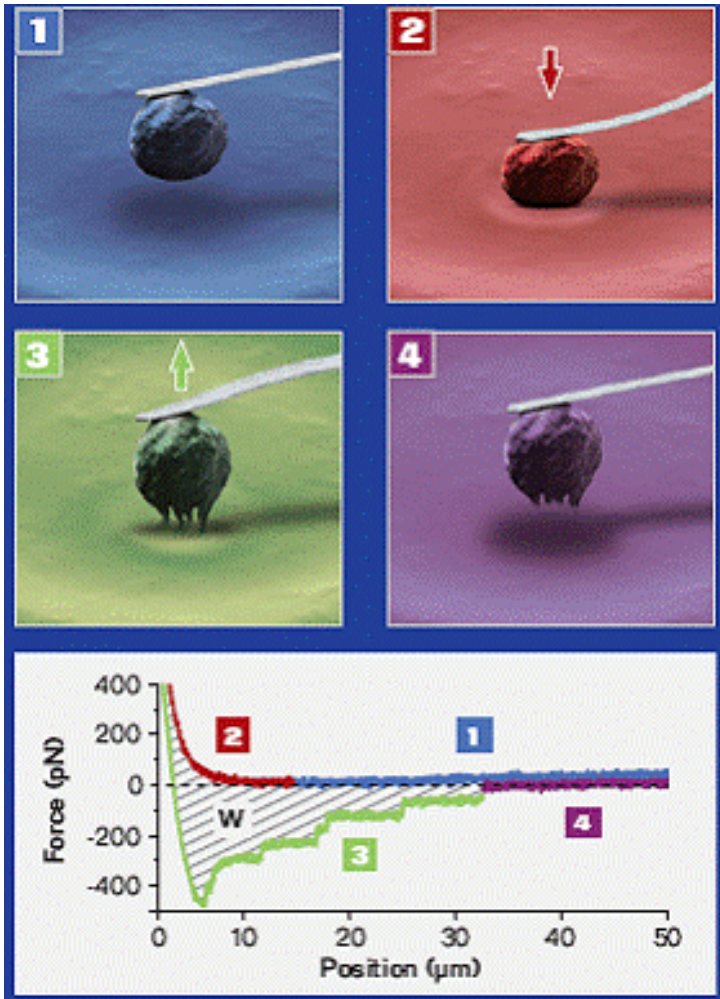
# Contexte: La Microscopie à Force Atomique (AFM)



G. Meyer, 1988,  
IBM Rüschlikon



Gerber et Lang, 2006

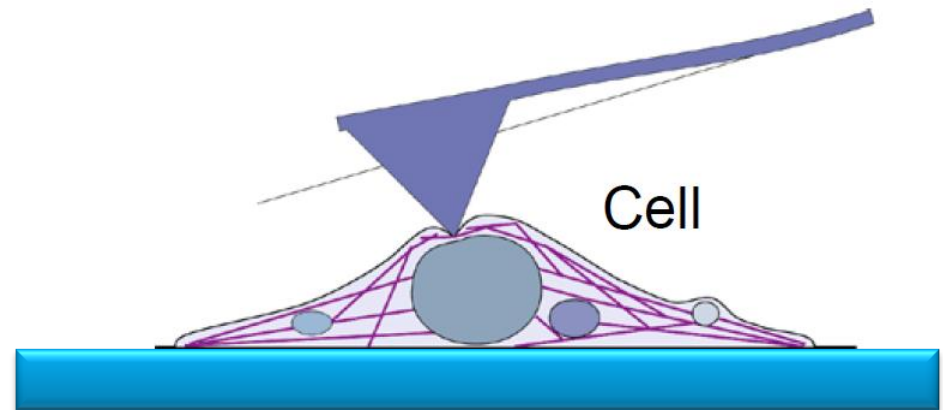


2

**Propriétés mécaniques:**

Module visco-élastique

3

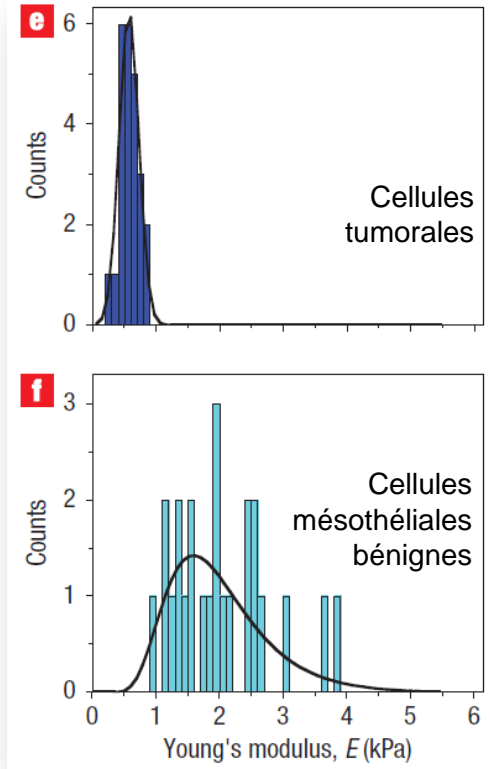
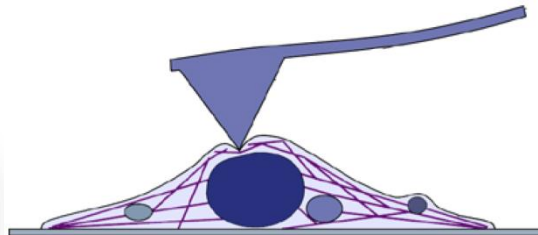
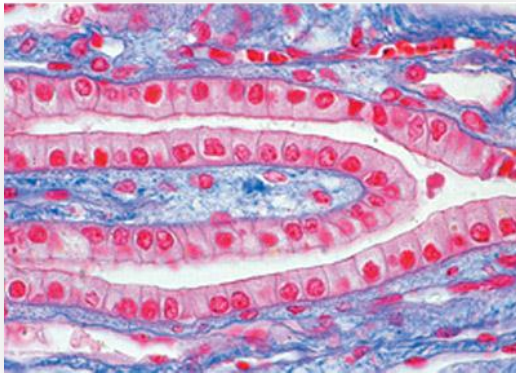
**Propriété d'adhésion :**Force maximum adhésion,  
Energies d'adhésion, ...

## LETTERS

## Nanomechanical analysis of cells from cancer patients

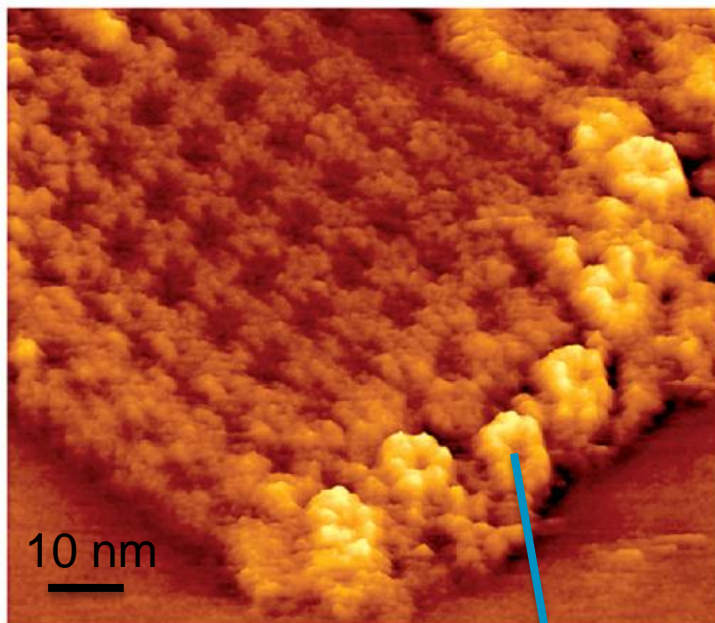
SARAH E. CROSS<sup>1,2†</sup>, YU-SHENG JIN<sup>3†</sup>, JIANYU RAO<sup>3\*†</sup> AND JAMES K. GIMZEWSKI<sup>1,2\*†</sup><sup>1</sup>Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, USA<sup>2</sup>California NanoSystems Institute, University of California, Los Angeles, California 90095, USA<sup>3</sup>Department of Pathology and Laboratory Medicine, University of California, Los Angeles, California 90095, USA<sup>†</sup>These authors contributed equally to this work.

\*e-mail: gim@chem.ucla.edu; JRao@mednet.ucla.edu

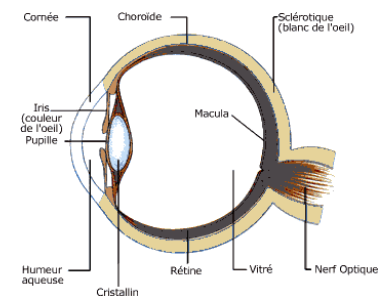
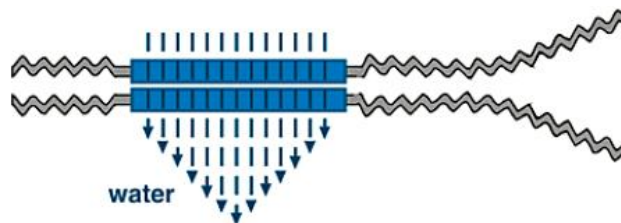
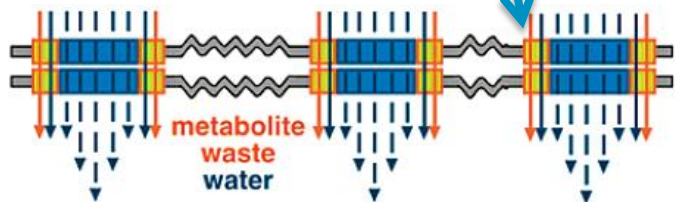
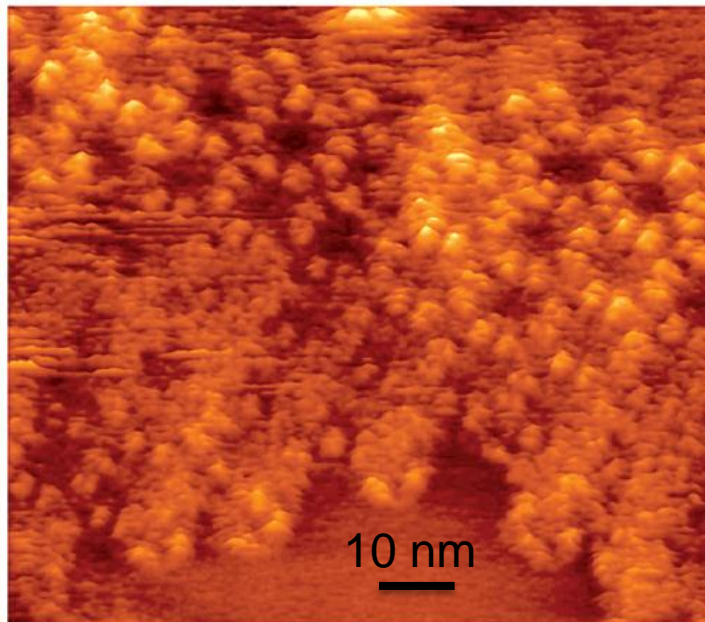
Cross et al., *Nature Nanotech.* 2007Diagnostic par histologie:

# Applications médicales

Junctional microdomain in healthy lens membranes



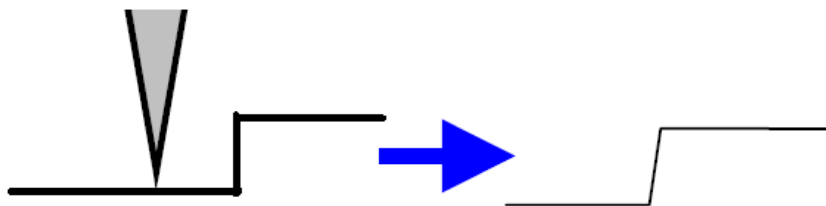
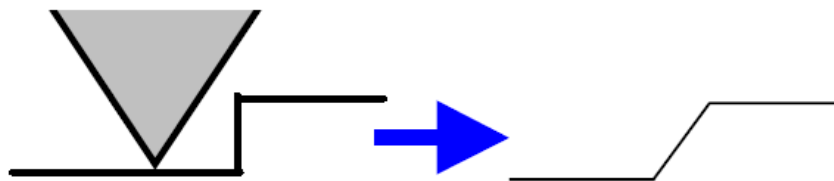
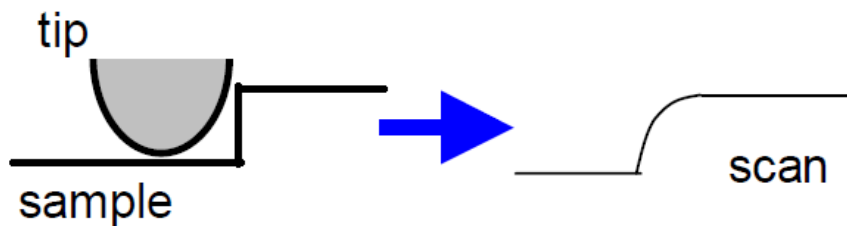
Junctional microdomain in cataract lens membranes



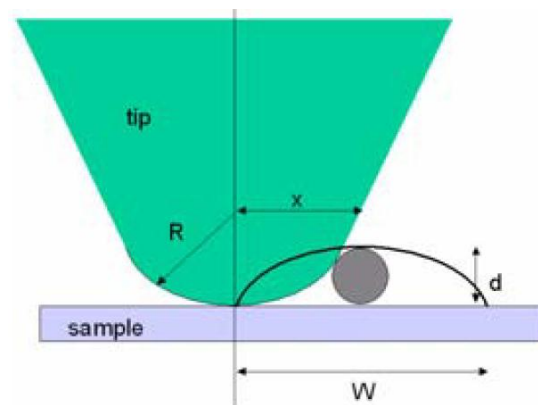
N. Buzhynskyy et al., J. Mol. Biol. 374, 162-169 (2007)



# Artefacts en AFM: l'apex de la pointe



Effect de convolution !



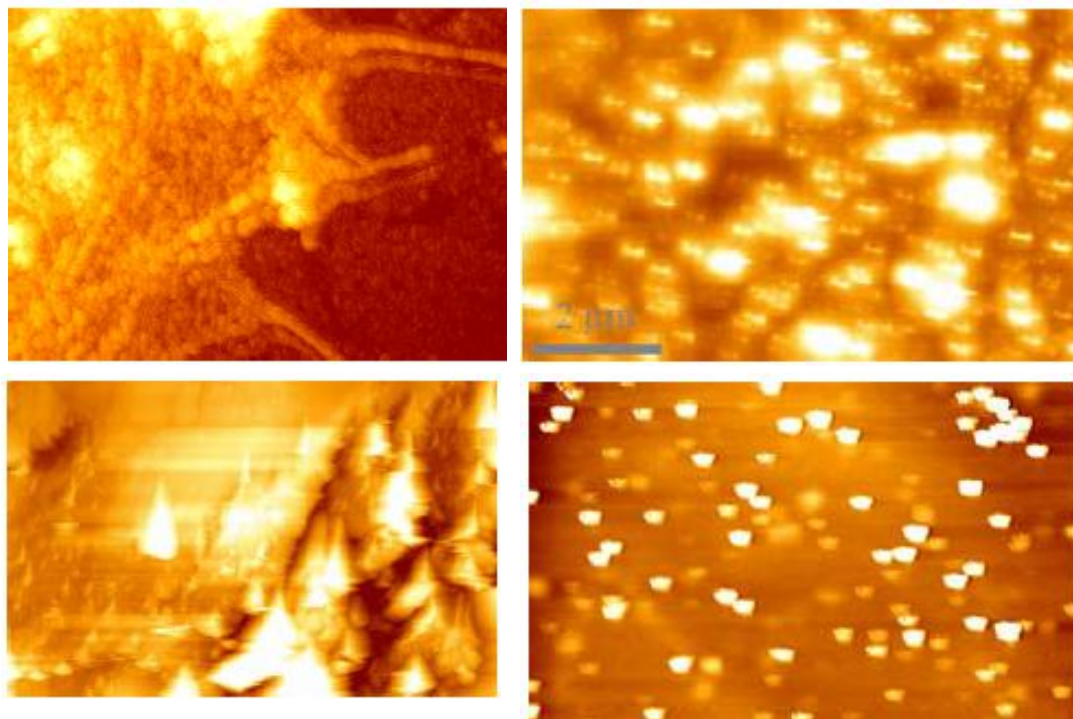
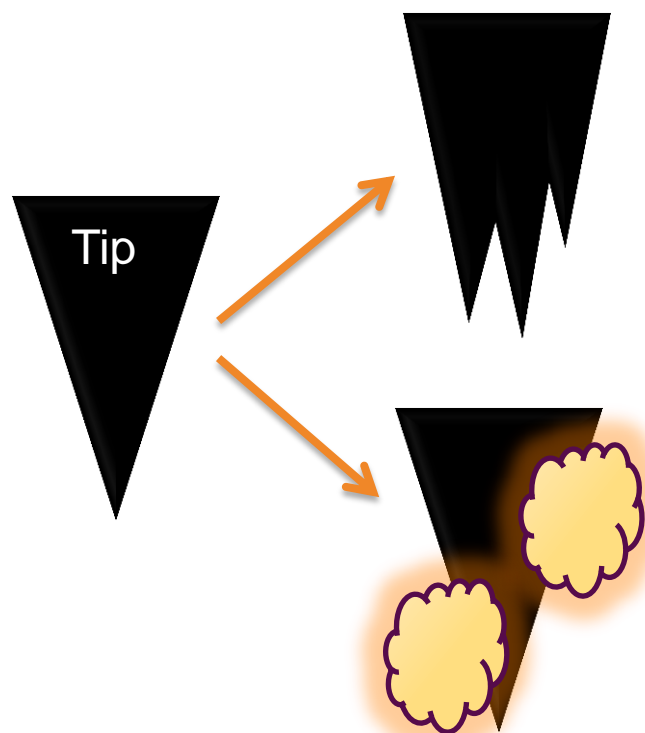
Pour  $R \gg d$

alors

$$W = \sqrt{8 \cdot d \cdot R}$$

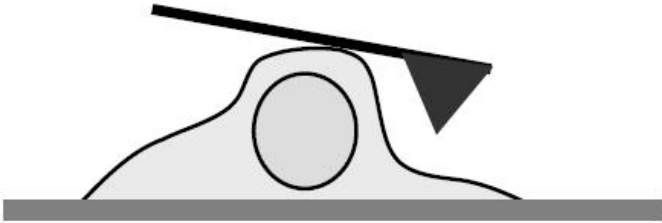
Pour  $R=10 \text{ nm}$  et  $d=5 \text{ nm}$ ,  
alors  $W=20 \text{ nm}$

# Artefacts en AFM: la pointe



Pointe multiples et contamination !

# Artefacts en AFM: la hauteur de la pointe



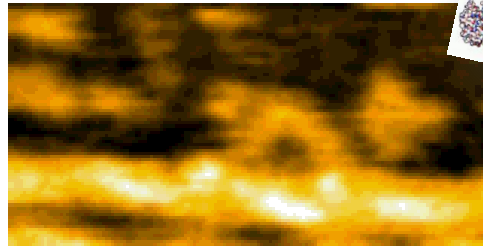
La hauteur de la  
pointe trop petite !

# Vers l'imagerie Ultra Rapide et la Haute Résolution

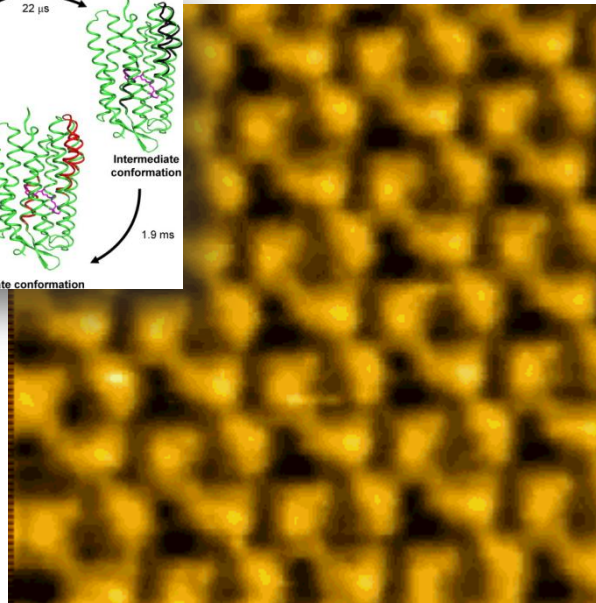
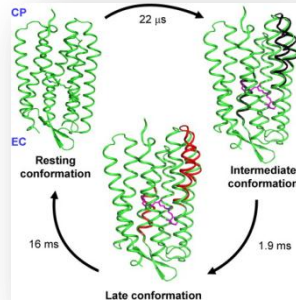
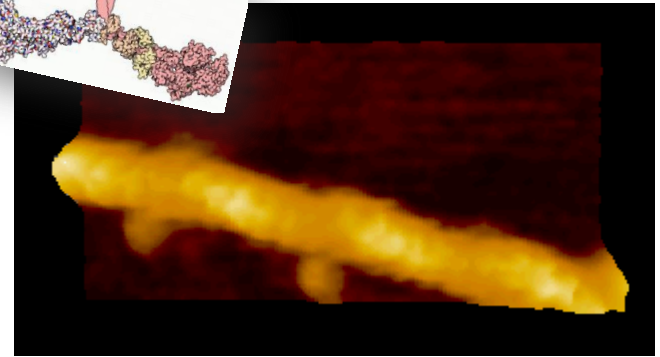
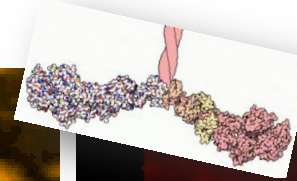


Toshio Ando

10 images AFM par seconde !

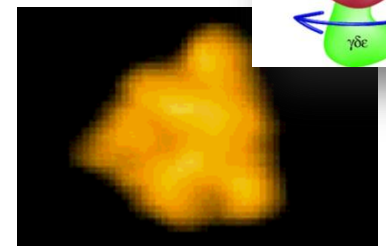
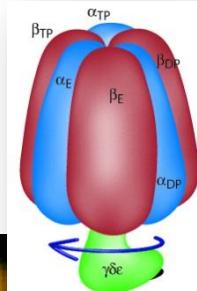


walking myosin V



bacteriorhodopsin (D96N) (réponse à la lumière)

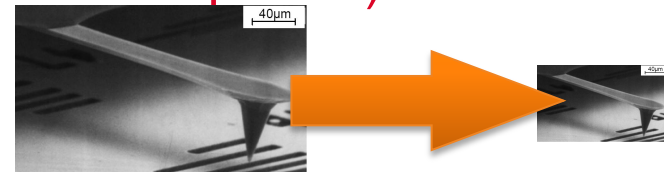
5 nm



rotorless F1-ATPase

## Des limitations à résoudre ...

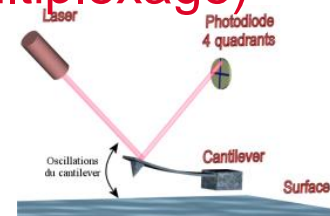
- Temps d'acquisition long sur de larges zones explorées ( $> \text{qq} \mu\text{m}$ ), ou spectroscopie cellulaire (plusieurs minutes à plusieurs heures !)
- Nécessité d'utiliser des leviers de plus en plus proche de la limite de diffraction optique de détection (faible  $k$ , et haute fréquence)



- Présence du laser : sources d'artefacts optiques, problèmes en milieux turbides, incompatible avec l'étude d'échantillons thermosensibles ou photosensibles

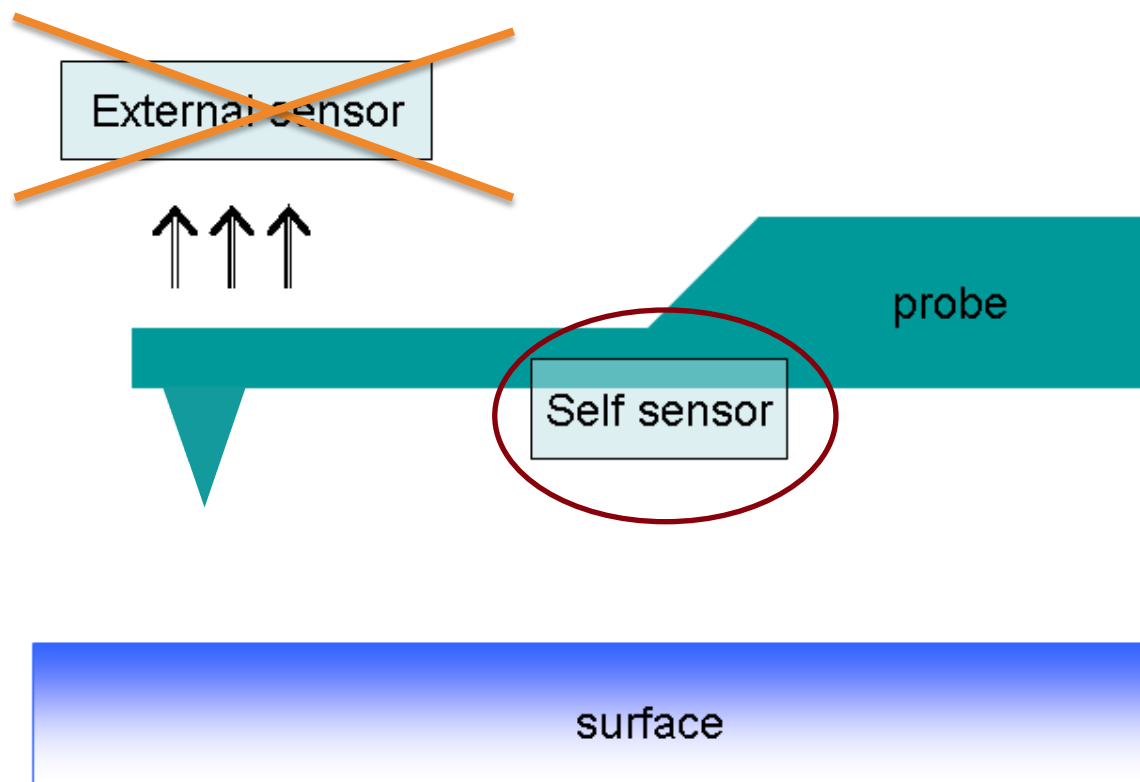


- Intégration du « levier optique » difficile pour une architecture multisondes fonctionnant en parallèle (nano-robotique, multiplexage)

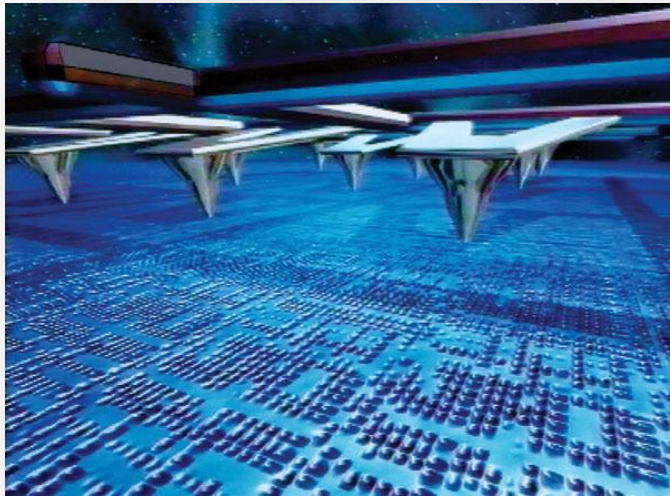


## IV. Vers de nouveaux dispositifs à sonde locale ...

# Capteurs intégrés et sondes AFM

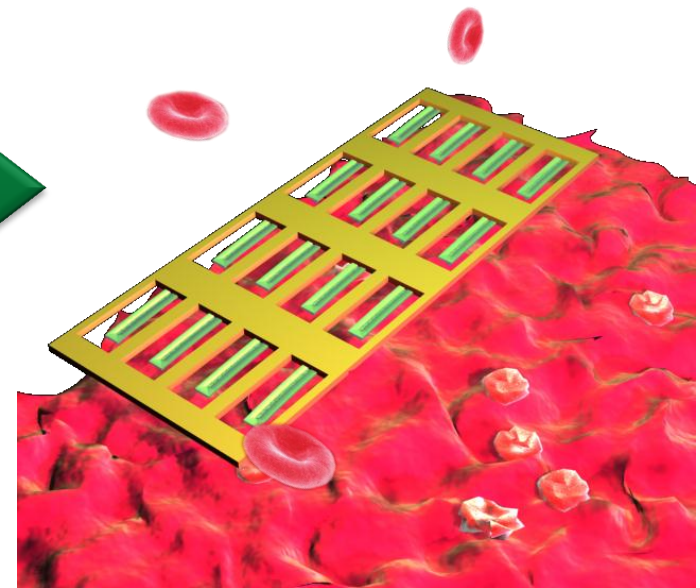
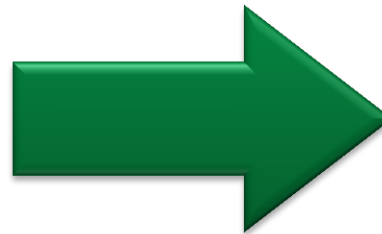


Le « Millipede » : du stockage de données ...

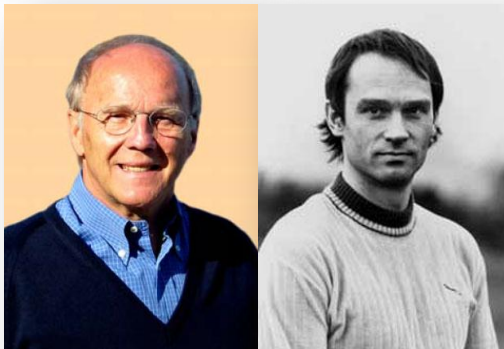


(1998)

... vers la biologie cellulaire !



(2005)

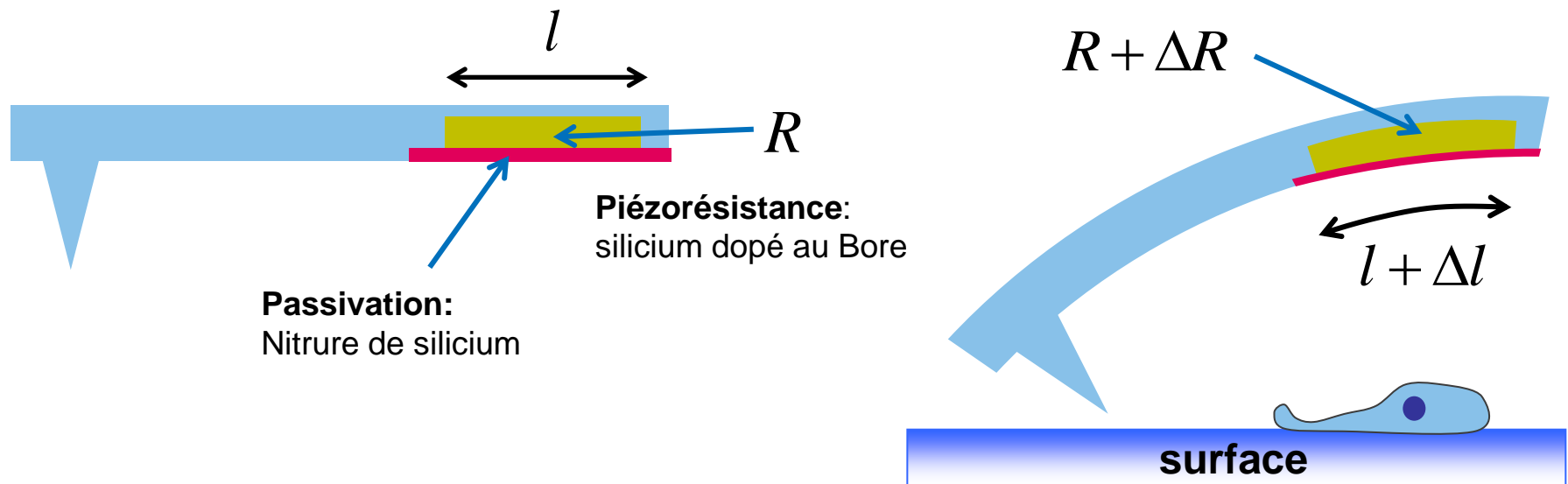


P. Vettiger,  
IBM Zürich

G. Binnig,  
IBM Zürich

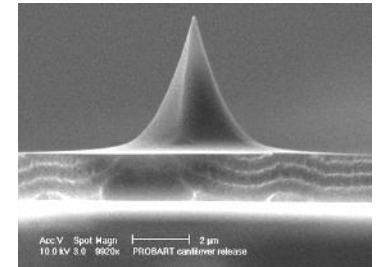
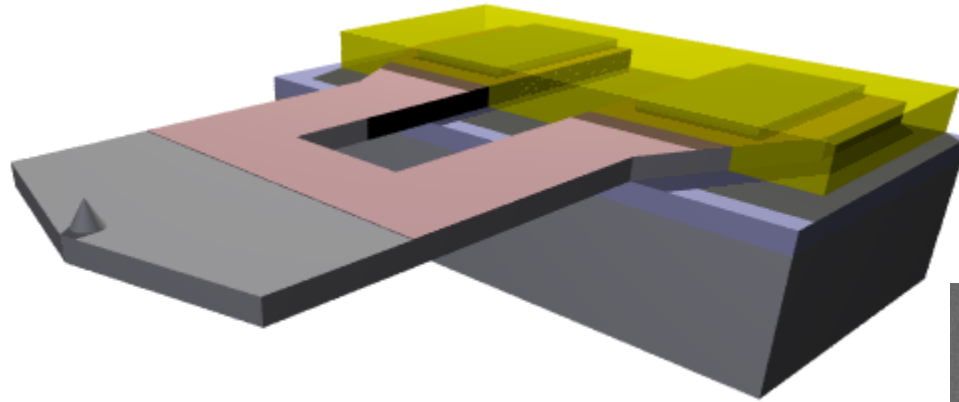
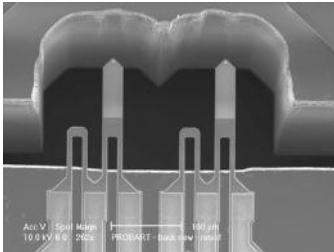
# Sondes piézorésistives: principe

- Proportionnalité entre l'élongation relative et la variation de résistance électrique

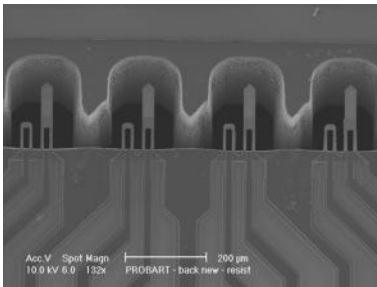


$$\frac{\Delta R}{R} = K \cdot \frac{\Delta l}{l}$$

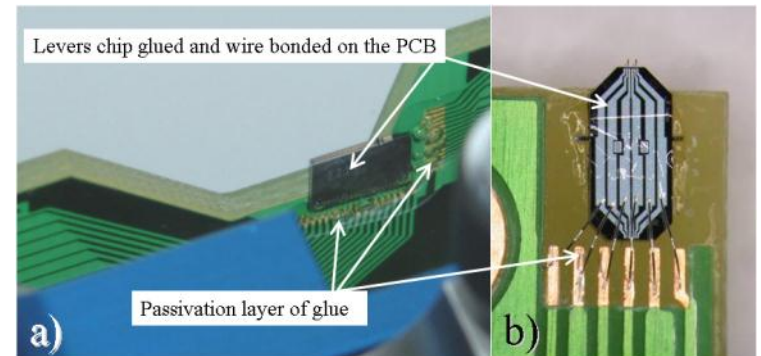
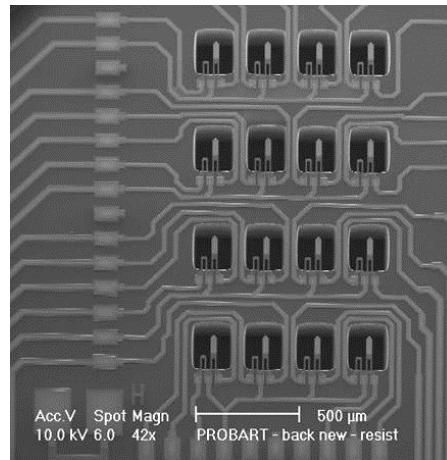
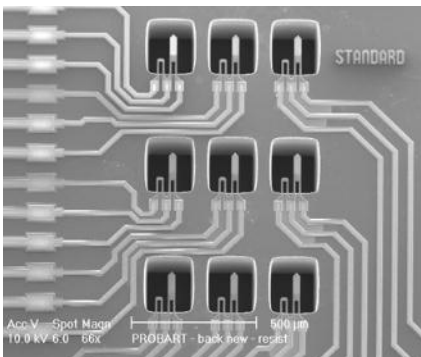
# Sondes piézorésistives: fabrication et matrices



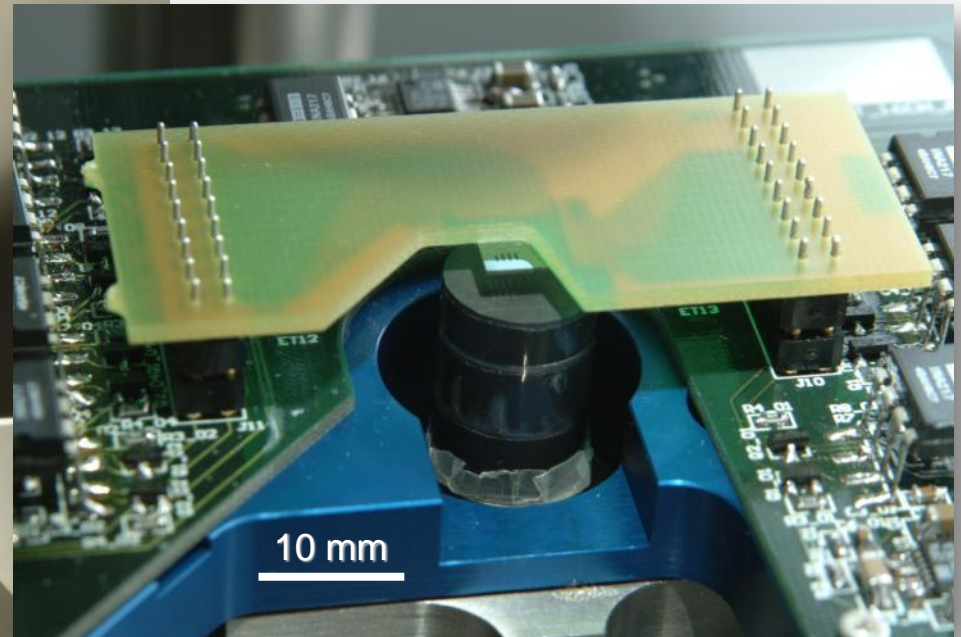
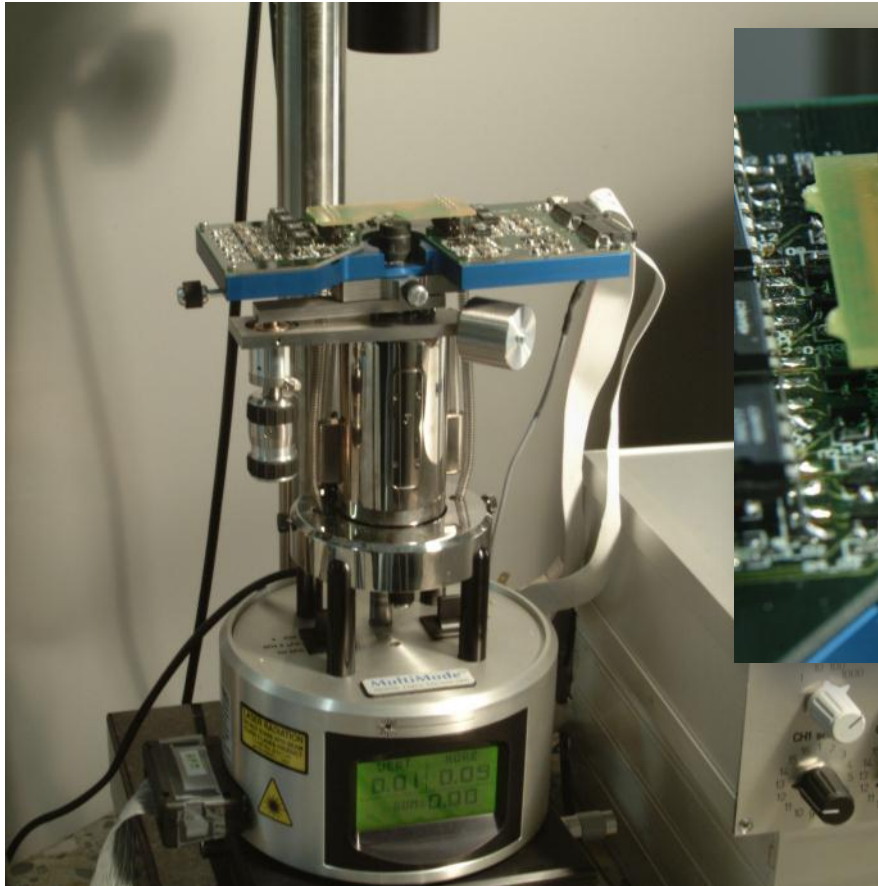
Apex= 20 nm  
k=0.06 N/m



csem imt

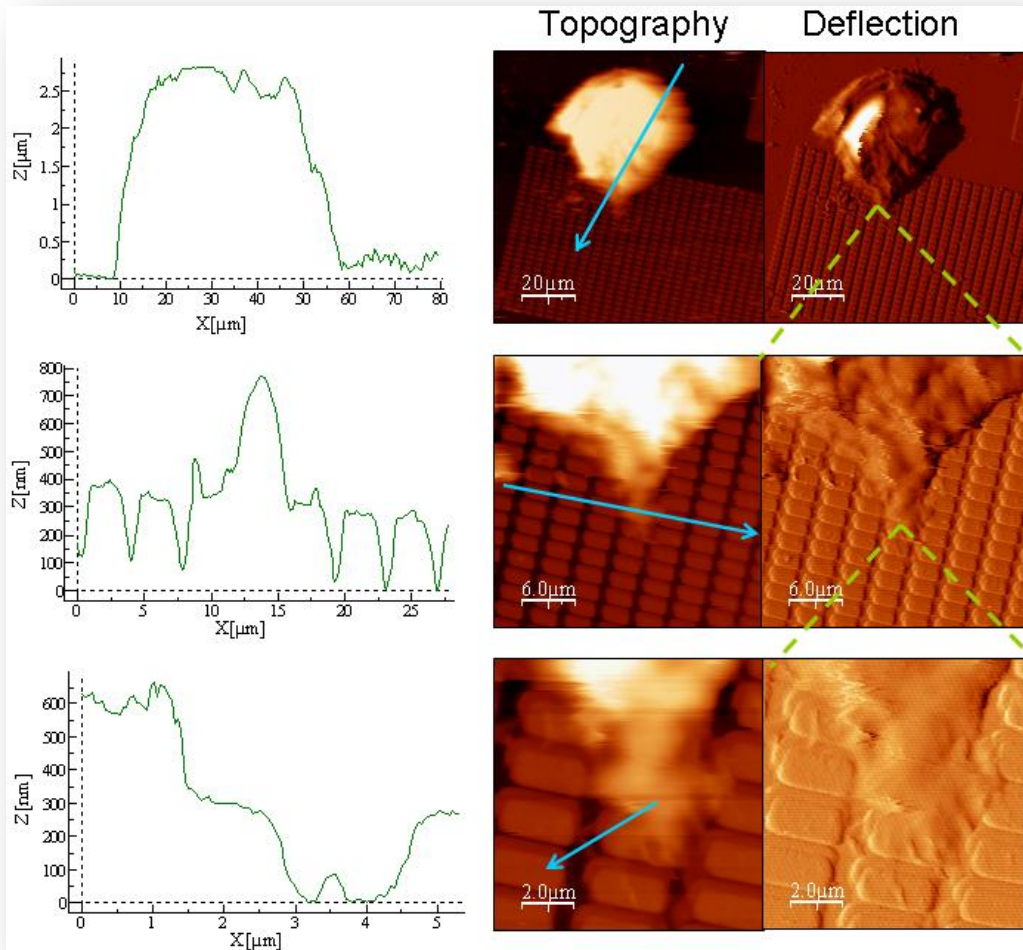


# Sondes piézorésistives: intégration

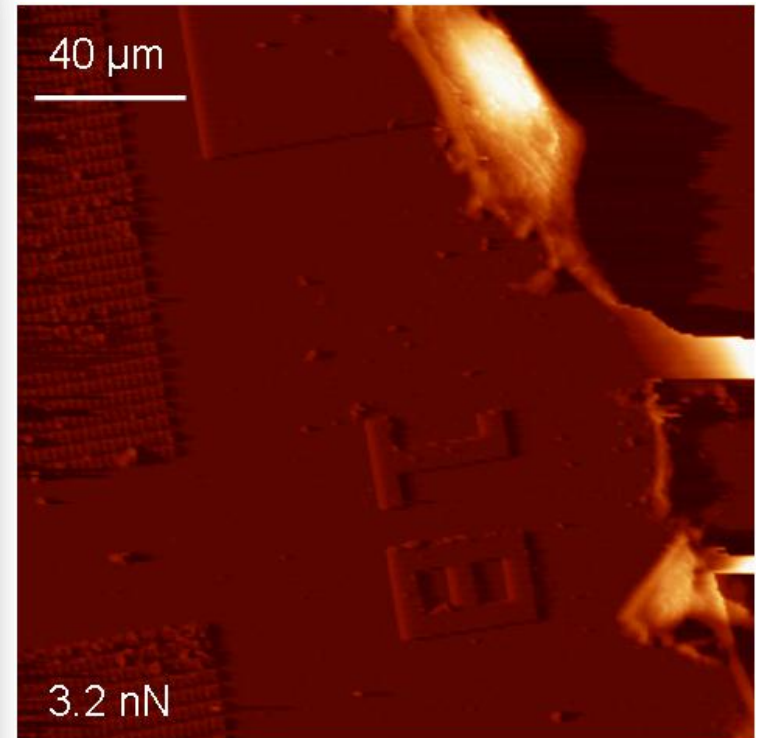


- Pilotage de matrice de 16 microleviers en parallèle

# Imagerie stable en milieu biologique

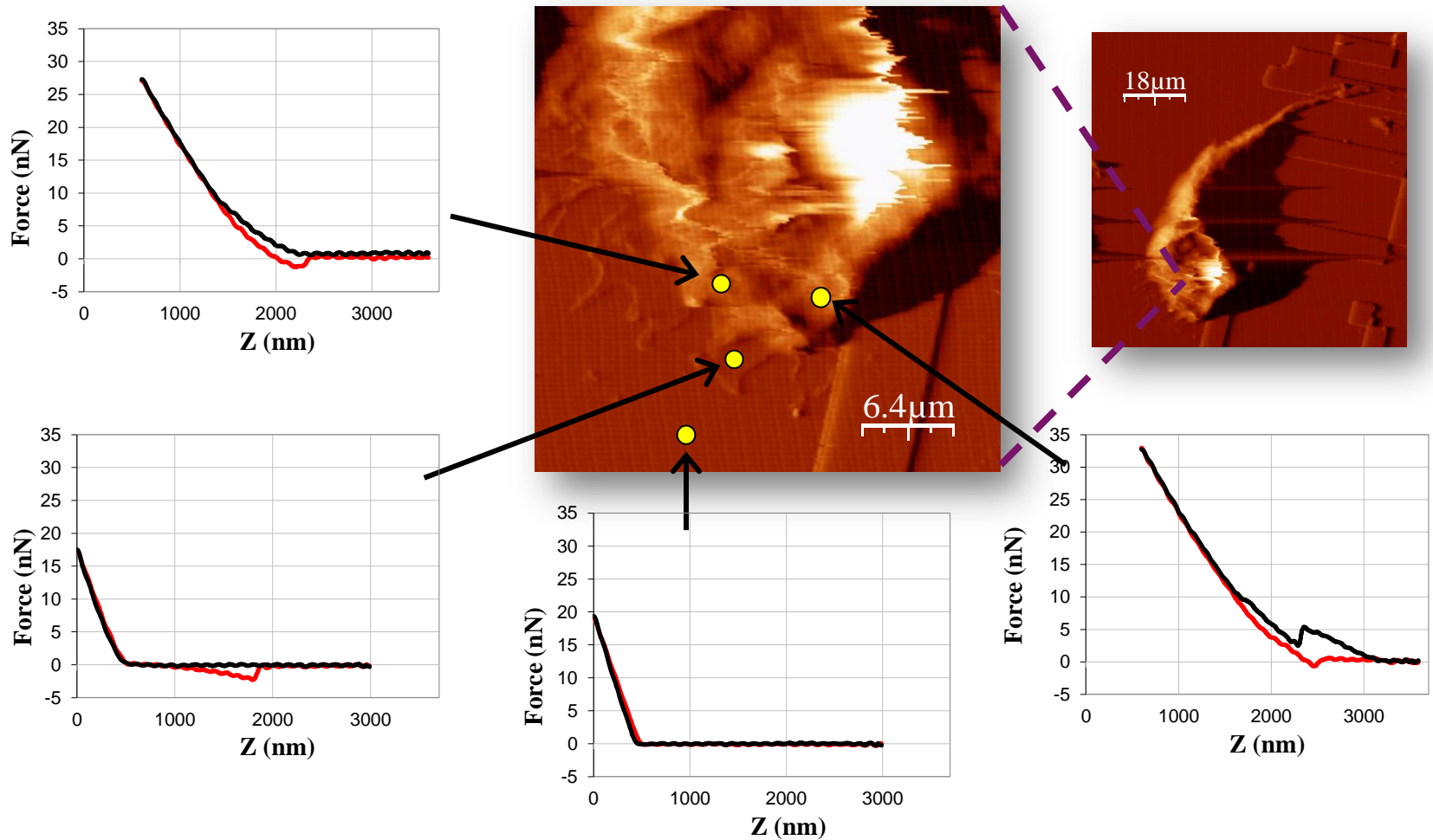


HTERT BJ1 human fibroblast cell



Incrément de la force appliquée:  
étude de l'adhérence

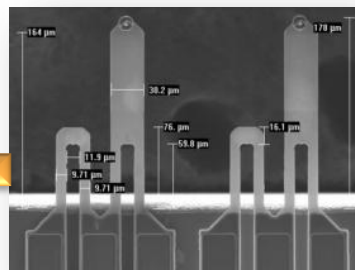
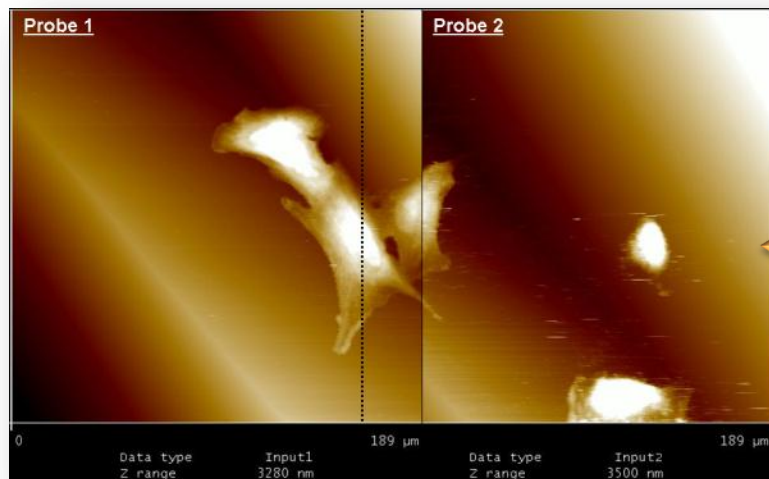
# Spectroscopie de force sur cellule



Analyse du module d'élasticité E donné par le modèle de Hertz-Sneddon :

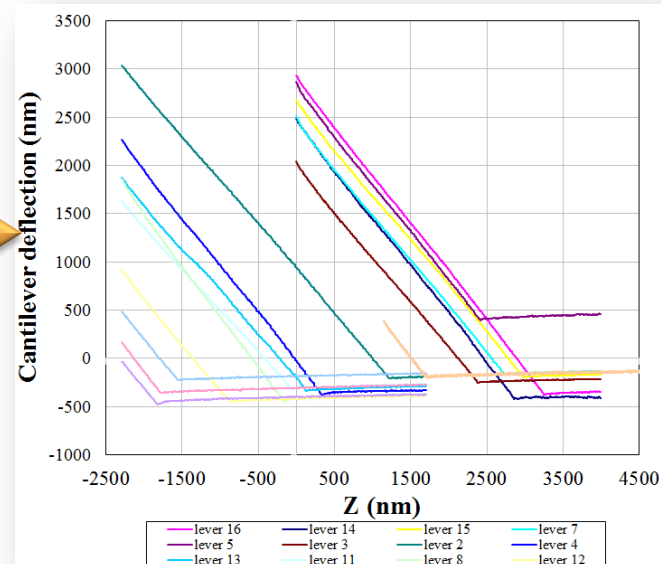
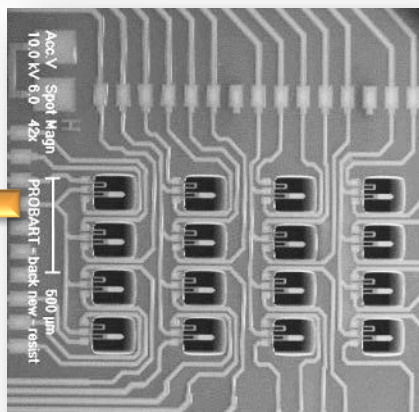
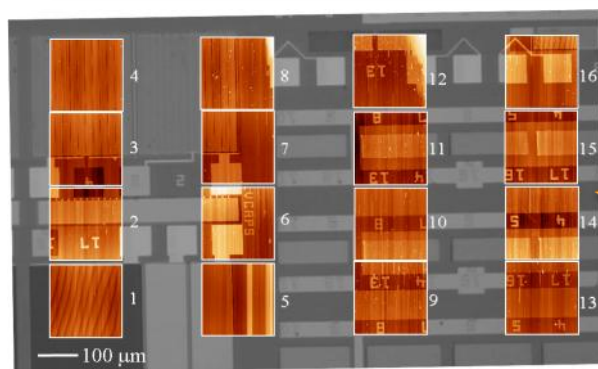
$$F = \frac{2}{\pi} \frac{E}{(1-\nu^2)} \cdot \tan \alpha \cdot \delta^2$$

# Acquisition d'images et de courbes en parallèle



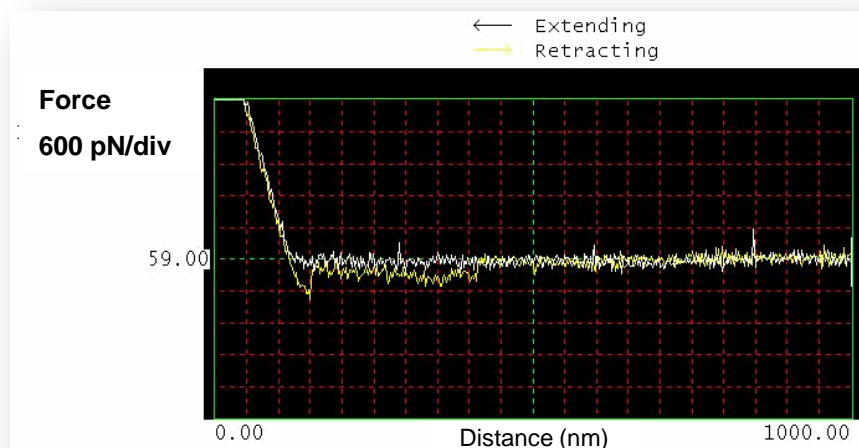
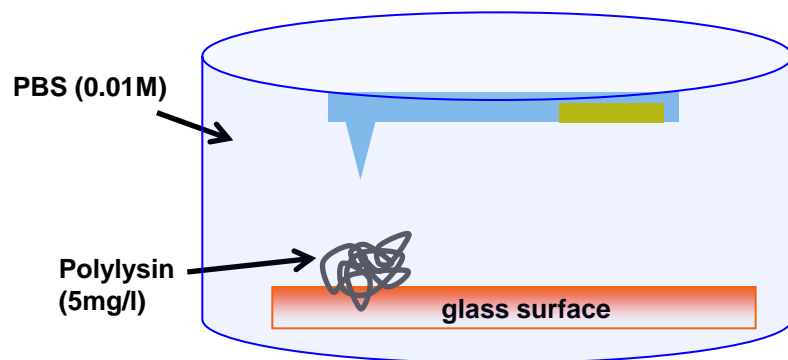
$k=160$  mN/m compatible avec l'imagerie AFM à hauteur constante

J. Polesel-Maris et al., "Piezoresistive cantilever array for life sciences applications", [Journal of Physics: Conference Series 61, 955-959 \(2007\)](#)



- Zone de l'ordre du  $\text{mm}^2$
- Résolution de l'ordre de 200 nm

# Spectroscopie de force et limite de résolution



## Détection minimale de force = 160 pN

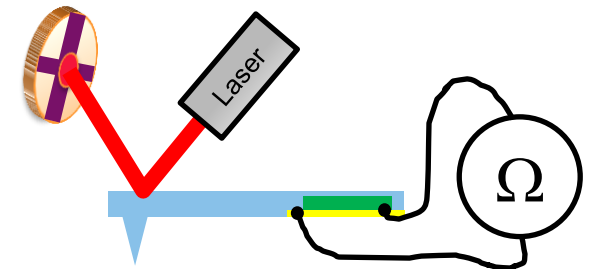
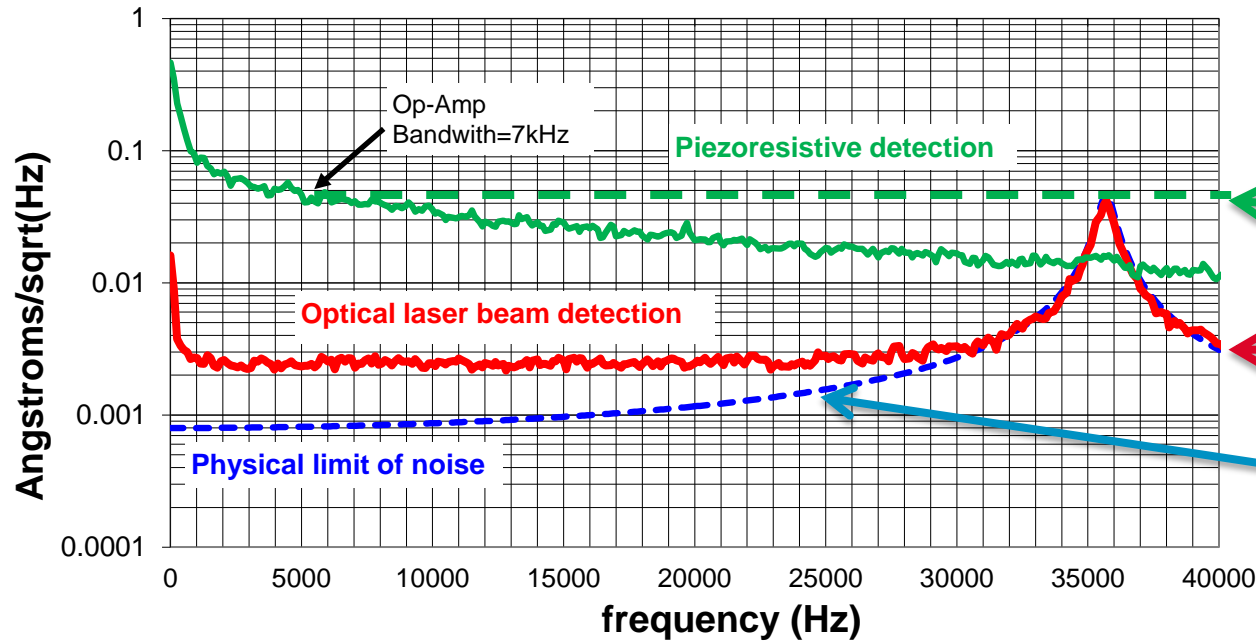
**Table 2. Examples of the interactive forces between specific molecules**

Ligand–receptor or cell-structure interactions	Force (pN)	
<b>Proteins</b>		
Meromyosin and actin	15–25	
Avidin and biotin	160–200	✓
Streptavidin and biotin	200–257	✓
Cell-adhesion proteoglycans	40–125	
Antibiotin antibody and biotin	60	
Anti-HAS and HAS; other antibodies and antigen	49–244	✓
P-selectin and glycoprotein ligand-1	165	✓
<b>Cells</b>		
RGD and cell receptor	35–120	
Trophoblast and uterine epithelium	1000–16 000	✓
Cell and uncoated surface	19 000–100 000	✓
Cell and coated surface	100 000–220 000	✓

Abbreviations used: HAS = human serum albumin; RGD = arginine–glycine–asparagine peptide

in “expert reviews in molecular medicine”,  
<http://www-ermm.cbcu.cam.ac.uk>

## Comparatif : détection optique et piézorésistive



$$\sqrt{S_{Johnson} + S_{1/f}}$$

$$\sqrt{S_{Johnson} + S_{A_{thermal}}}$$

$$\sqrt{S_{A_{thermal}}}$$

$$S_{Johnson} = 4 \cdot k_B \cdot T \cdot R$$

$$S_{1/f}(f) = i_{load}^2 \cdot \frac{\alpha \cdot R^2}{f \cdot N}$$

- Prédominance du bruit Johnson-Nyquist et du bruit en  $1/f$  pour la détection piézorésistive

- Origine du bruit en  $1/f$  ?

$$S_{A_{thermal}}(f) = \frac{4k_B T}{Q_c \omega_0 k_c} \frac{1}{\left(1 - \frac{(2\pi f)^2}{\omega_0^2}\right)^2 + \left(\frac{2\pi f}{Q_c \cdot \omega_0}\right)^2}$$

# Détection optique par interférométrie

# Détection interférométrique: principe

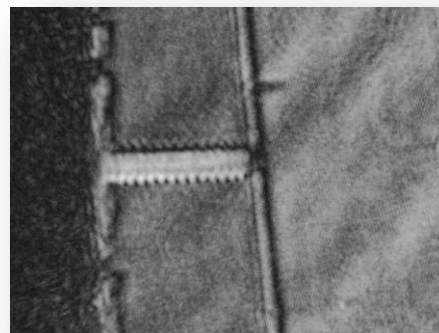
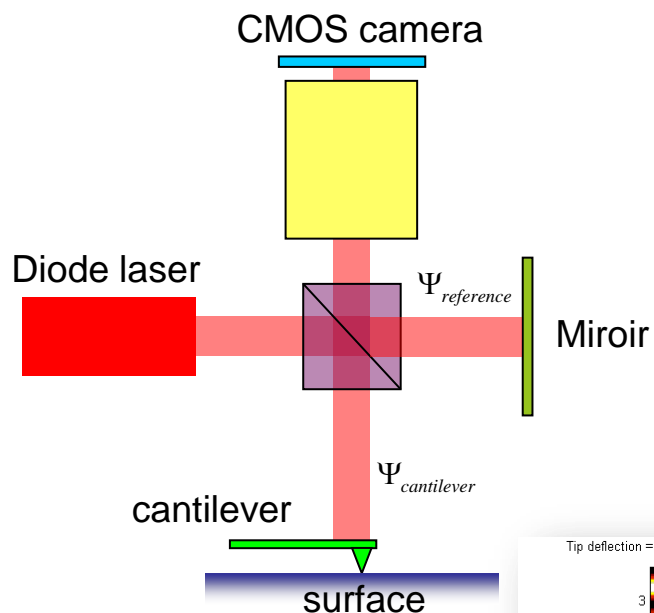
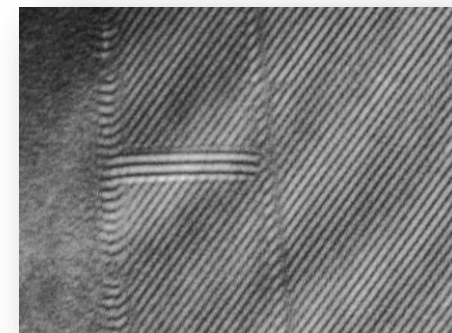
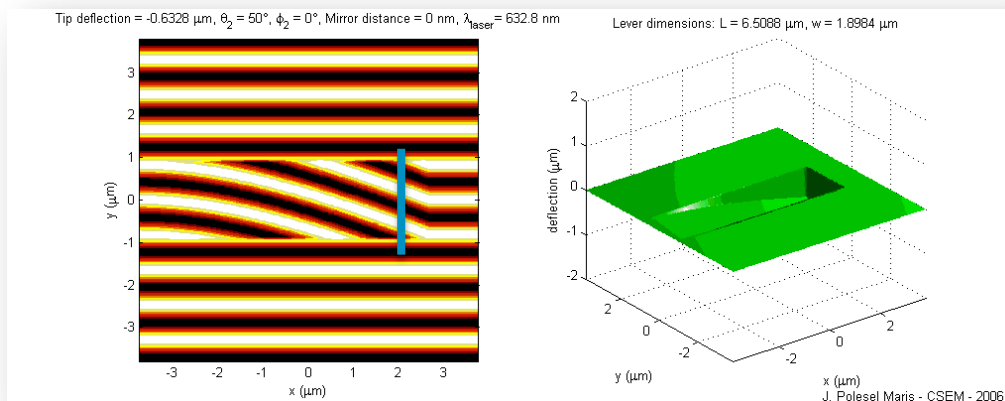


Image en intensité =  $(\Psi_{cantilever})^2$



Interférogramme =  $(\Psi_{cantilever} + \Psi_{reference})^2$

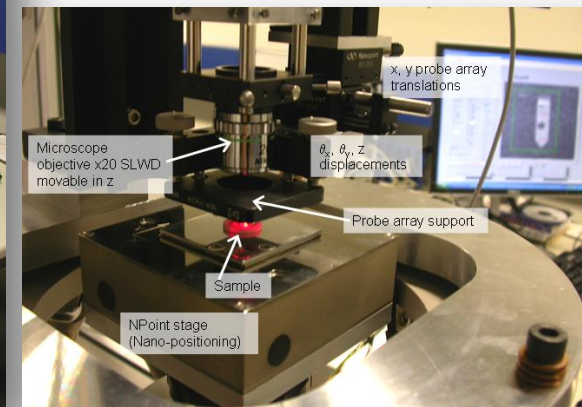
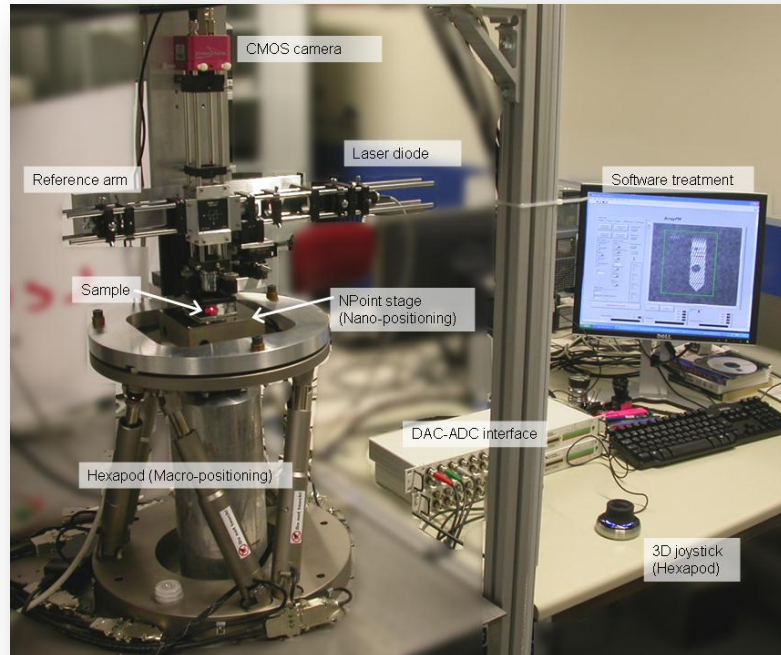
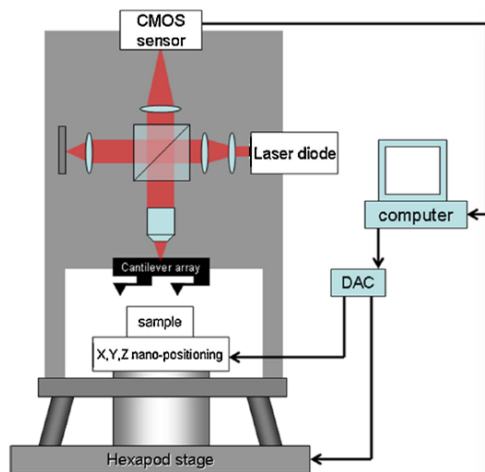


$$I(\Delta z) = I_1 + I_2 + 2\sqrt{I_1 \cdot I_2} \cos(\Delta \varphi)$$

avec:

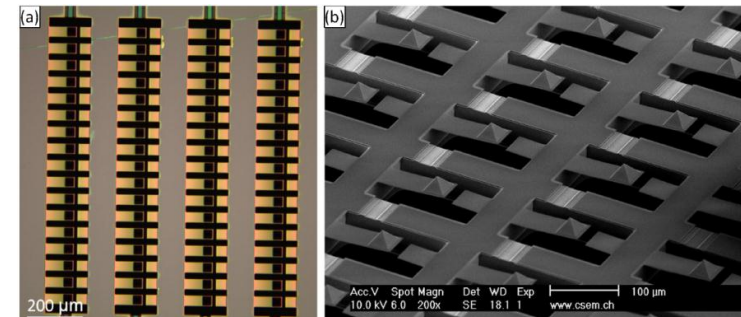
$$\Delta z = \frac{\lambda}{4\pi n} \Delta \varphi$$

# Détection interférométrique: intégration

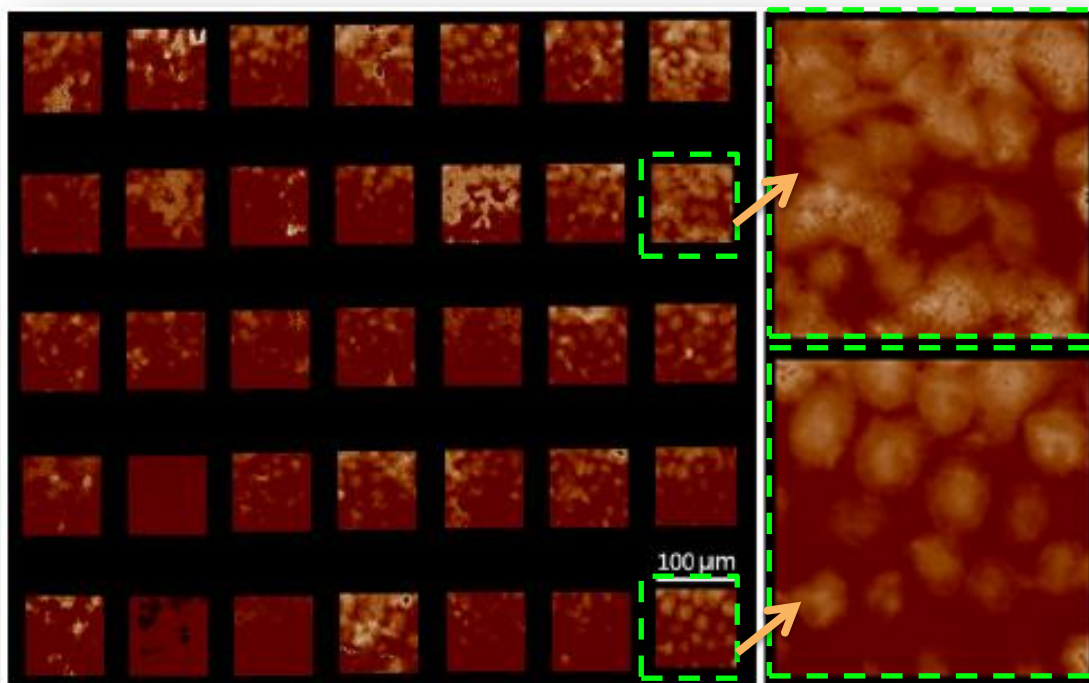


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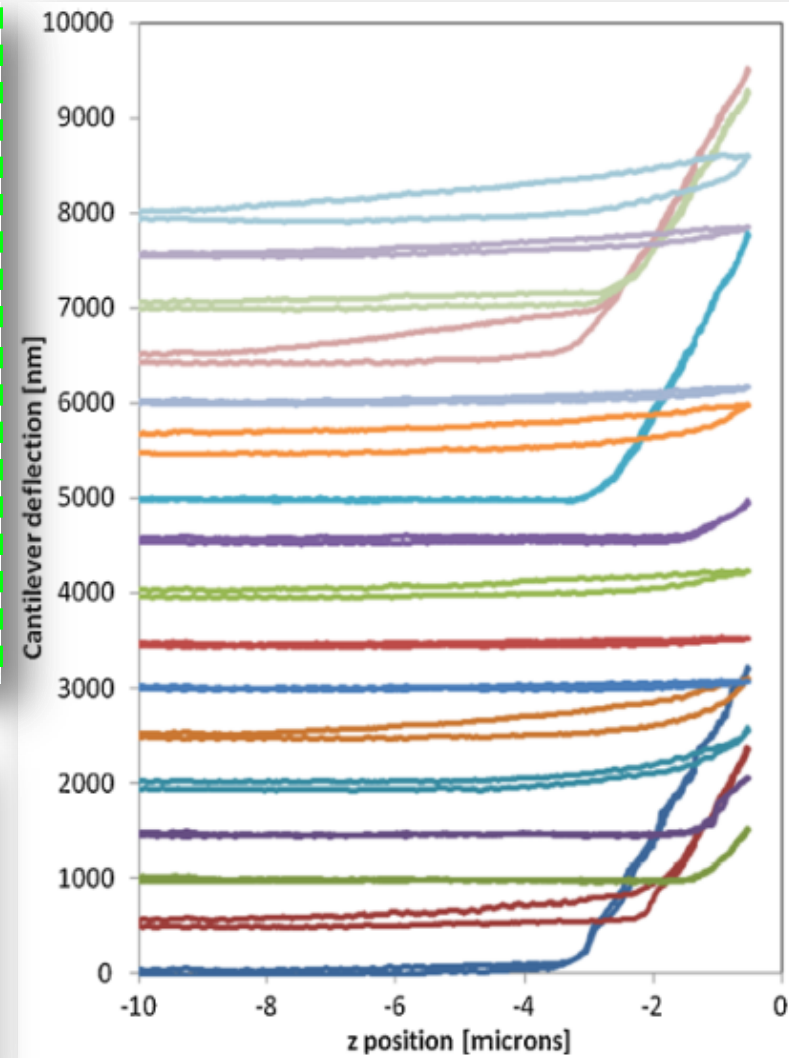
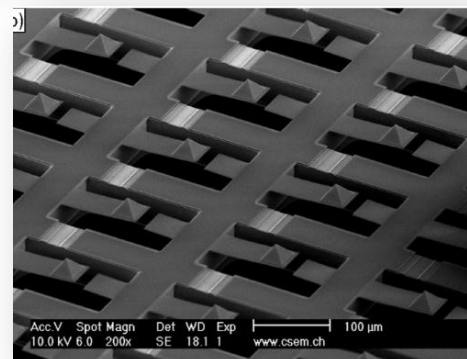
- M. Favre, J. Polesel-Maris, et al. , "Parallel AFM imaging and force spectroscopy using two-dimensional probe arrays for applications in cell biology", J.I of Molecular Recognition 24(3), 446-452 (2011)
- Brevet EP2336789, J. Polesel-Maris, A. Meister, G. Gruener, "Parallel cantilever deflection measurement"



# Mesures élasticité cellulaire avec réseaux de microleviers



3T3 cells



- Sensibilité de l'ordre de 10 pN avec  $k = 0.01$  N/m

# Agir sur la cellule unique

- Des cellules individuelles, bien que génétiquement identiques, montrent des phénotypes différents et des profils d'expression différents des gènes ...



James Eberwine's ingenuity in pioneering single-cell protocols has led to dozens of patents.

**Nature**, december 2011

deploys the vortex trap to fuse droplets and change the concentration of reagents<sup>11</sup>.

Chiu's lab has also developed microfluidic devices for quantifying fluorescently tagged molecules, and for detecting and analysing cells that are rare in a population, such as tumour cells circulating in the blood (see 'Beyond amplification').

Ultimately, a combination of techniques will be necessary for researchers to attain their goal of measuring multiple parameters in a single, living cell. "The more parameters you can define — the transcriptome, the peptide-ome, how a cell looks, how it responds to drugs — the more information you are going to get out," says Eberwine.

Eberwine is confident that these methods will emerge, even if it takes years. "I think we will be successful," he says, "and if we are not, somebody else will be." ■

## The NIH gets singular

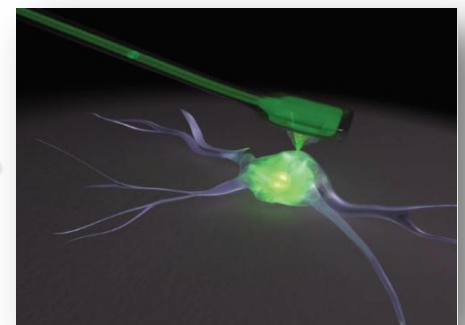
The challenges of single-cell analysis have caught the attention of the US National Institutes of Health (NIH). The agency has launched a programme to fund advances in single-cell research, with a budget of around US\$90 million over five years from the NIH Common Fund, which backs science that crosses disciplines. Grant applications are due early next year, and the NIH expects to make the first awards by September 2012, says Andrea Beckel-Mitchener, a programme officer at the NIH campus in Bethesda,

Maryland. The programme will fund new techniques in areas ranging from microscopy to biochemistry, and foster their commercialization. The NIH also sees a big need for tools to examine cells in their natural environment.

Many of the techniques need an extra push. "It's still really difficult for individual labs to move into that area; the group of researchers who work on this is still highly specialized," says Beckel-Mitchener. "If you want to reach the next level you really have to push the envelope." C.S.

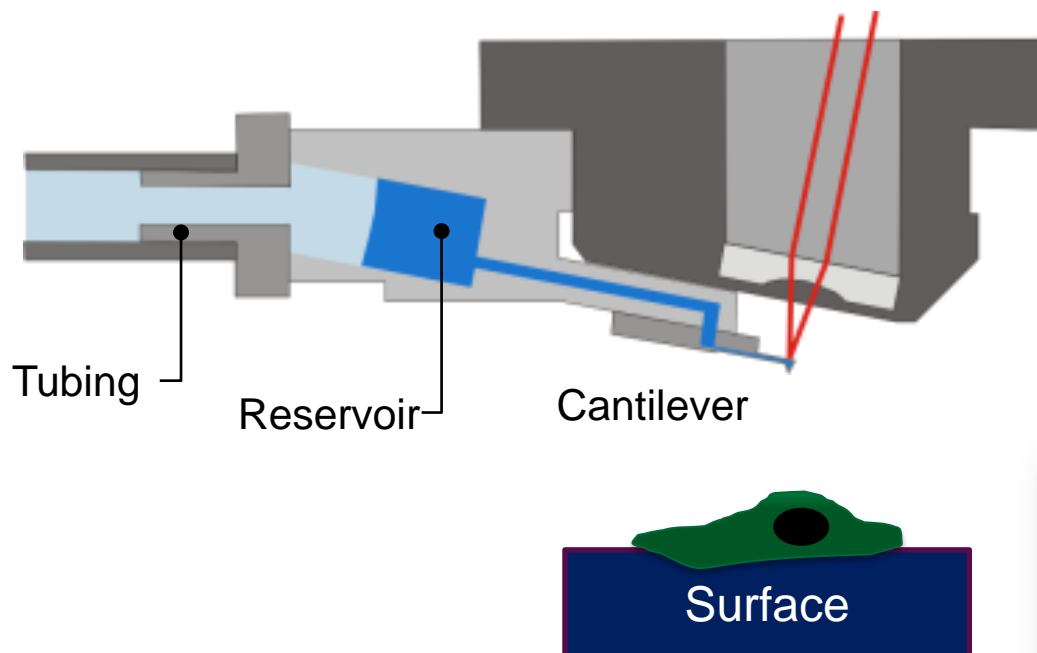


T. K. Chowdhury, 1969



2006

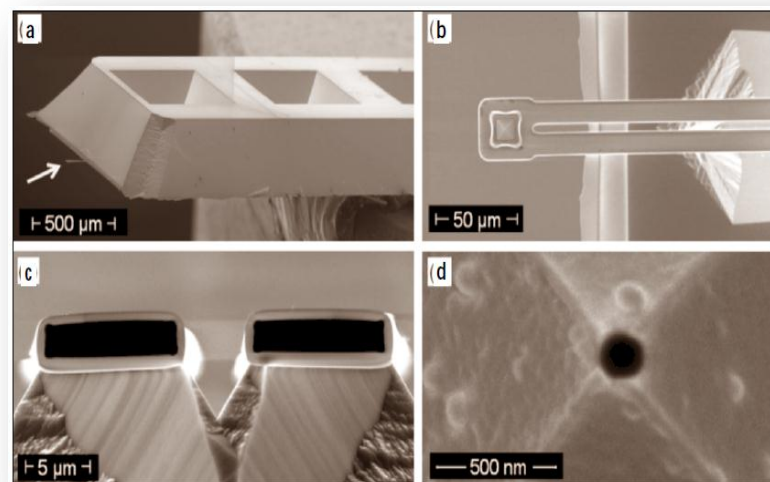
# FluidFM: principe et intégration



AFM Scan head

Un microlevier creux:

**csem**

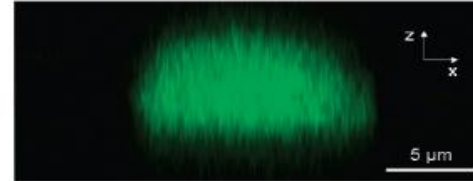
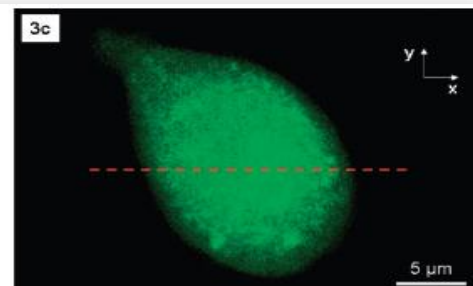
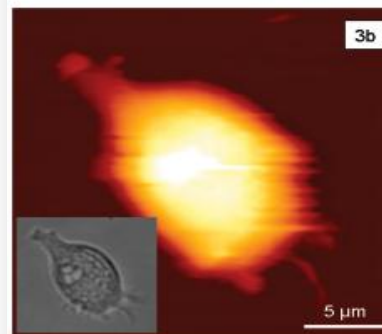
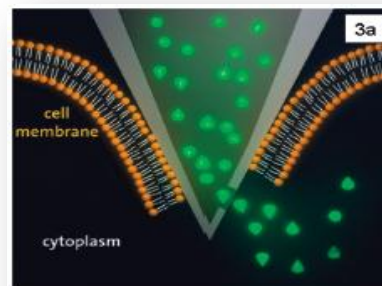
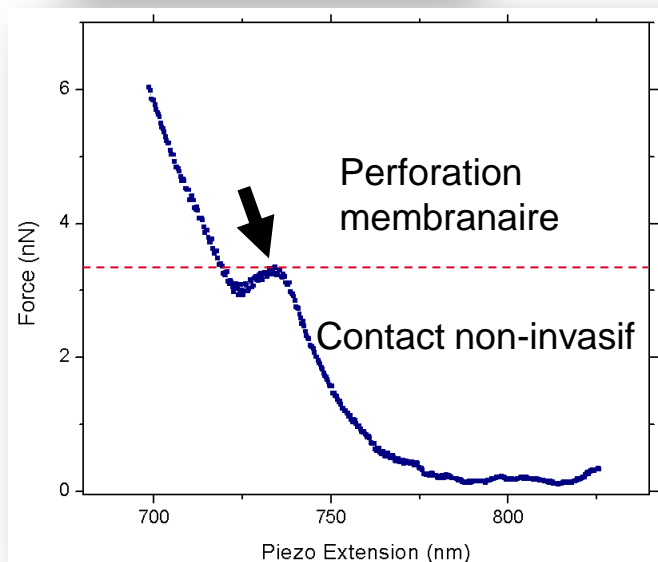
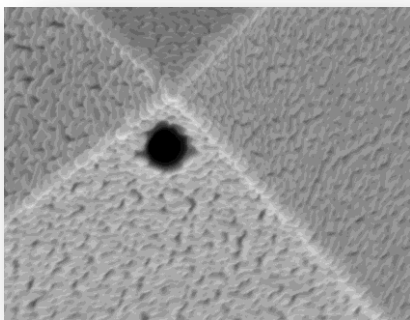


**ETH**

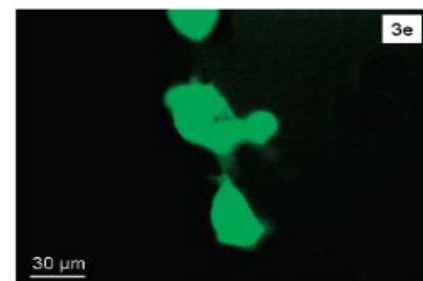
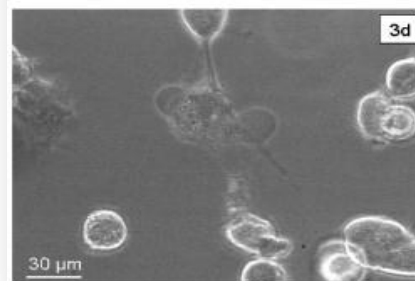
Eidgenössische Technische Hochschule Zürich  
Swiss Federal Institute of Technology Zurich

A. Meister, J. Polesel-Maris et al., "Nanoscale dispensing in liquid environment of streptavidin on a biotin-functionalized surface using hollow atomic force microscopy probes," [Microelectronic Engineering 86, 1481 \(2009\)](#)

## Perforation et injection/extraction contrôlée:

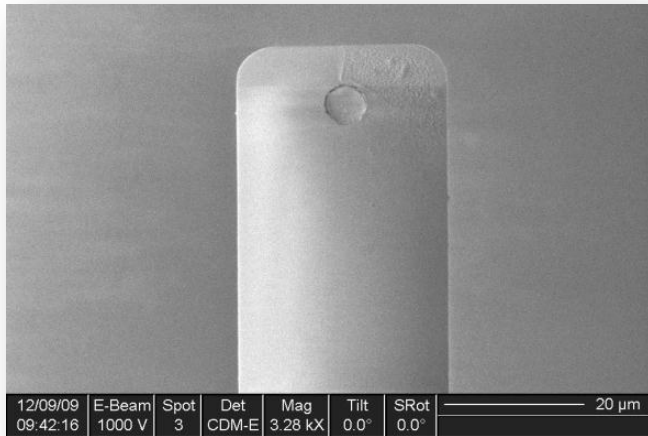


Myoblastes C2C12

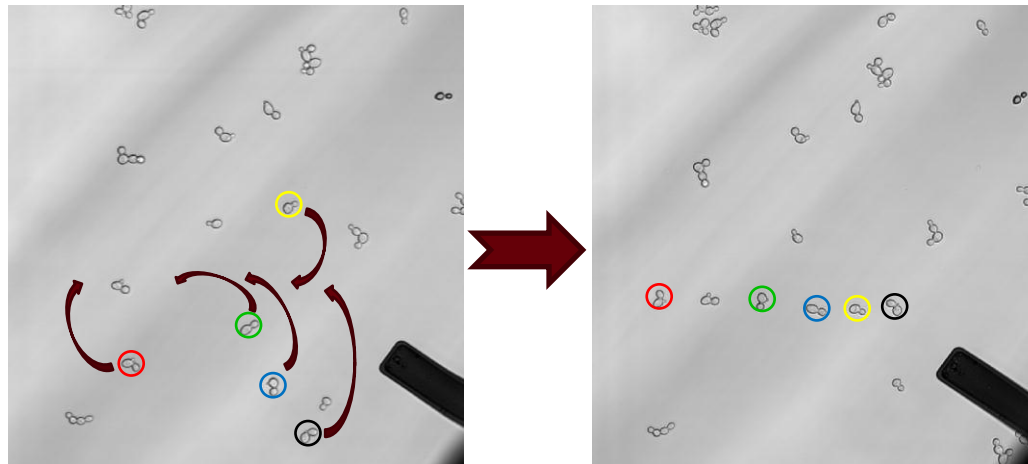
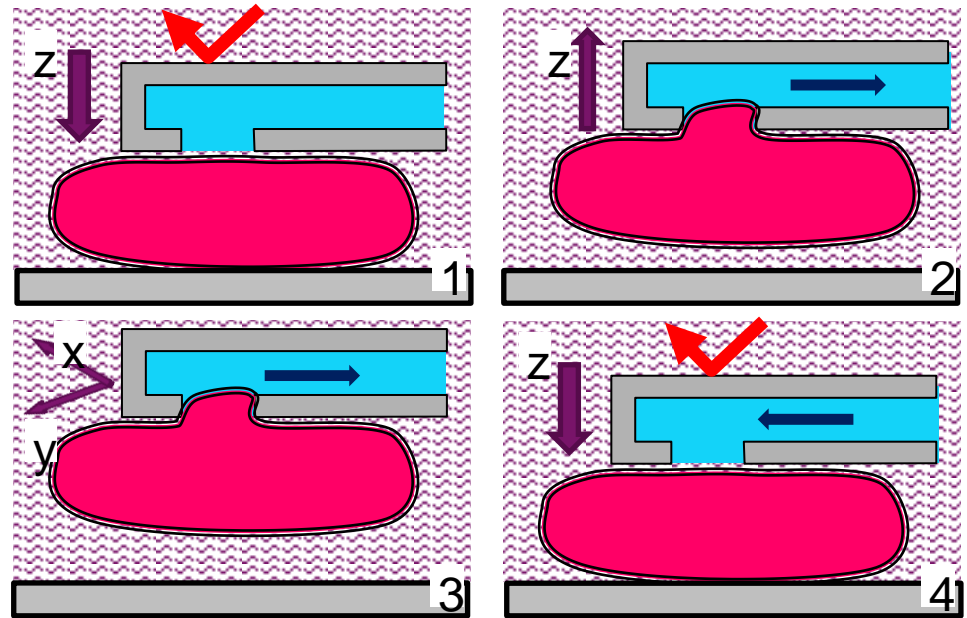


A. Meister, M. Gabi, J. Polesel-Mariset al., "FluidFM: combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond", [Nano Letters 9\(6\), 2501 \(2009\)](#)

# Manipulation de cellules

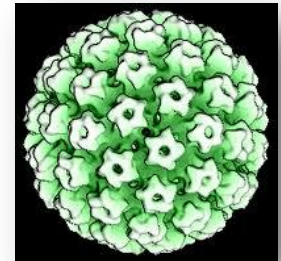
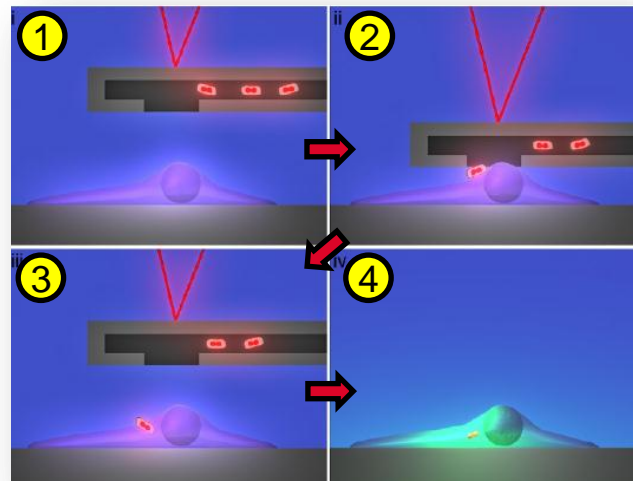


P. Dörig et al., Appl. Phys. Lett. 97(2), 023701 (2010)

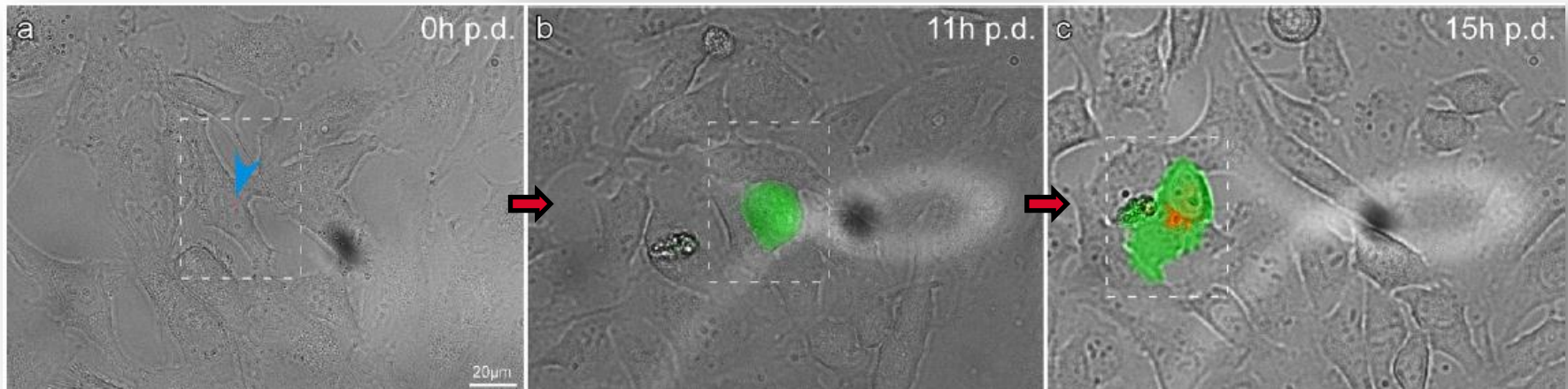


*Saccharomyce cerevisiae*

# Injection ciblée de virus dans une cellule



Vaccinia A5-GFP

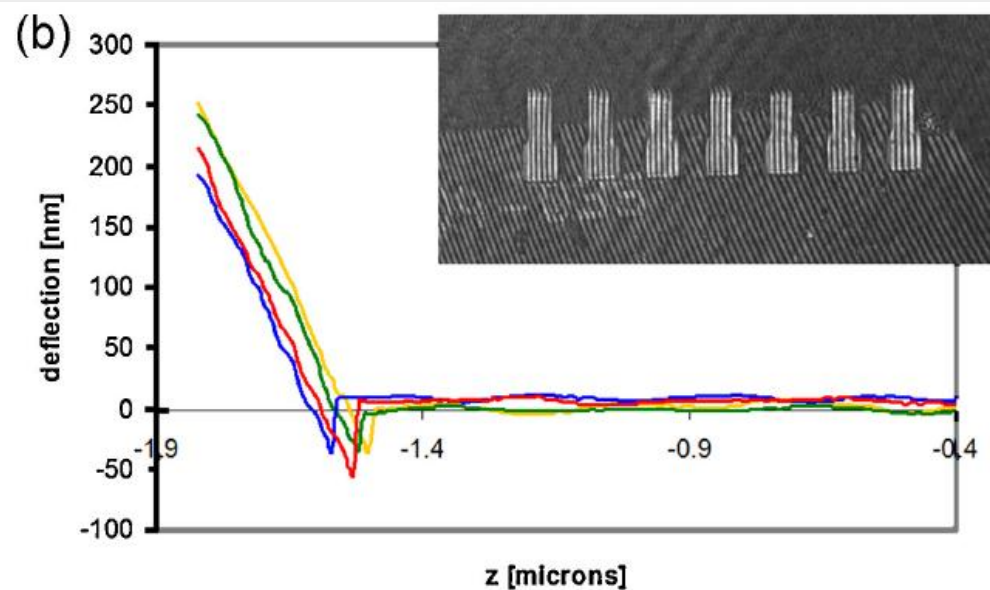
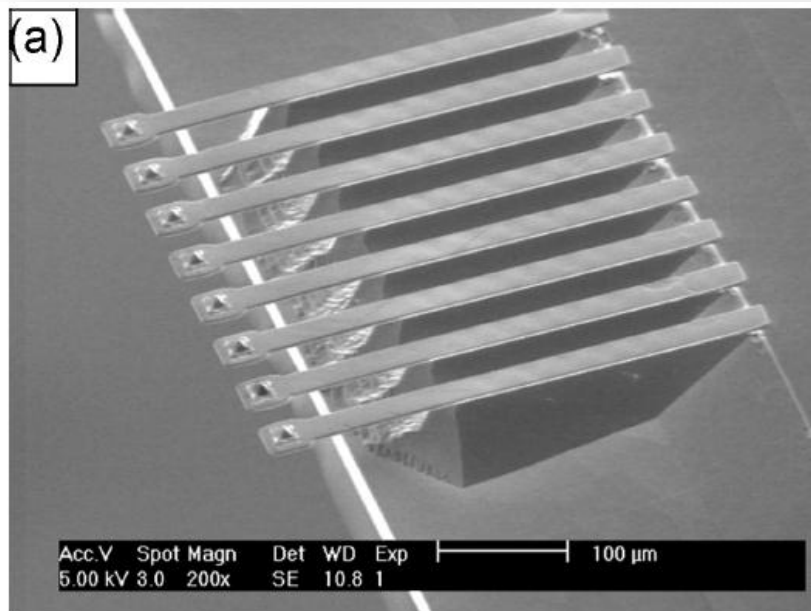


P. Stiefel et al., NanoLetters (2012)

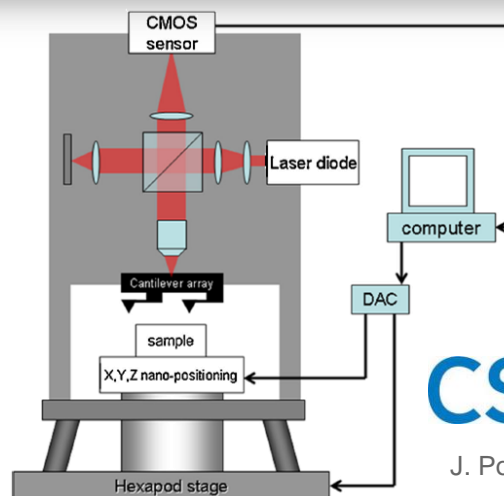
**ETH**

Eidgenössische Technische Hochschule Zürich  
Swiss Federal Institute of Technology Zurich

# Matrice de microleviers creux



- Détection interférométrique de réseau de microleviers creux
- Méthode sérielle augmentant la statistique d'analyse

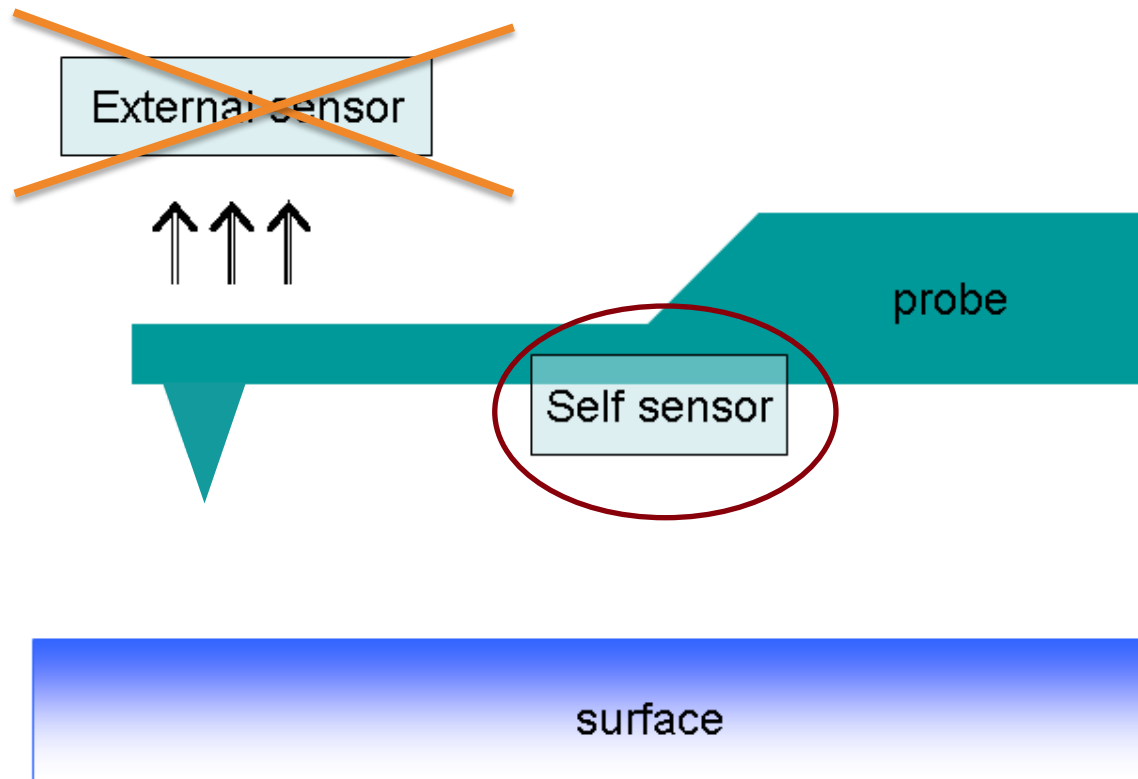


csem

J. Polesel | 21 septembre 2012

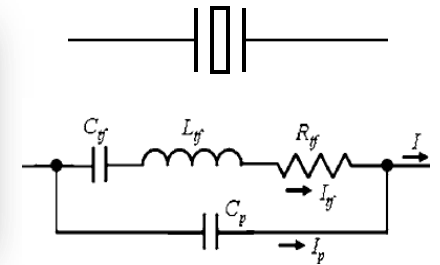
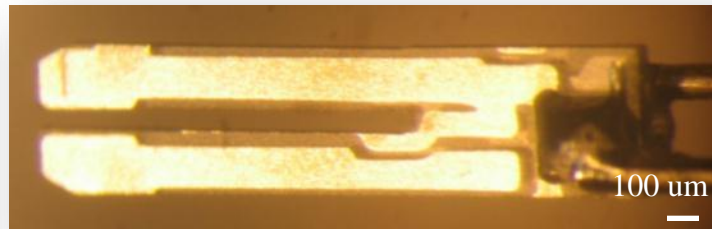
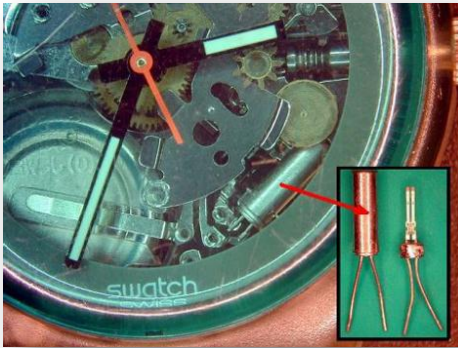
73

# Capteurs intégrés et Haute Résolution



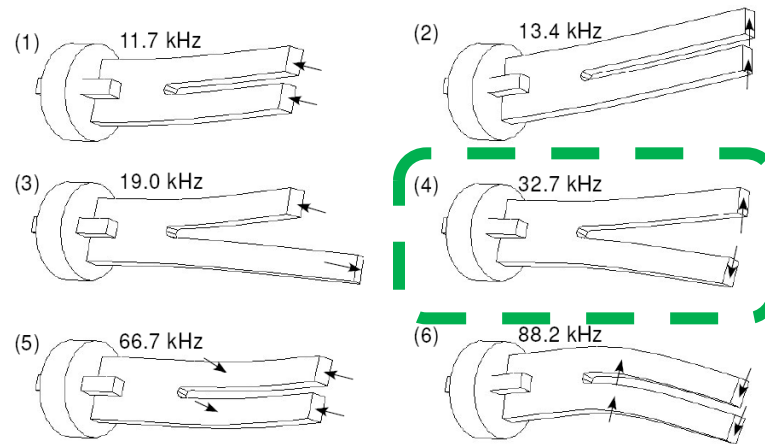
# Sonde piézoélectrique: principe

## Diapason en quartz avec électrodes (Cr, Au, Ni, Al) de pilotage



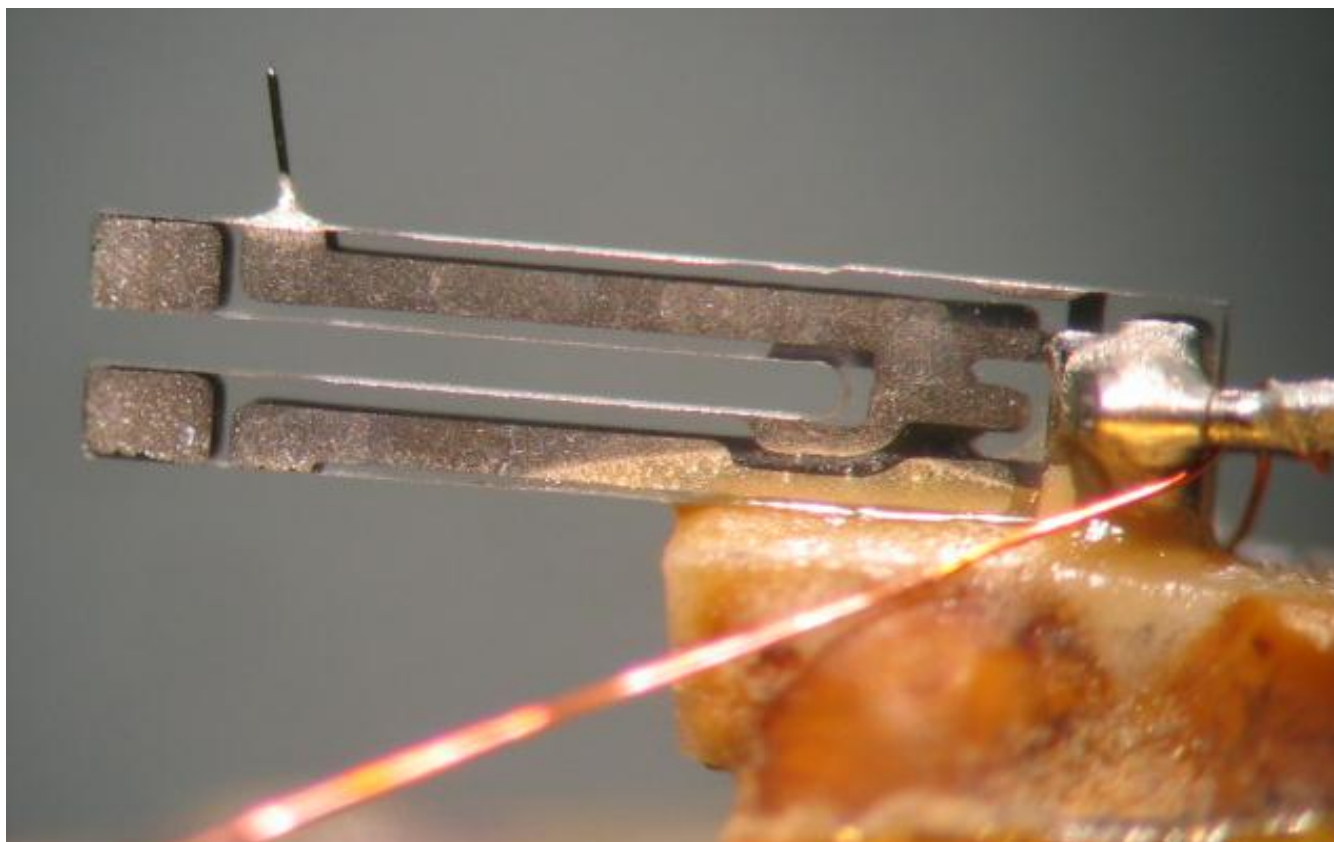
Electrical *Butterworth-Van Dyke* model

- Facteur de qualité élevé  $Q > 10000$  à l'air
- $500 \text{ N/m} < k < 10'000 \text{ N/m}$
- Utilisé en mode AC



F. Giessibl, Rev. Mod. Phys. 75, 949 (2003)

# Sonde AFM à diapason piézoélectrique



# Avantage du diapason

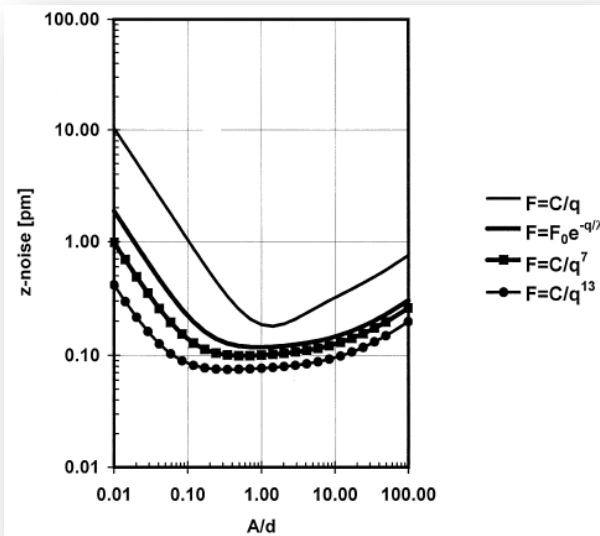
- Force de rappel nécessaire pour oscillation entretenue:

$$k.A > F_{tip-surface}$$

- L'énergie d'entretien de l'oscillateur:

$$\frac{\pi}{Q} k.A^2 > E_{tip-surface}$$

- Le rapport *signal-sur-bruit* le plus favorable si  $A=\lambda$  portée de l'interaction:



$$(\delta z)^2 = \left( \frac{\delta f}{\frac{\partial f}{\partial z}} \right)^2 + (\delta A)^2 + (\delta Z_{mechanical})^2$$

F. Giessibl et al., Appl. Surf. Sci. 140, 352 (1999)

Ainsi, pour  $\lambda \sim \text{\AA}$ , on converge vers une sonde tel que :

$$A \approx \text{Angström}$$

$$k \approx 2000 \text{ N/m}$$



F. Giessibl, 1999

# Le bruit d'origine thermique et modes d'utilisation

$$S_F^{th} = 4 \cdot k_B \cdot T \cdot \frac{K_{probe}}{Q \cdot \omega_0}$$

$$S_{\Delta f}^{th} = \frac{k_B \cdot T}{2\pi} \cdot \frac{f_0}{K_{probe} \cdot Q \cdot A^2}$$

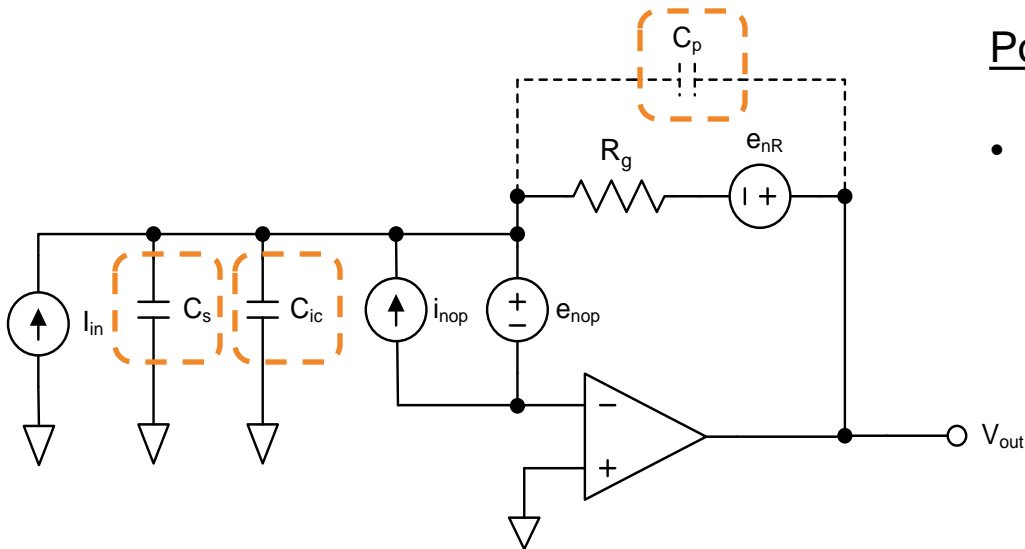
$$S_A^{th} = \frac{1}{K_{probe}^2} \cdot \frac{S_{Fth}}{\left(1 - \frac{f^2}{f_0^2}\right)^2 + \left(\frac{f}{Q \cdot f_0}\right)^2}$$

$$S_{\varphi}^{th} = \frac{4 \cdot S_A^{th}}{A^2}$$

Sonde	Bruit en Force (fN/√Hz)	Bruit en fréquence (mHz/√Hz) FM-AFM	Bruit en amplitude (pm/√Hz) AM-AFM	Bruit de phase (mrad/√Hz) PM-AFM
<b>Cantilever silicium standard</b> (k=30 N/m, Q=10000, f=150 kHz, A=5nm)	8.4	3.6	2.42	1
<b>Tuning Fork</b> (k=8000 N/m, Q=5000, f=32 kHz, A=0.1nm)	363	7.3	0.23	4.6

J. Polesel-Maris et al., Phys. Rev. B 79, 235401 (2009)  
N. Kobayashi et al., Jpn. J. Appl. Phys. 45, L793 (2006)

# Le bruit du préamplificateur

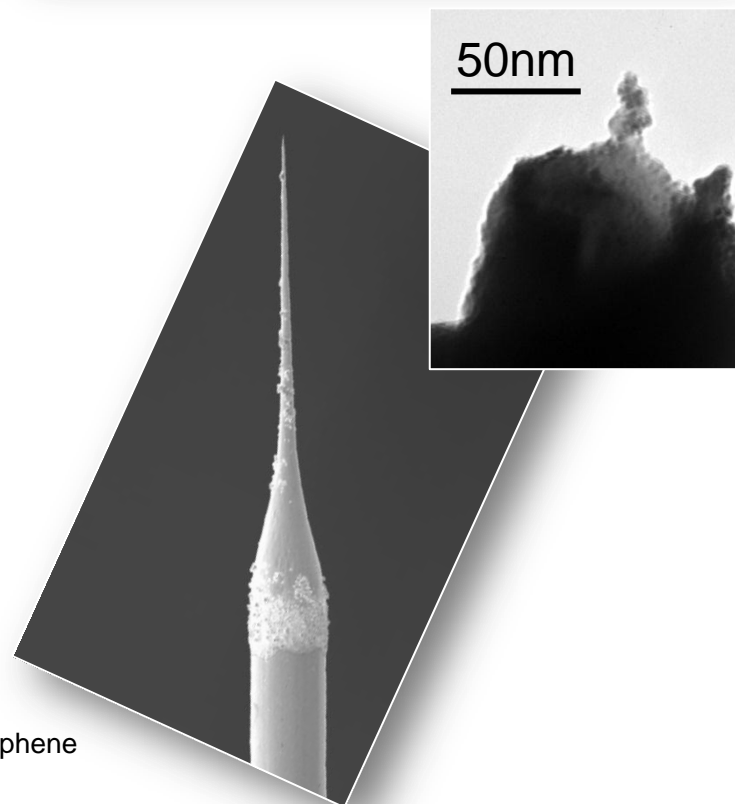
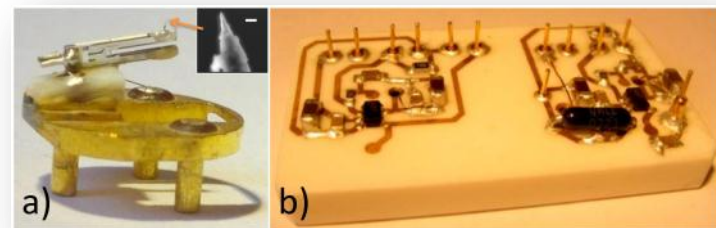
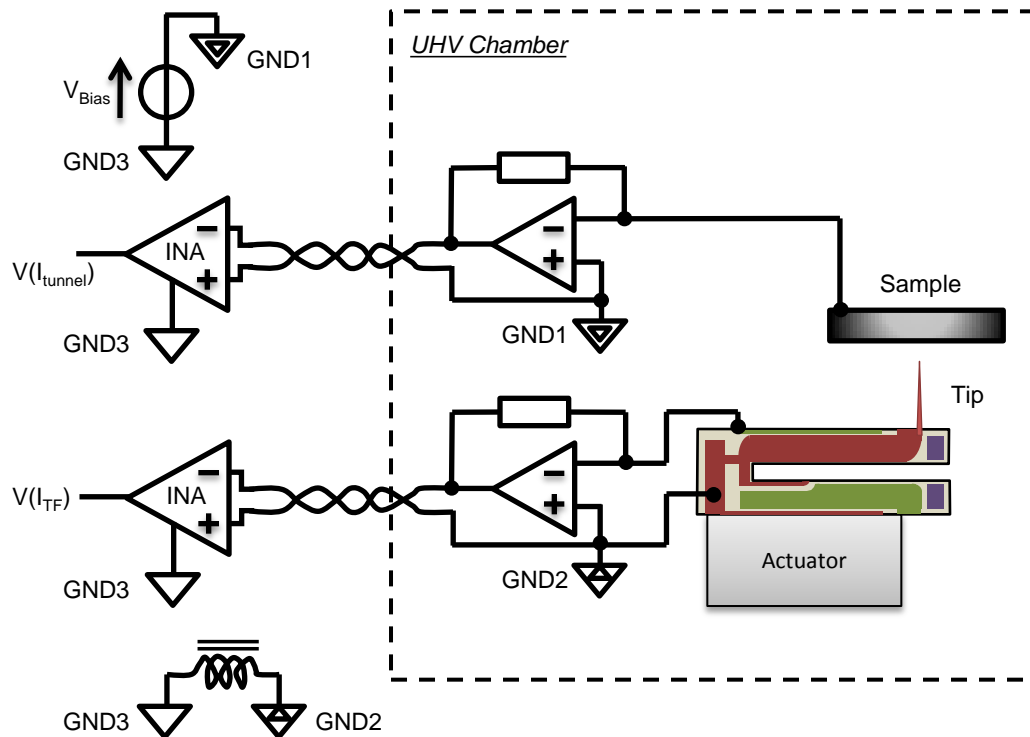


Point critique dans la limite de détection:

- La capacité d'entrée, **dépendance quadratique de la densité de bruit avec la fréquence !**

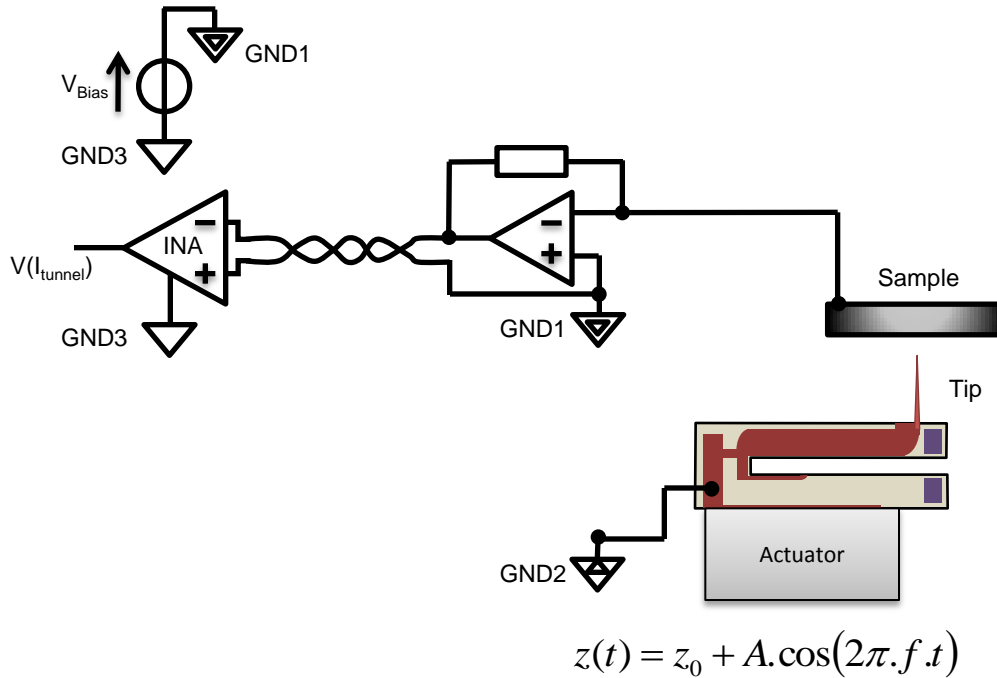
$$S_{out}^{noise} = \left[ i_{nop}^2 + \frac{4 \cdot k_B \cdot T}{R_g} + e_{nop}^2 \cdot \left( \frac{1}{R_g} + \omega \cdot \underbrace{(C_s + C_{ic} + C_p)} \right)^2 \right] \cdot \frac{R_g^2}{1 + R_g^2 \cdot C_p^2 \cdot \omega^2}$$

# Sondes piézoélectrique: intégration



J. Polesel-Maris et al., "Combined dynamic STM and FM-AFM investigations on polythiophene chains on graphite with a tuning fork sensor", [J. Appl. Phys. 109, 074320 \(2011\)](#)

# Sondes piézoélectrique: intégration



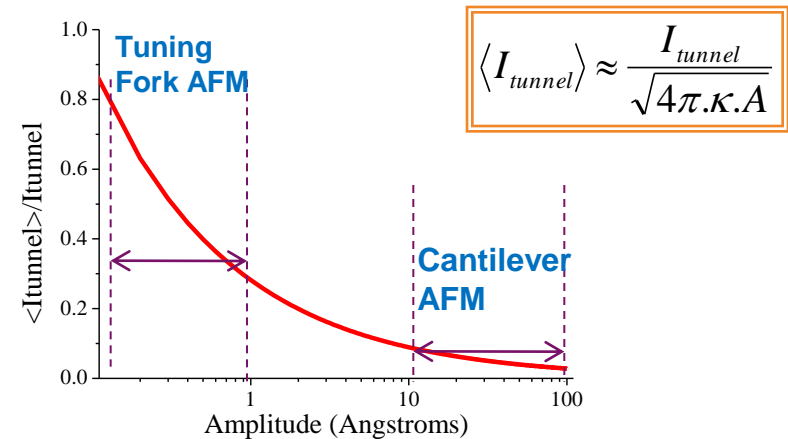
## STM standard:

$$I_{tunnel} = I_0 \cdot \exp(-2 \cdot \kappa \cdot z) \quad \text{avec} \quad \kappa = \frac{\sqrt{2 \cdot m \cdot \phi}}{\hbar}$$

## STM oscillant:

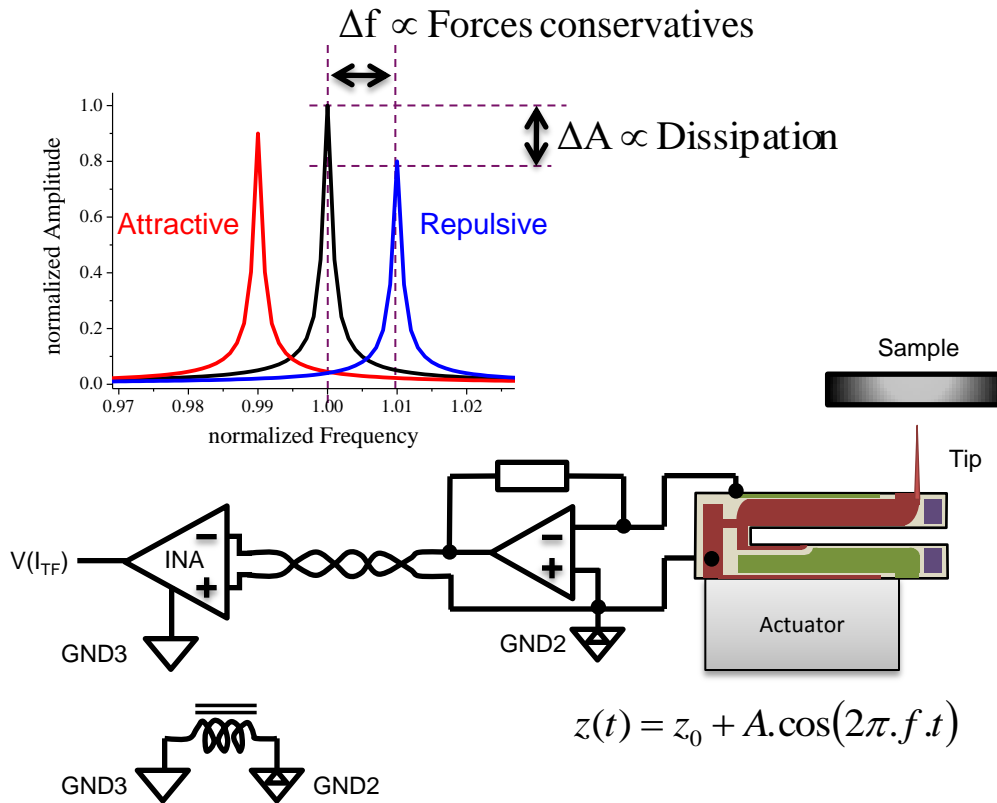
$$\langle I_{tunnel} \rangle = \frac{1}{T} \int_0^T I_0 \cdot \exp(-2 \cdot \kappa \cdot z(t)) \cdot dt$$

$$\langle I_{tunnel} \rangle = I_0 \cdot \exp(-2 \cdot \kappa \cdot z_0) \cdot \mathfrak{J}_0(2 \cdot \kappa \cdot A)$$



Gain d'un facteur 6 à 16 sur  $\langle I_{tunnel} \rangle$  !

# Sondes piézoélectrique: intégration



## FM-AFM (Modulation de Fréquence):

$$\text{Force} = F(z(t), \dot{z}(t))$$

$$\Delta f = \frac{f_0^2}{A.k_{TF}} \int_0^{1/f_0} F(z(t), \dot{z}(t)) \cdot \sin(2\pi.f_0.t) dt$$

$$\Delta f = \frac{f_0}{2.k_{TF}} \cdot \frac{dF}{dz}$$

$$\text{Energy Dissipation} = \frac{\pi.k.A_0^2}{Q} \cdot \left( \frac{Exc}{Exc_0} - \frac{f}{f_0} \right)$$

$$\mathbf{U} = \mathbf{R} \cdot \mathbf{I}$$

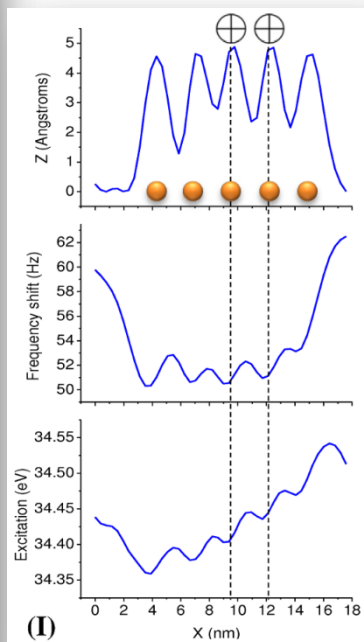
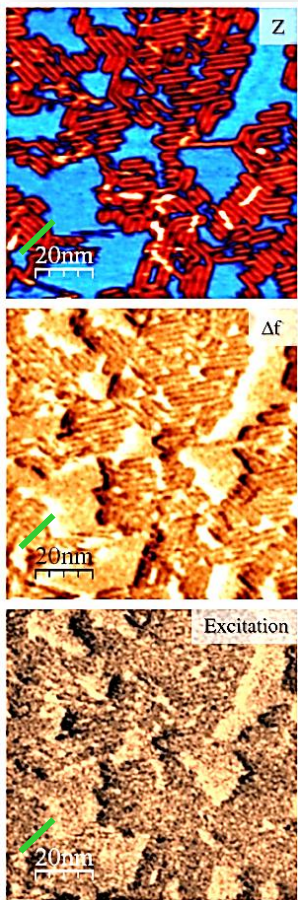
$$\sum \vec{F} = k \cdot \vec{x}$$

$$\Delta E$$

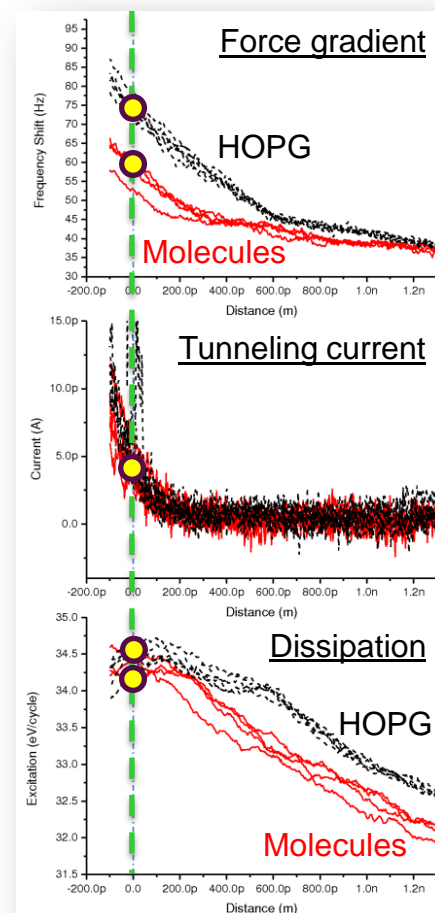
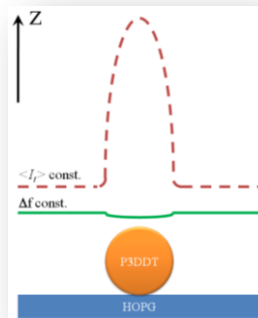
# Molécules faiblement adsorbées: P3DDT sur HOPG

## STM regulation

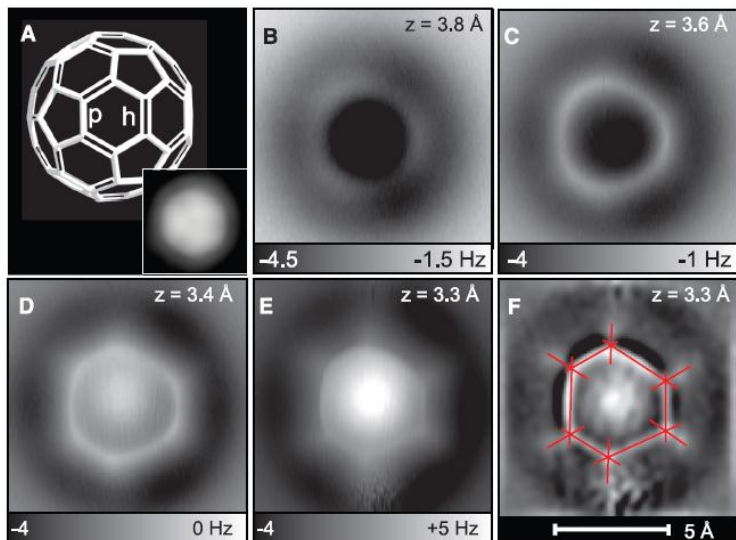
$\langle I_t \rangle = 4.3 \text{ pA}$ ,  
 $V = -1 \text{ Volt}$ ,  $A = 92 \text{ pm}$   
 $T = 300 \text{ K}$



- Stabilité d'imagerie sur molécules faiblement adsorbées
- Inversion de contraste lié à un effet de trajectoire de la pointe
- La spectroscopie en distance démontre un régime de force répulsif durant l'imagerie STM, à petit courant (pA)



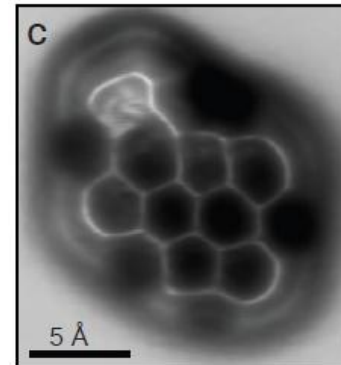
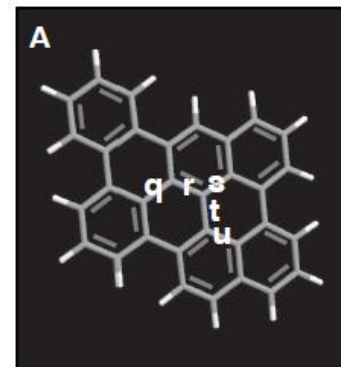
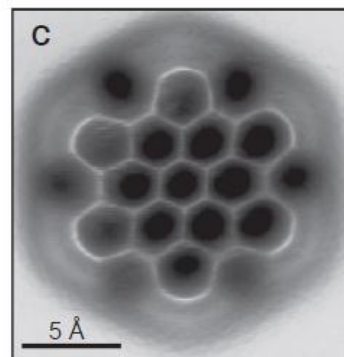
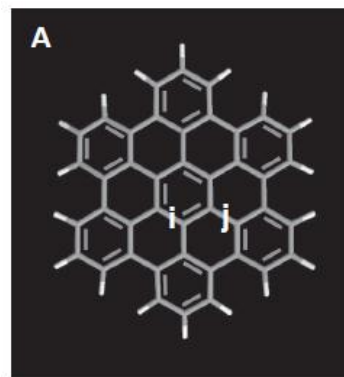
J. Polesel-Maris et al., "Combined dynamic STM and FM-AFM investigations on polythiophene chains on graphite with a tuning fork sensor", [J. Appl. Phys. 109, 074320 \(2011\)](#)



*Science*, Septembre 2012

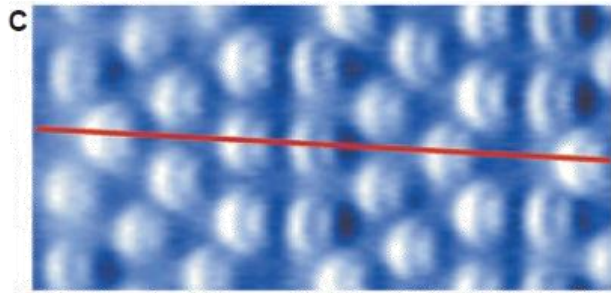


G. Meyer, 2012,  
IBM Rüschlikon

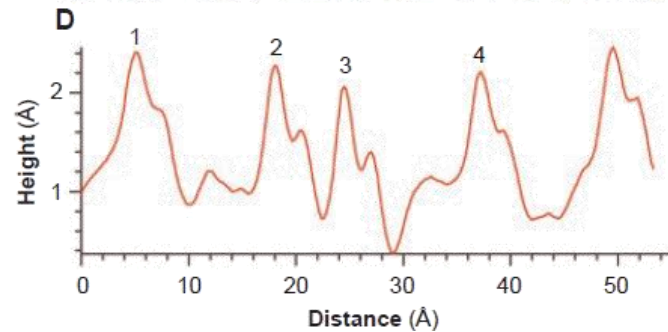


Ordre de liaisons distingué à l'échelle de deux atomes  
d'une molécule !

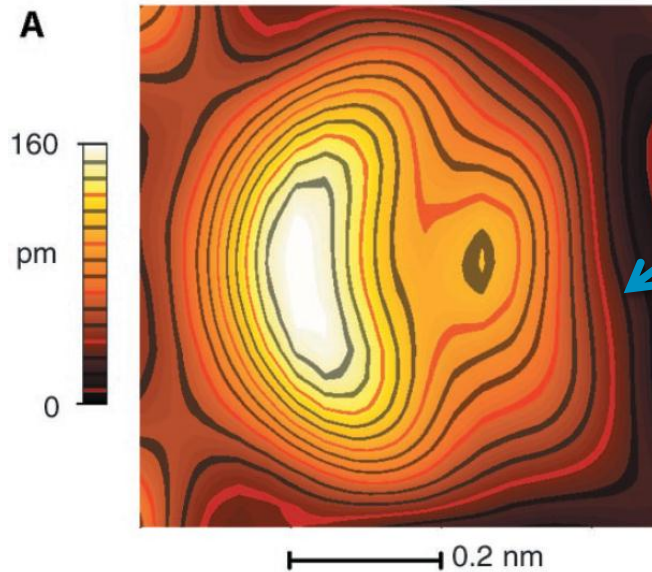




Silicium (111)-(7x7)



*Science, 2000*

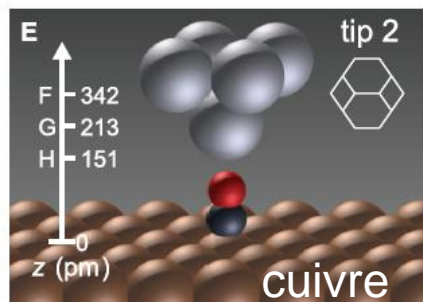


Un seul atome  
de silicium !



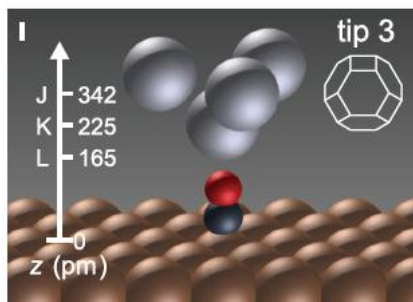
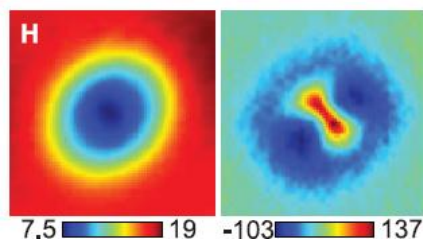
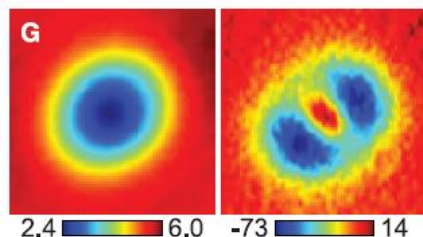
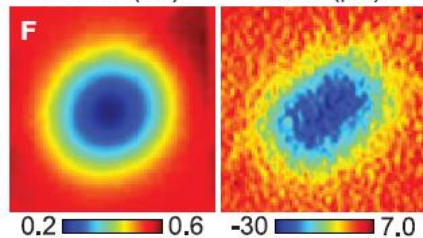
F. Giessibl, 2000

- Cartographie sub-atomique en gradient de force !



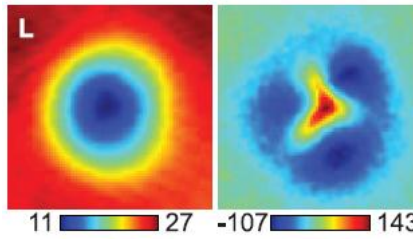
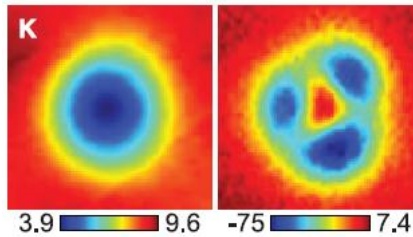
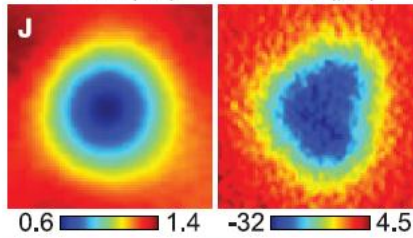
current (nA)

force (pN)

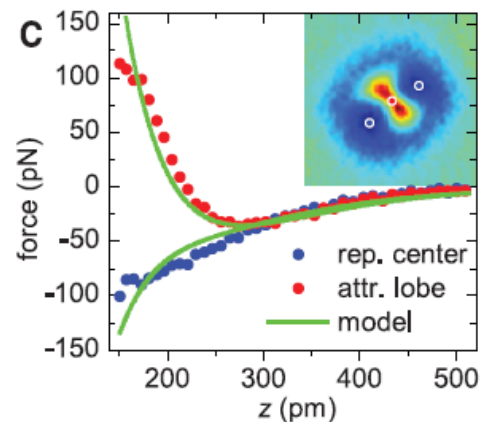


current (nA)

force (pN)



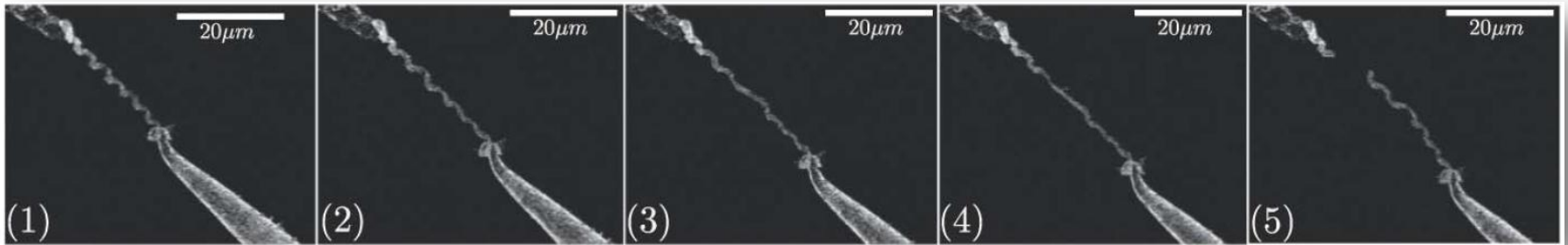
F. Giessibl, 2012



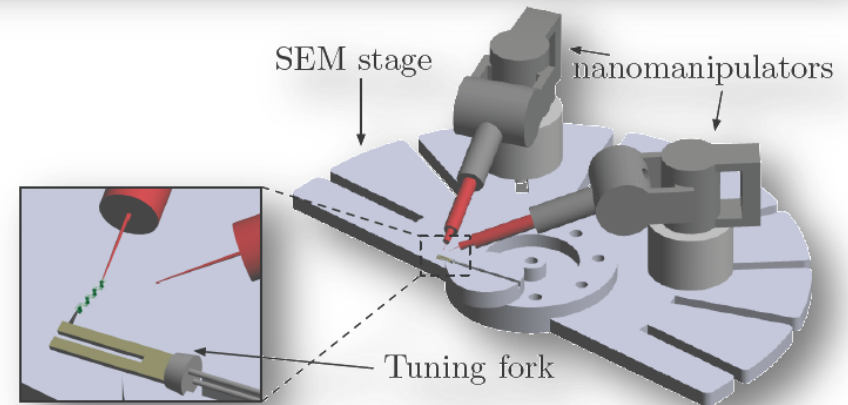
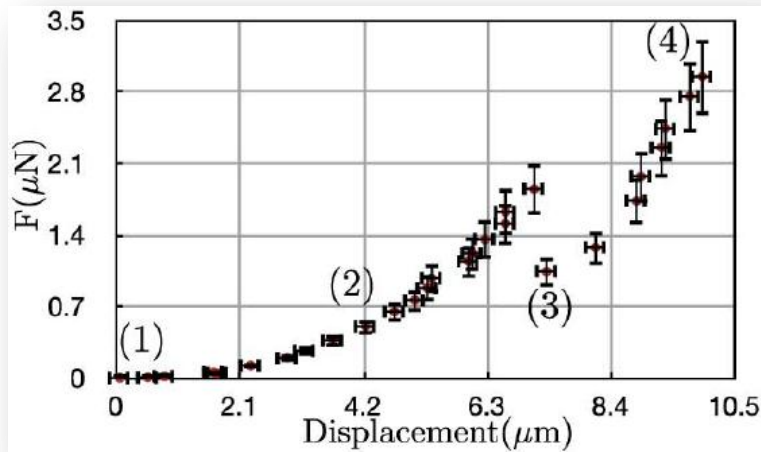
- Cartographie des propriétés électroniques (STM) et mécaniques (AFM) sur une molécule

Science, 2012

- Etirement contrôlé d'un « nanoressort » GaAs :

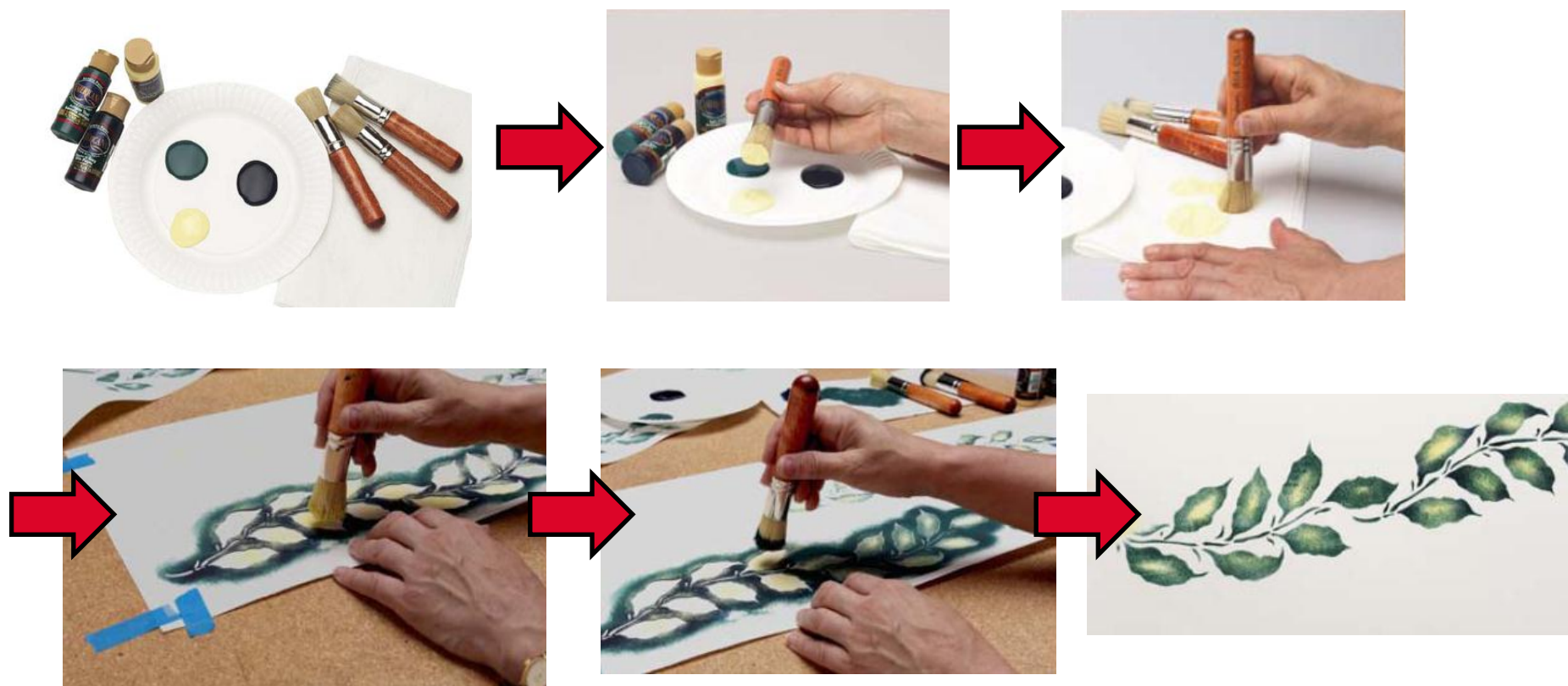


- Courbe de force acquise en parallèle :



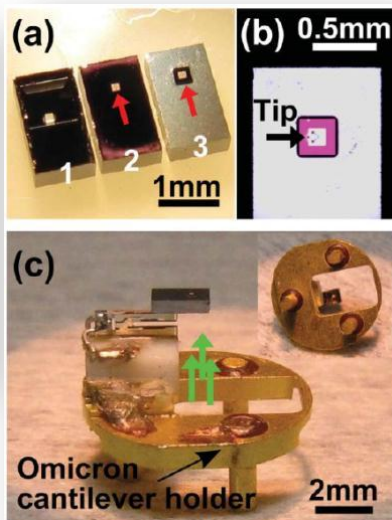
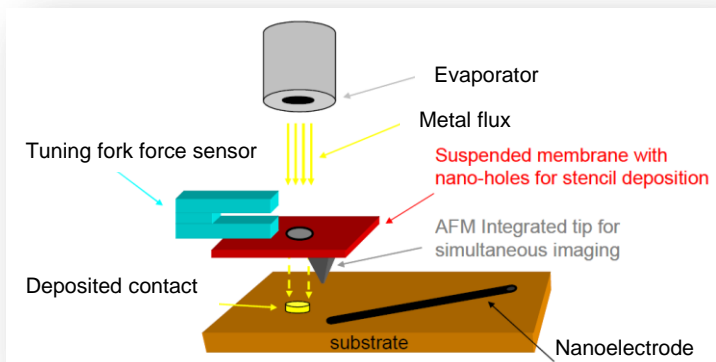
J. C. Acosta, G. Hwang, J. Polesel-Maris, S. Régnier, "A tuning fork based wide range mechanical characterization tool with nanorobotic manipulators inside a scanning electron microscope", [Rev. Sci. Instr. 82, 035116 \(2011\)](#)

# NanoStencil – fabrication de nano-électrodes

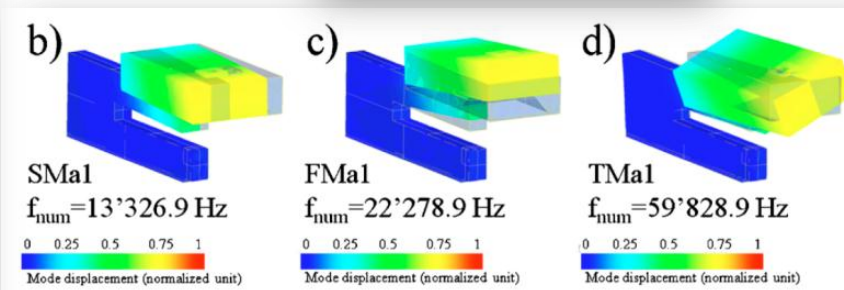
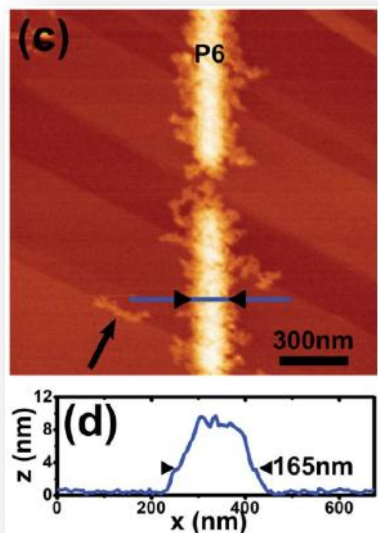


<http://www.stencilrevolution.com>

# NanoStencil – fabrication de nano-électrodes



- « Atterrissage » de la sonde en AM-AFM et FM-AFM
- Rapport des masses sonde/puce  $\sim 1$

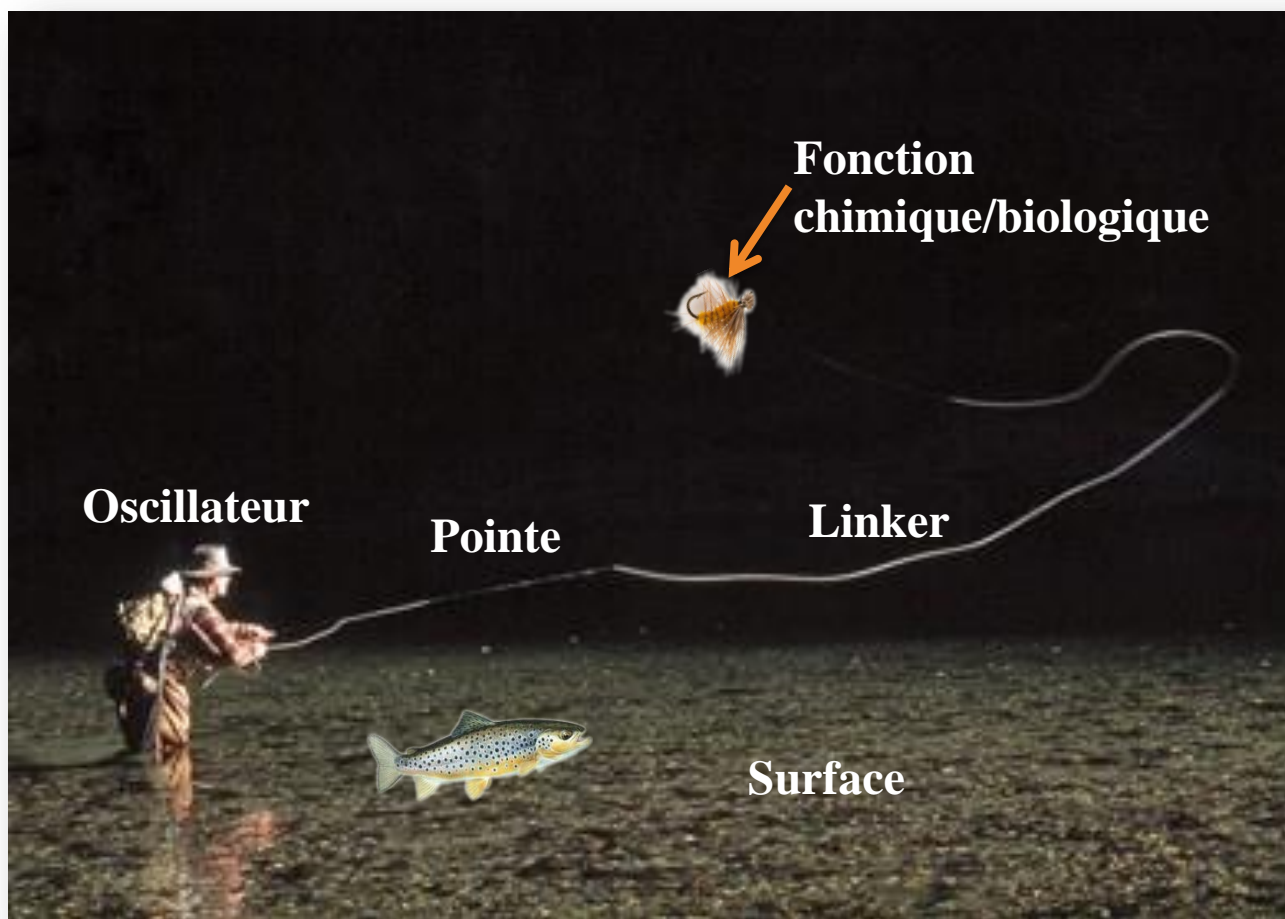


J. Hayton, J. Polesel-Maris et al., "Atomic force microscopy imaging using a tip-on-chip: Opening the door to integrated near field nanotools", [R. Sci. Instr. 81, 093707 \(2010\)](#)

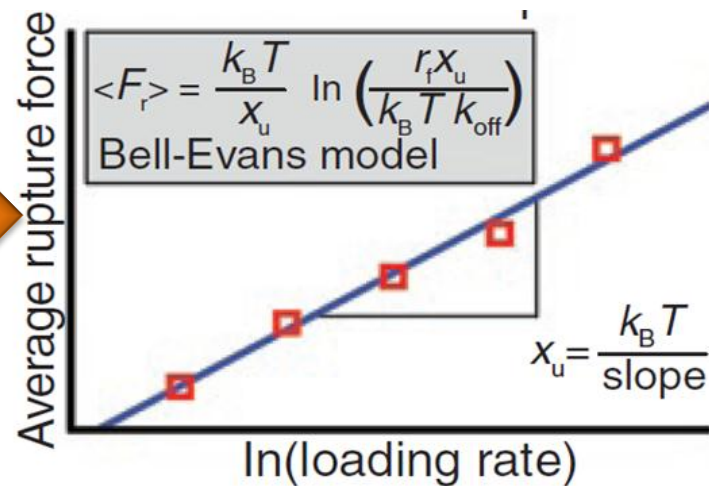
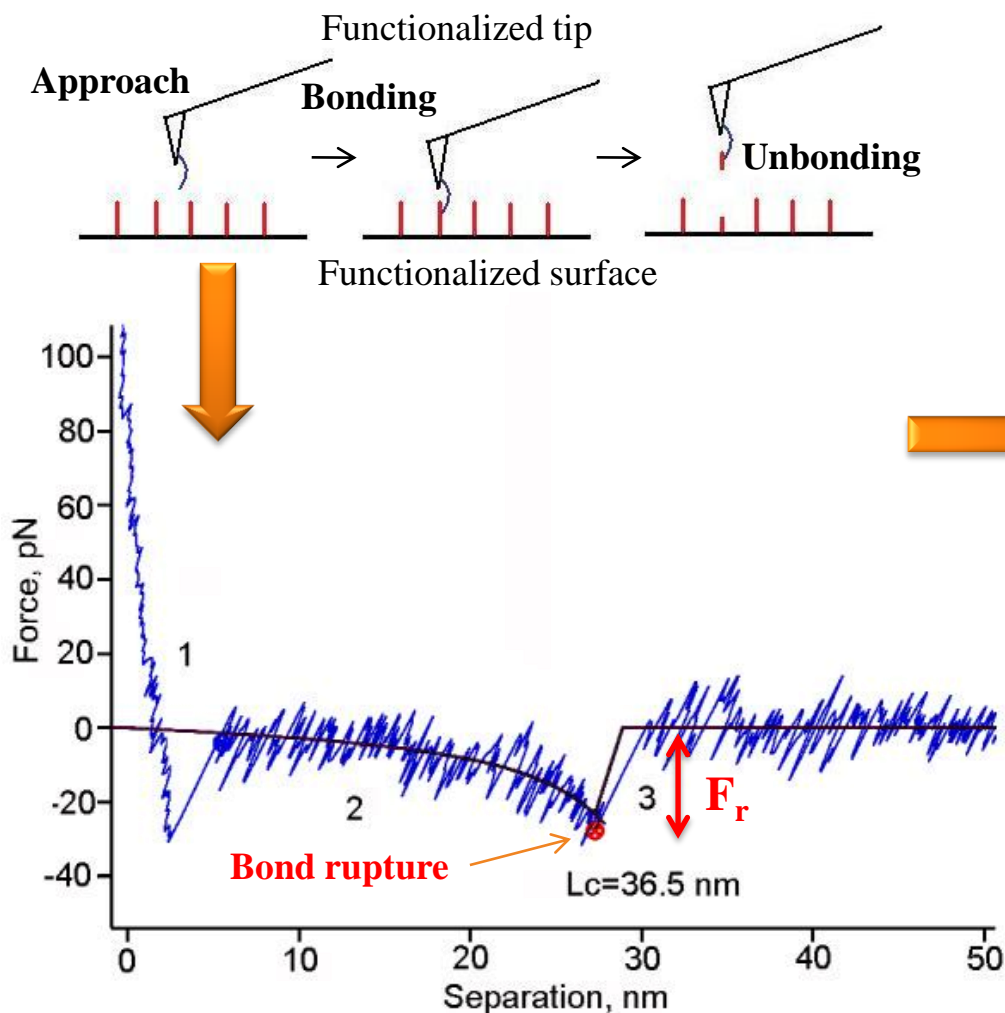
Nanoélectrodes d'or sur  $\text{TiO}_2$

# Spectroscopie de Force à l'échelle de la protéine

# Spectroscopie de Force à l'échelle de la protéine



# Spectroscopie de force dynamique (DFS): principe et théorie



# Spectroscopie de force dynamique (DFS): principe et théorie

$$\langle F \rangle = \frac{k_B T}{x_u} \ln \left( \frac{r x_u}{k_{off} k_B T} \right)$$

$F$  : Force (N)

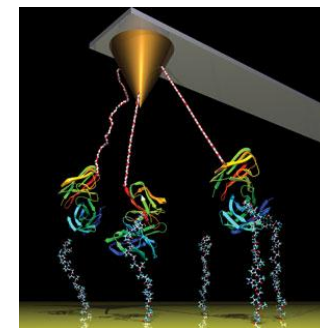
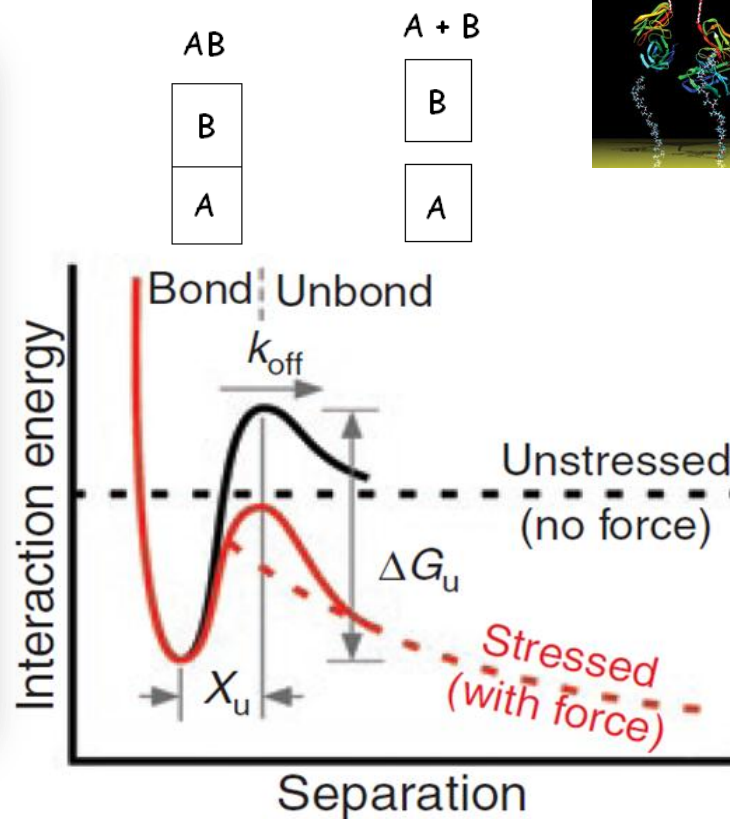
$T$  : température (K)

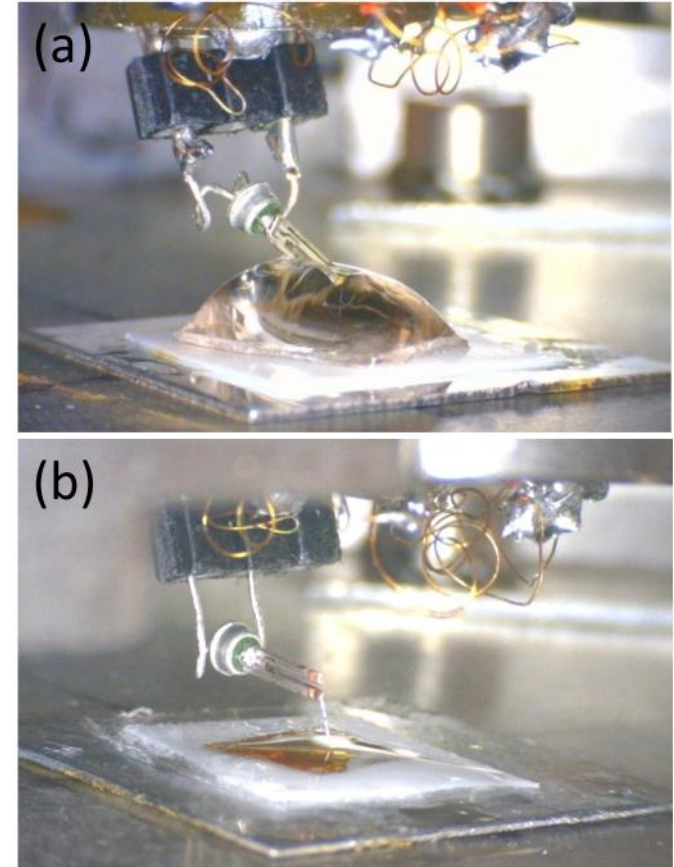
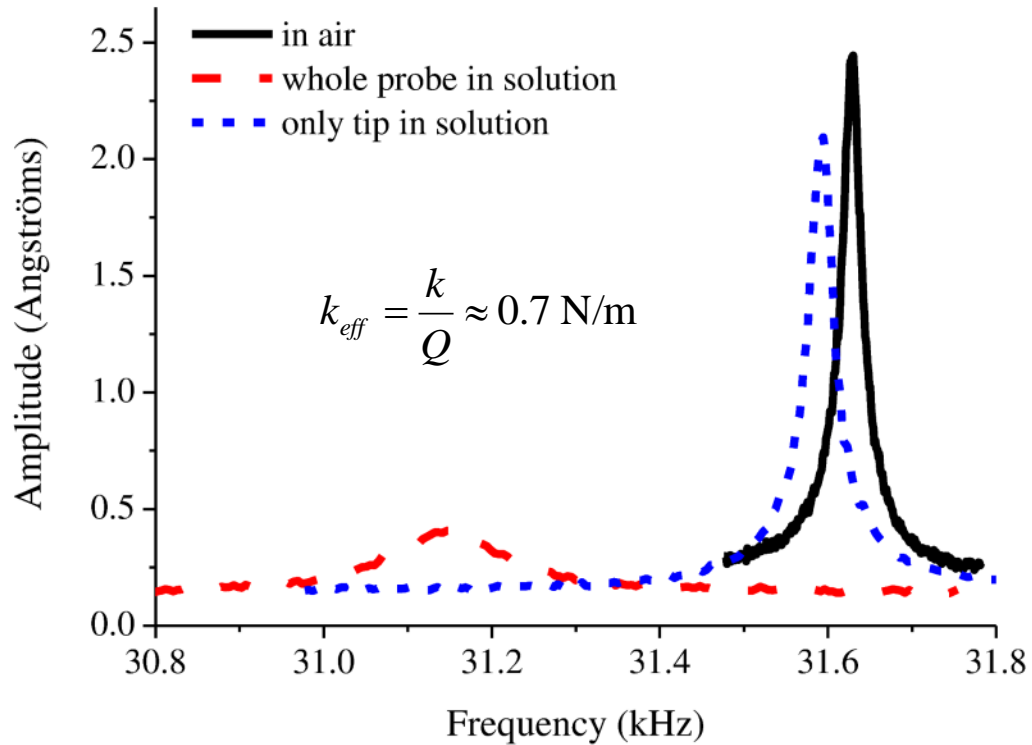
$k_B$  : constante de Boltzmann ( $J \cdot K^{-1}$ )

$x_u$  : distance de l'état de transition (m)

$r$  : taux de charge ( $N \cdot s^{-1}$ )

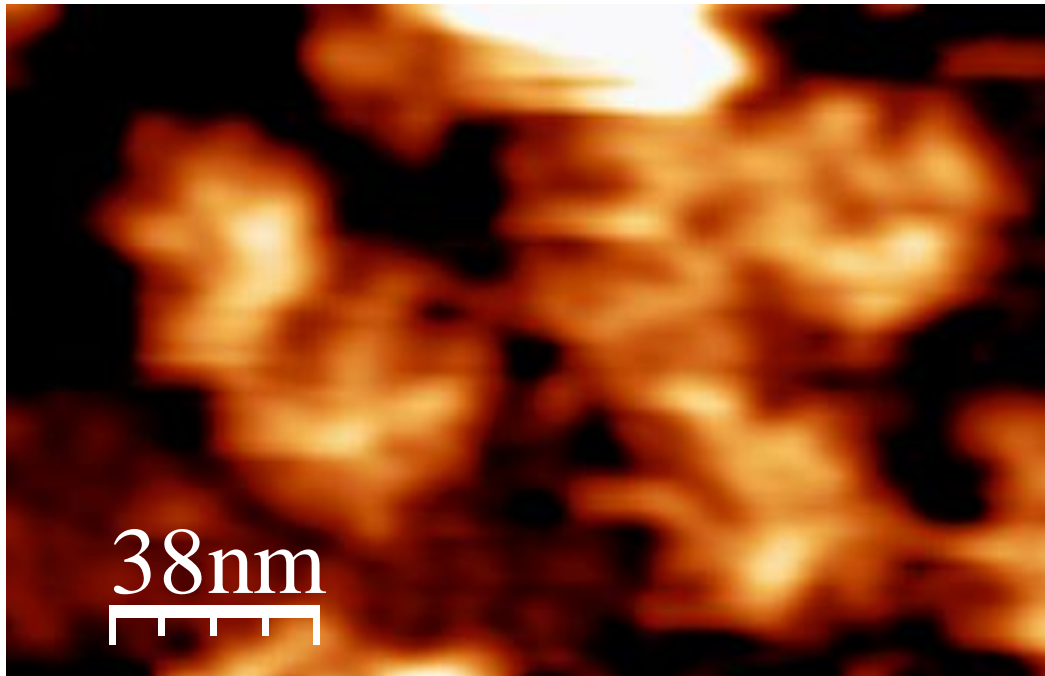
$k_{off}$  : taux de dissociation moléculaire ( $s^{-1}$ )





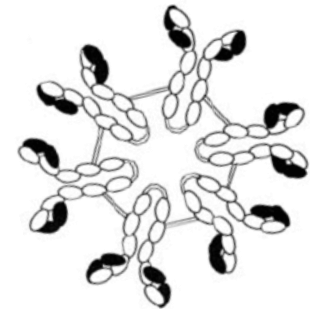
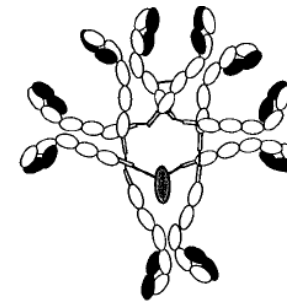
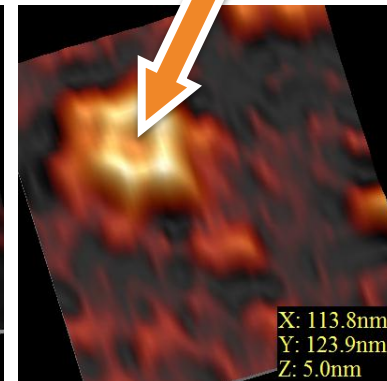
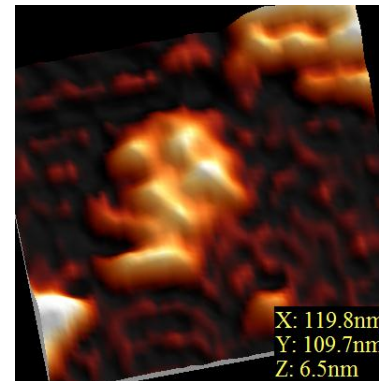
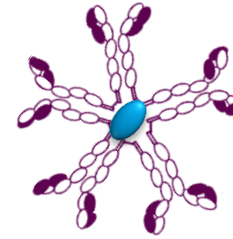
J. Polesel-Maris et al., "Force Spectroscopy by Dynamic Atomic Force Microscopy on Bovine Serum Albumin proteins changing the tip hydrophobicity, with piezoelectric tuning fork self-sensing scanning probe", [Sensors and Actuators B: Chemical 161\(1\), 775-783 \(2012\)](#)

# Image de protéines isolées

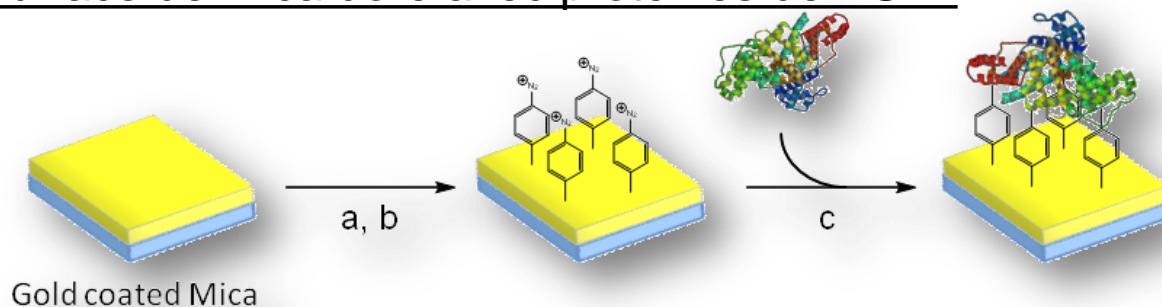


Anticorps IgM

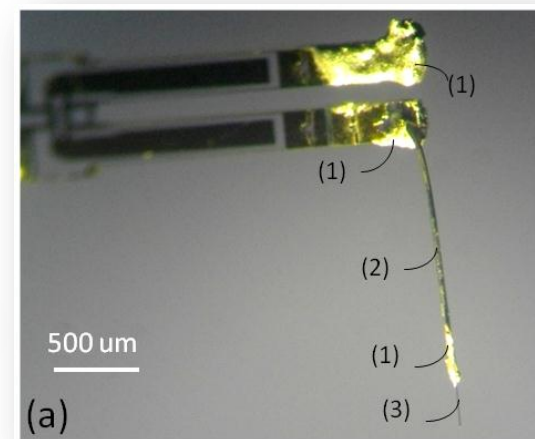
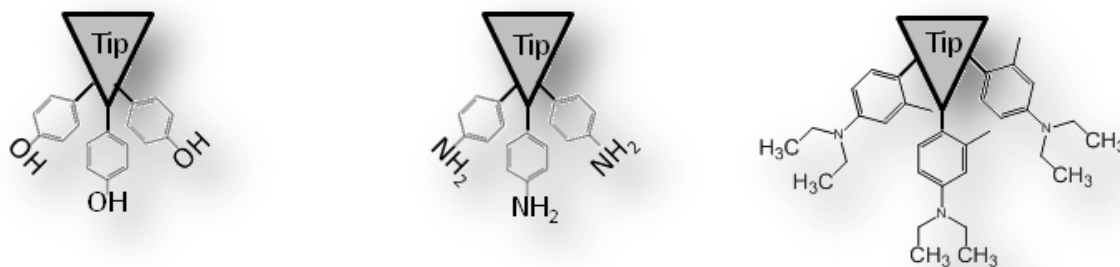
A. Makky, Th. Berthelot, C. Feraudet-Tarisse, H. Volland, P. Viel, J. Polesel-Maris, "Substructures High Resolution Imaging of Individual IgG and IgM Antibodies with Piezoelectric Tuning Fork Atomic Force Microscopy", [Sensors and Actuators B: Chemical 162\(1\), 269-277 \(2012\)](#)



## Surface de mica doré avec protéines de BSA :

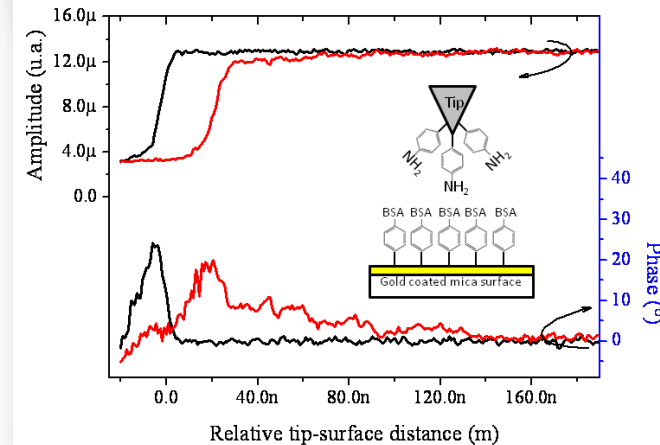
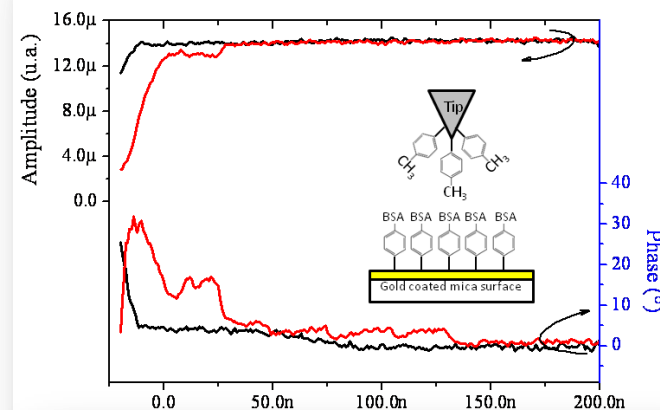
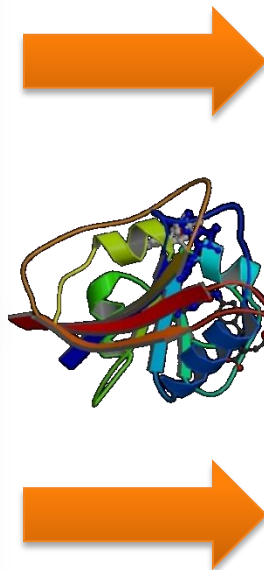
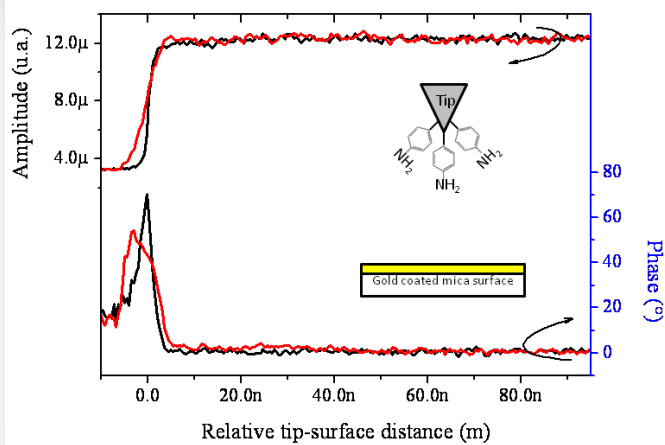
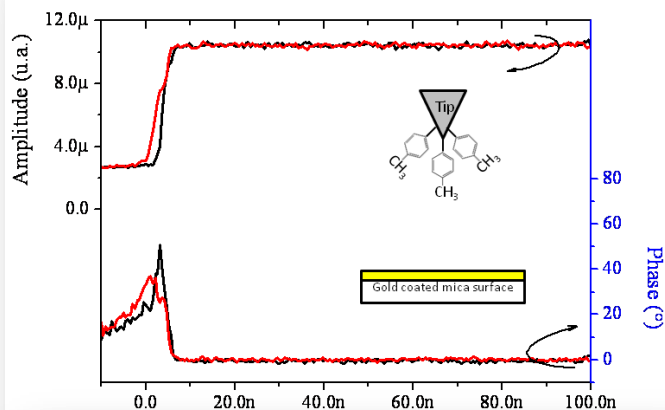


## Pointe en graphite (3 fonctionnalisations différentes):

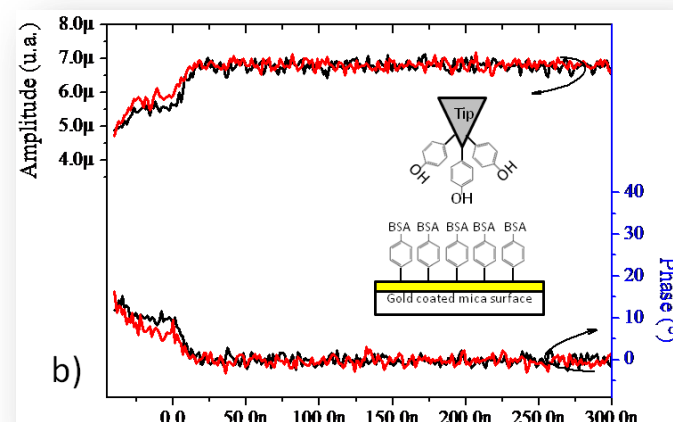
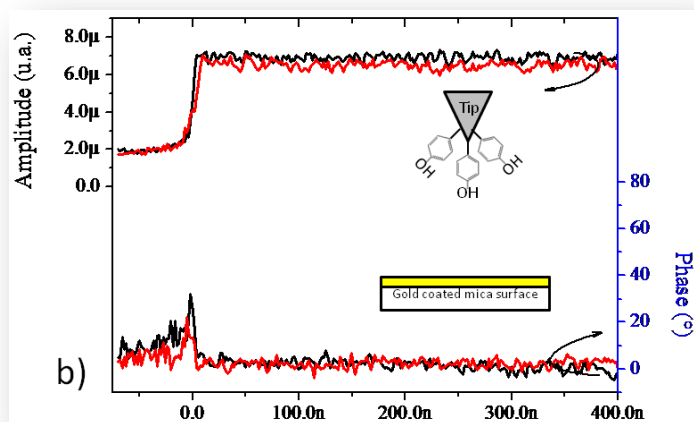


J. Polesel-Maris et al., [Sensors and Actuators B: Chemical 161\(1\), 775-783 \(2012\)](#)

## Spectroscopie AFM



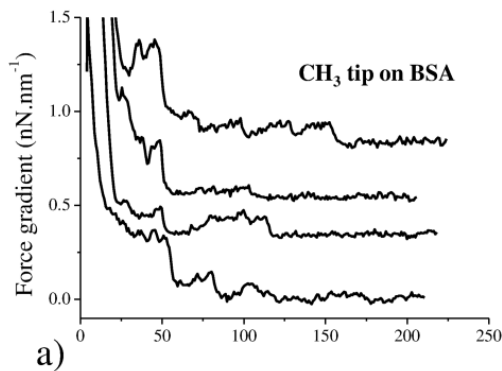
## Spectroscopie AFM



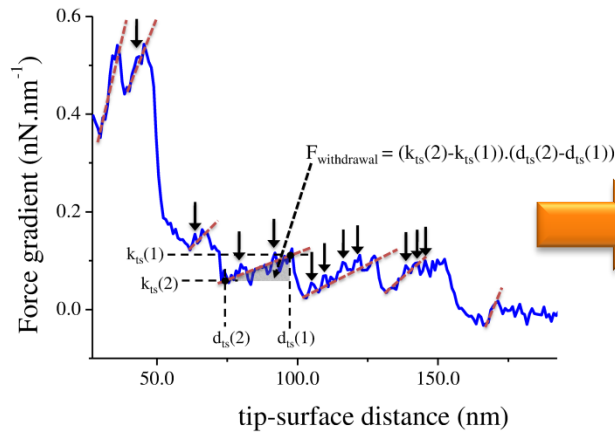
## AFM *Amplitude* and *Phase* curves



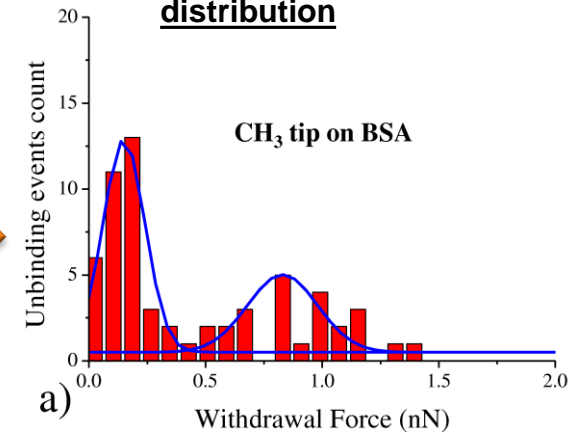
### Proteins stretching



### Data treatment



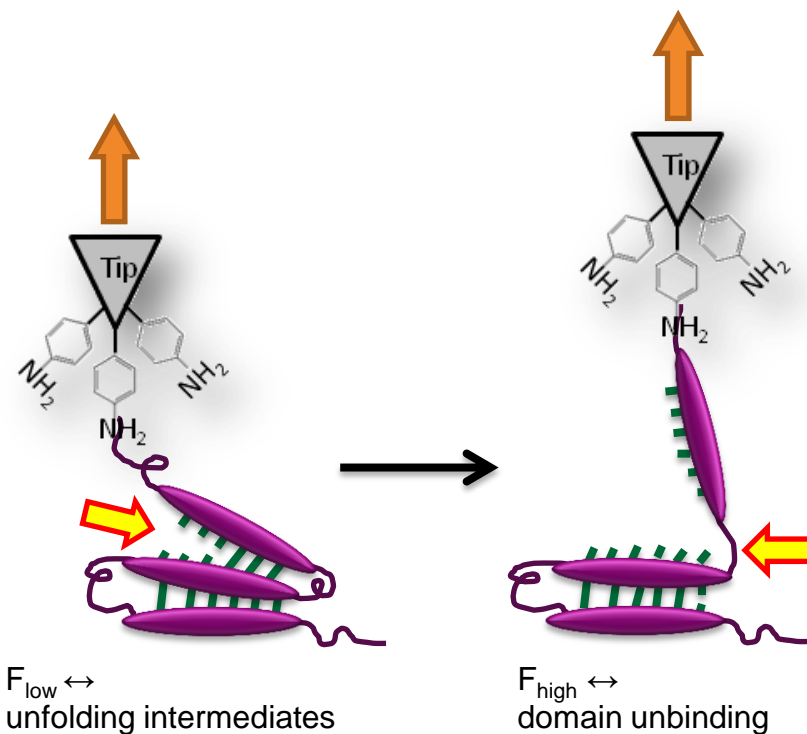
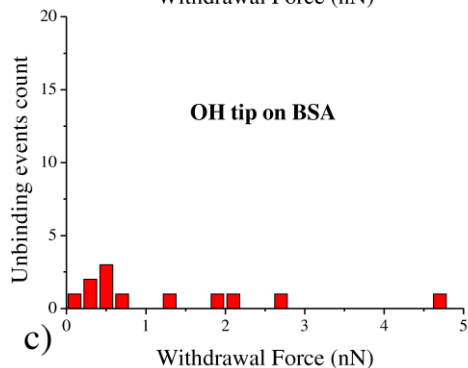
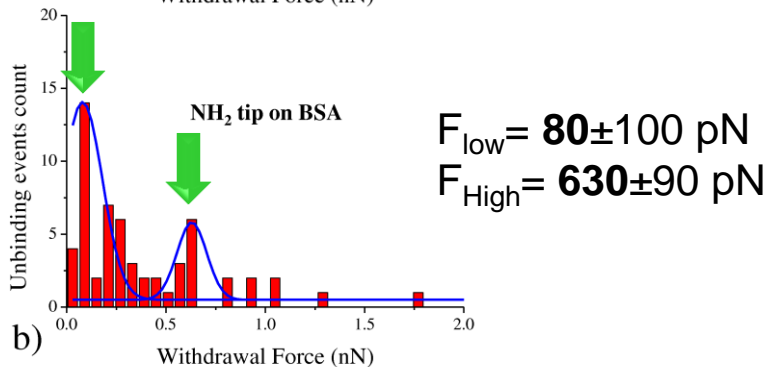
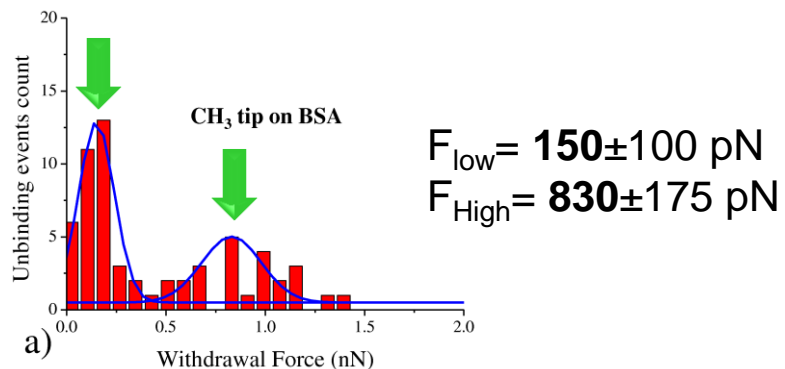
### Unbinding forces distribution



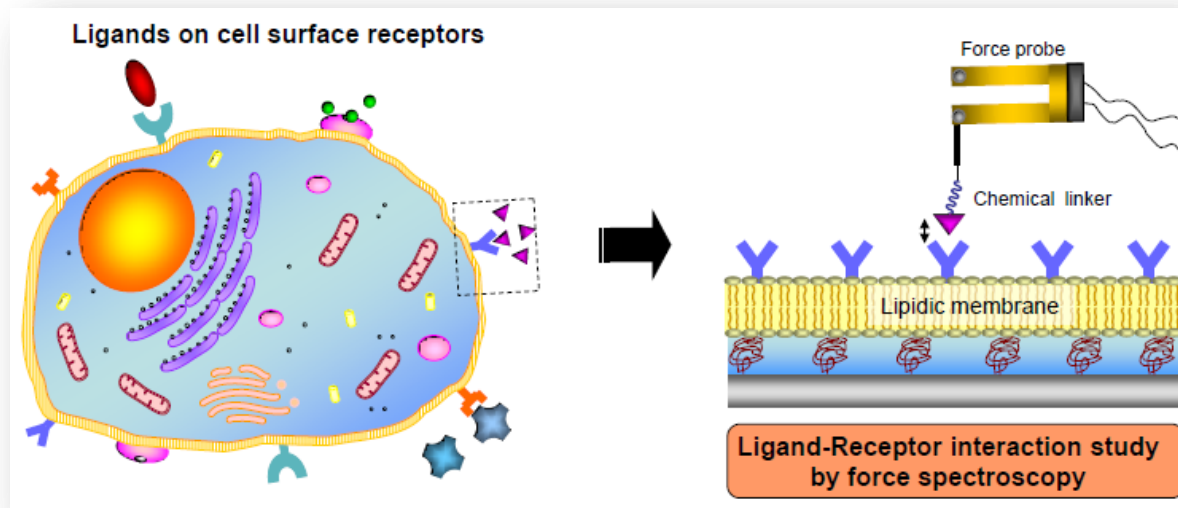
$$F_{ts} = 2.k_{eff} \cdot \int_d^{\infty} \left( \left( 1 + \frac{a^{1/2}}{8\sqrt{\pi(u-d)}} \right) \Omega(u) - \frac{a^{3/2}}{\sqrt{2(u-d)}} \frac{d\Omega(u)}{du} \right) du$$

**Sensibilité ~ 100 picoNewtons**

# Mises en évidence de deux évènements distincts



- Information énergétique de l'interaction ligand-protéines
- Etudes sur modèles membranaires
- Etude de systèmes biologiques photosensibles



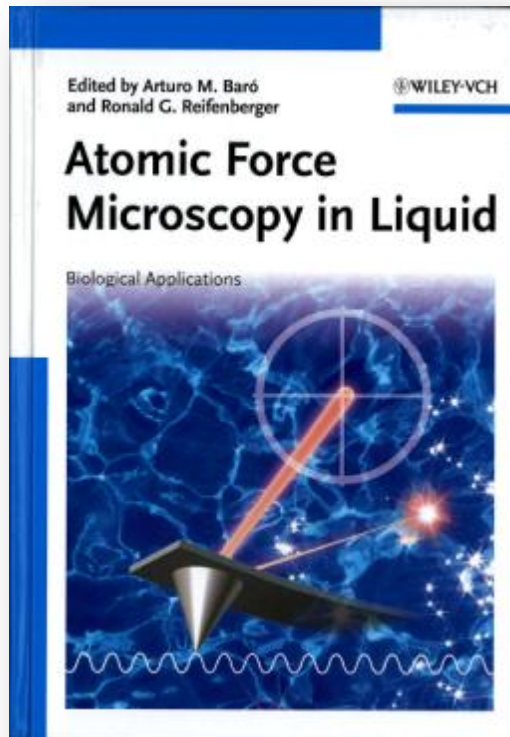
# Les sondes AFM piézoélectriques prometteuses ...

## 4. Dynamic-Mode AFM in liquid

*Takeshi Fukuma, Michael J. Higgins*



References | 117



Février 2012

There are several issues that are currently under investigation or expected to become important subjects in the near future. An example of such subjects is an understanding of atomic-scale interactions between a tip and surface (or water). While experimental techniques reached the level required for an atomic-resolution imaging in liquid, atomic-scale simulation of AFM in liquid has yet to be achieved. Such a study is particularly important for understanding the correlation between the water distribution and 3D force field obtained by 3D-SFM.

Another important subject is an improvement of the force sensor. There are two approaches that have been considered. One of them is to reduce the size of the cantilever, which enhances  $f_0$  without changing  $k$  and  $Q$ . Another approach is to use a self-sensing quartz sensor, which enhances both  $Q$  and  $k$ , while  $f_0$  is determined by the size of the sensor. Although both sensors seem to be very promising, their applicability and performance are yet to be experimentally verified.

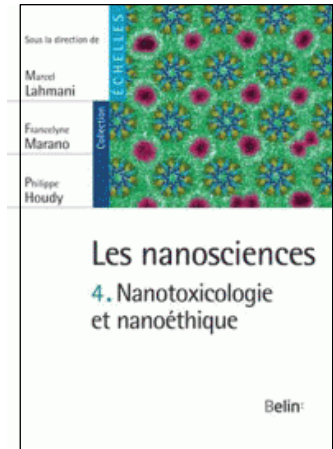
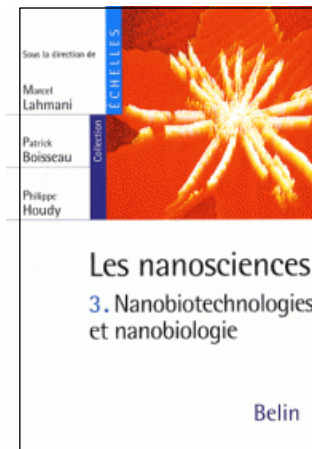
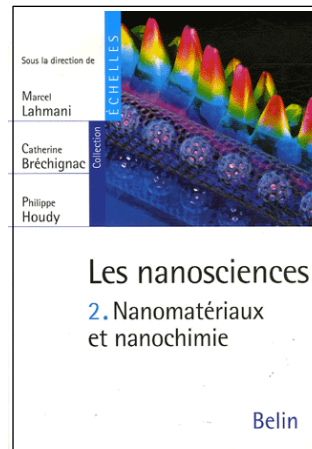
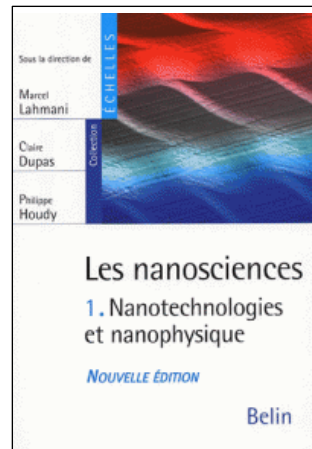
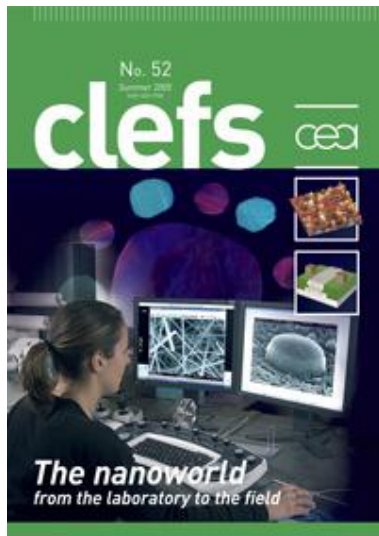
The major application areas of liquid-environment dynamic-mode AFM include biology and electrochemistry. So far, biological applications have been intensively explored, which has been driven by the rapid growth of the nanobioscience field. On the contrary, applications in electrochemistry are still in their infancy. However, considering the increasing interests in the so-called green science (e.g., solar power and water splitting), we expect that its applications to the studies on catalysis and batteries will rapidly increase in the near future.



# Pour aller plus loin

## Journal Clefs CEA numéro 52 "Nanomonde":

[http://www.cea.fr/technologies/le\\_nanomonde\\_de\\_la\\_sciences\\_aux\\_applications](http://www.cea.fr/technologies/le_nanomonde_de_la_sciences_aux_applications)



nanotechnology nanobiotechnology

- Les sondes locales: **énorme** polyvalence d'applications
- Beaucoup de curiosité, un peu d'ingéniosité, un soupçon d'instrumentation, un zeste de traitement du signal permettent d'élaborer des systèmes originaux à sondes locales !

## **Sujets de stage au CEA :**

<http://iramis.cea.fr/Pisp/123/jerome.polesel.html>

## **Email:**

[jerome.polesel@cea.fr](mailto:jerome.polesel@cea.fr)

# MERCI DE VOTRE ATTENTION

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