Tópicos Avançados em Fluidos Complexos (PGF5286)

Microscopia de Força Atômica
Princípios, Aplicações e Derivações (não matemáticas)

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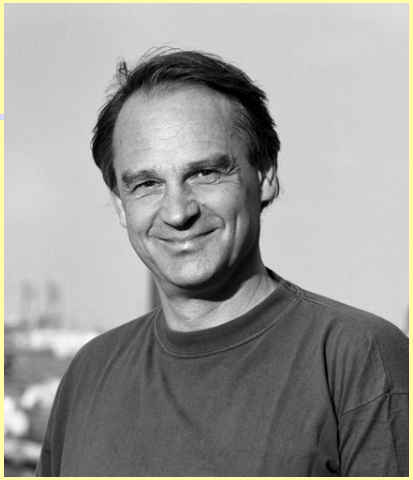
Instituto de Química – dfsp@usp.br

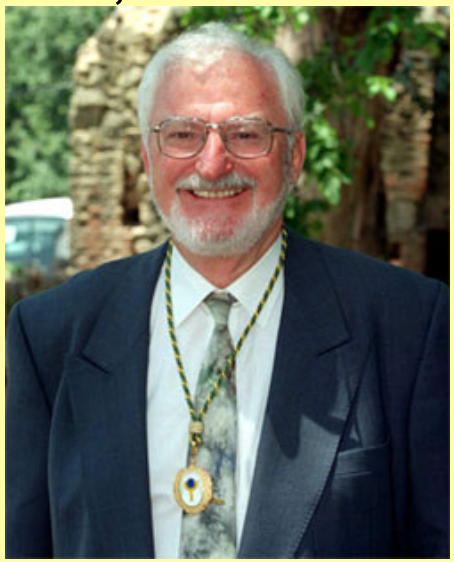
08/10/2015

Microscópio de varredura de tunelamento (STM), 1981: o equipamento permitiu medir a corrente de tunelamento entre uma ponta de tungstênio e uma <u>superfície condutora</u>

1986 – Prêmio Nobel de Física
Professor. **Ernst Ruska**, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin

Dr **Gerd Binnig** and Dr **Heinrich Rohrer**,
IBM Research Laboratory, Zurich, Switzerland,





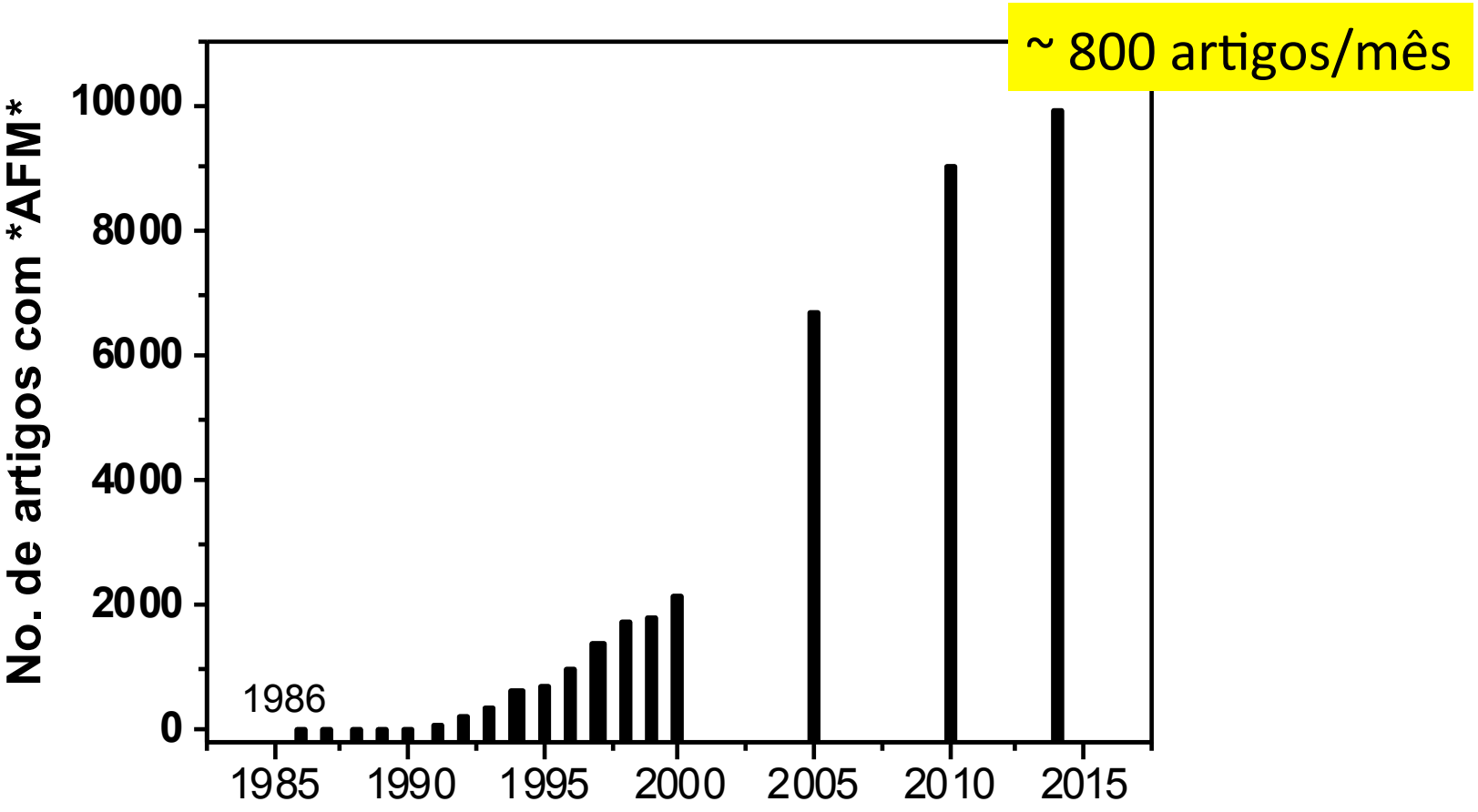
http://nobelprize.org/nobel_prizes/physics/laureates/1986/binnig-lecture.pdf

STM → corrente de tunelamento
AFM → forças de interação entre ponta e amostra

Microscopia eletrônica de varredura mostra vales e topos, mas não quantifica e ainda tem que recobrir com camada condutora
Microscopia confocal, depende muito da amostra e difícil de quantificar

AFM surgiu como uma ferramenta para quantificar a topografia na amostra "in natura"

****Materiais não condutores (polímeros, proteínas, partículas virais, bactérias, DNA)**


~ 800 artigos/mês
No. de artigos com *AFM*
10000
8000
6000
4000
2000
0
1986
1985
1990
1995
2000
2005
2010
2015

Tipo de interação	Potencial x Distância	Energia típica (kJ/mol)
Íon – íon	$\frac{(ze)_1\,(ze)_2}{4\pi\varepsilon_0\,x}$	250
Íon-dipolo permanente	$\frac{(ze)_1\mu_2\cos\theta}{4\pi\varepsilon_0\,x^2}$	15
Dipolo perman. - dipolo permanente (Keesom)	$-\frac{\mu_1^{\,2}\,\mu_2^{\,2}}{3(4\pi\varepsilon_0)^2 k_B\,T\,x^6}$	2
Dipolo perman. - dipolo induzido (Debye)	$-\frac{(\alpha_{0\,1}\mu_2^{\,2}+\alpha_{0\,2}\mu_1^{\,2})}{(4\pi\varepsilon_0)^2 x^6}$	2
Dipolo induzido -dipolo induzido (London)	$-\frac{3h}{2}\,\frac{\nu_1\nu_2}{(\nu_1+\nu_2)}\,\frac{(\alpha_{0\,1}\alpha_{0\,2})}{(4\pi\varepsilon_0)^2 x^6}$	1 a 2

Forças de van der Waals

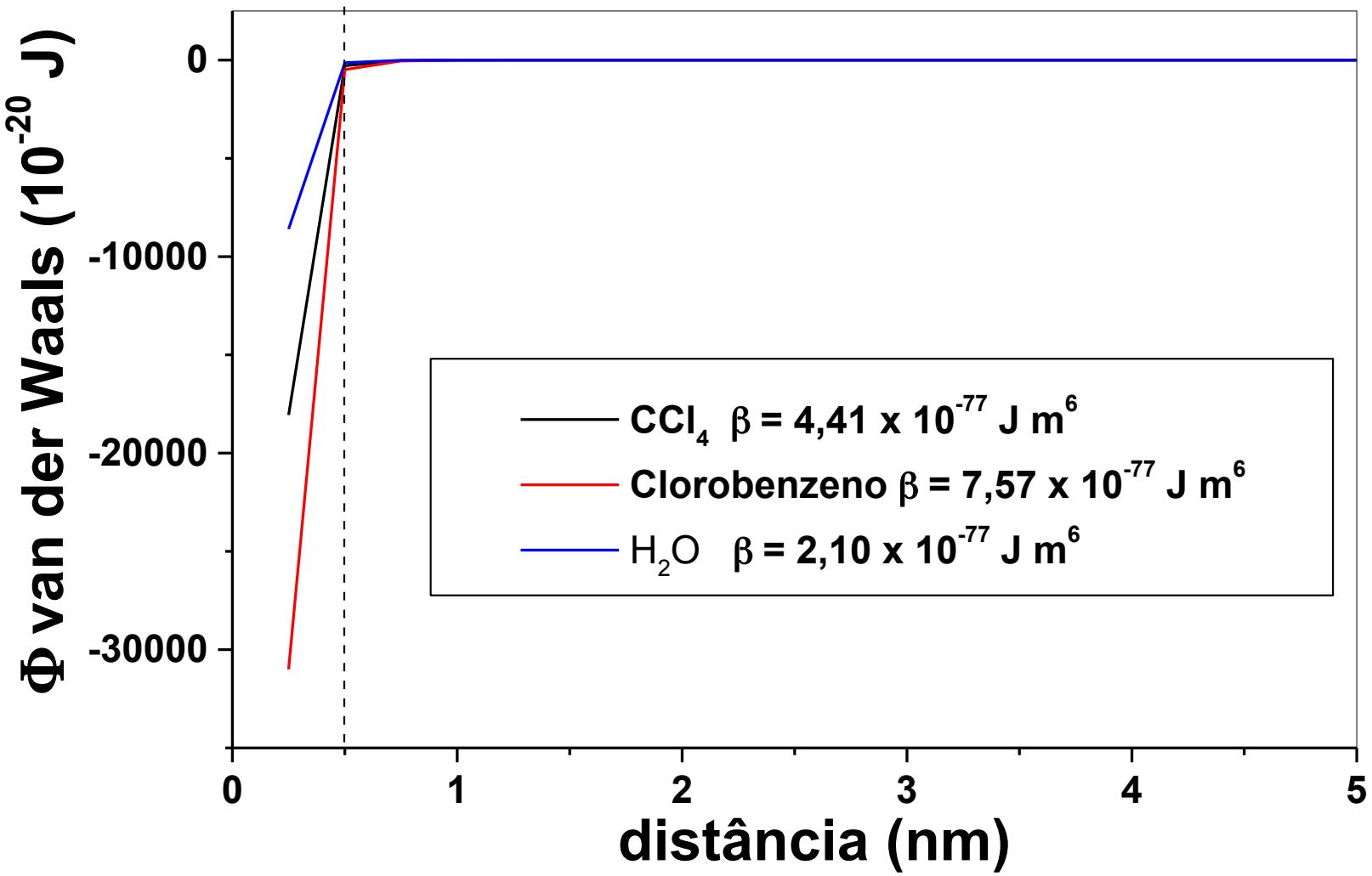
Ligações de H: ~20 kJ/mol

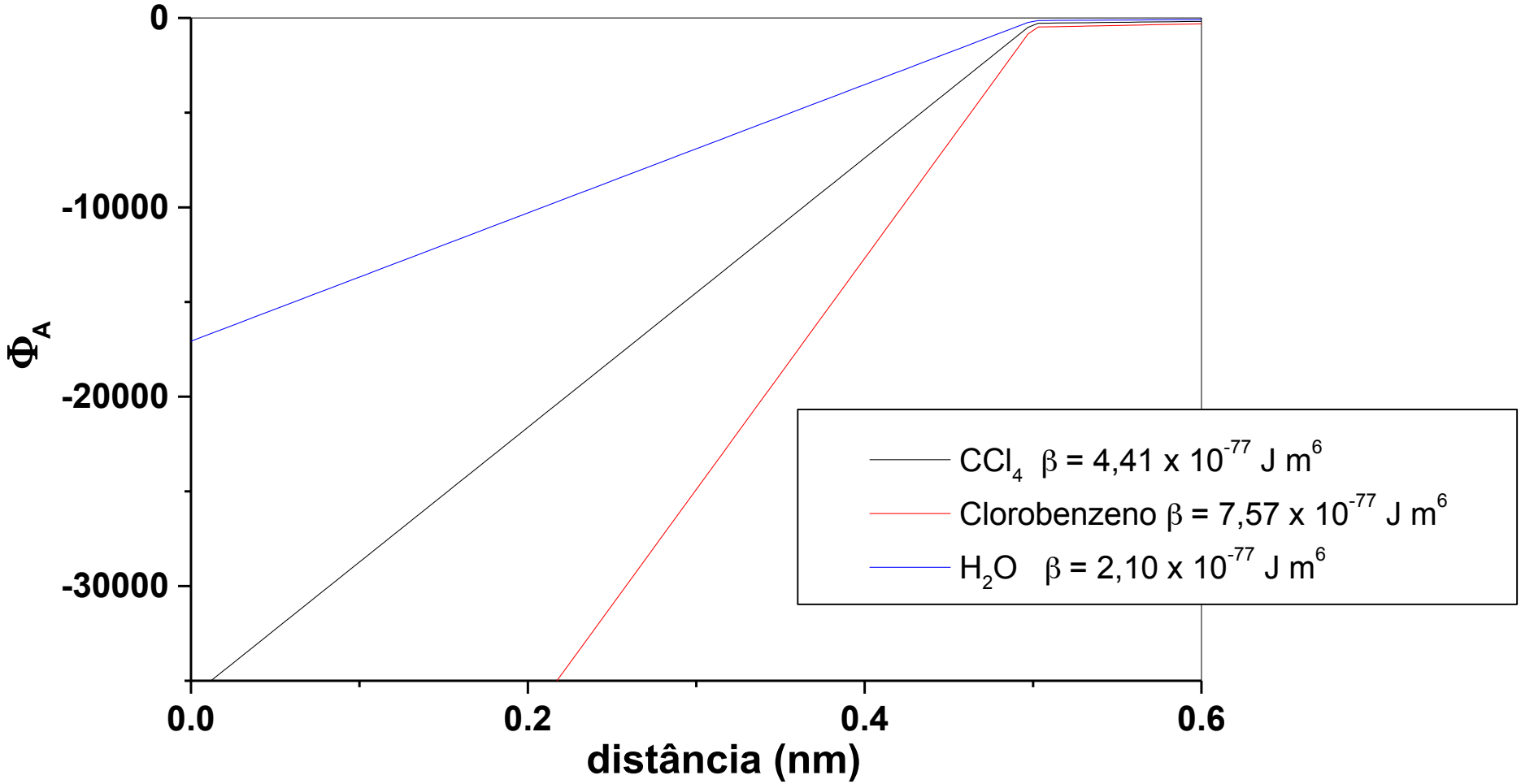
Para calcular como varia o potencial de atração de van der Waals (Φ_A) com a distância entre 2 moléculas iguais em função da distância (x):

$$\Phi_A = -\frac{1}{(4\pi\varepsilon_0)^2}\left(2\alpha_{0,1}\mu_1{}^2 + \frac{2\mu_1{}^4}{3k_BT} + \frac{3}{4}h\nu_1\alpha_{0,1}{}^2\right)x^{-6} = -\beta_{11}x^{-6}$$

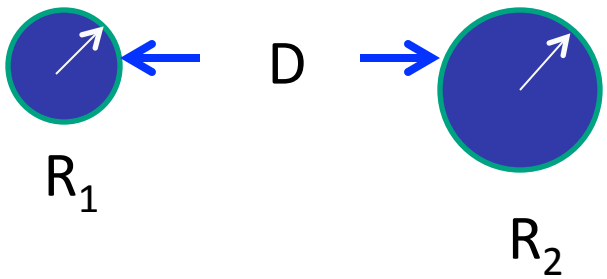
$$\downarrow$$

$$\beta_{11}$$

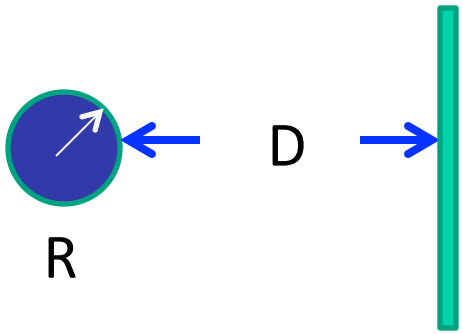

Φ van der Waals (10^{-20} J)
0
-10000
-20000
-30000
0
1
2
3
4
5
distância (nm)
CCl_4 β = 4,41 x 10^{-77} J m^6
Clorobenzeno β = 7,57 x 10^{-77} J m^6
H_2O β = 2,10 x 10^{-77} J m^6


0
-10000
-20000
-30000
Φ_A
0.0
0.2
0.4
0.6
distância (nm)
CCl_4 β = 4,41 x 10^{-77} J m^6
Clorobenzeno β = 7,57 x 10^{-77} J m^6
H_2O β = 2,10 x 10^{-77} J m^6

Forças de van der Waals entre 2 sólidos macroscópicos



$$F = -\frac{A_H}{6D^2} \cdot \frac{R_1 R_2}{R_1 + R_2}$$



$$F = -\frac{A_H R}{6D^2}$$

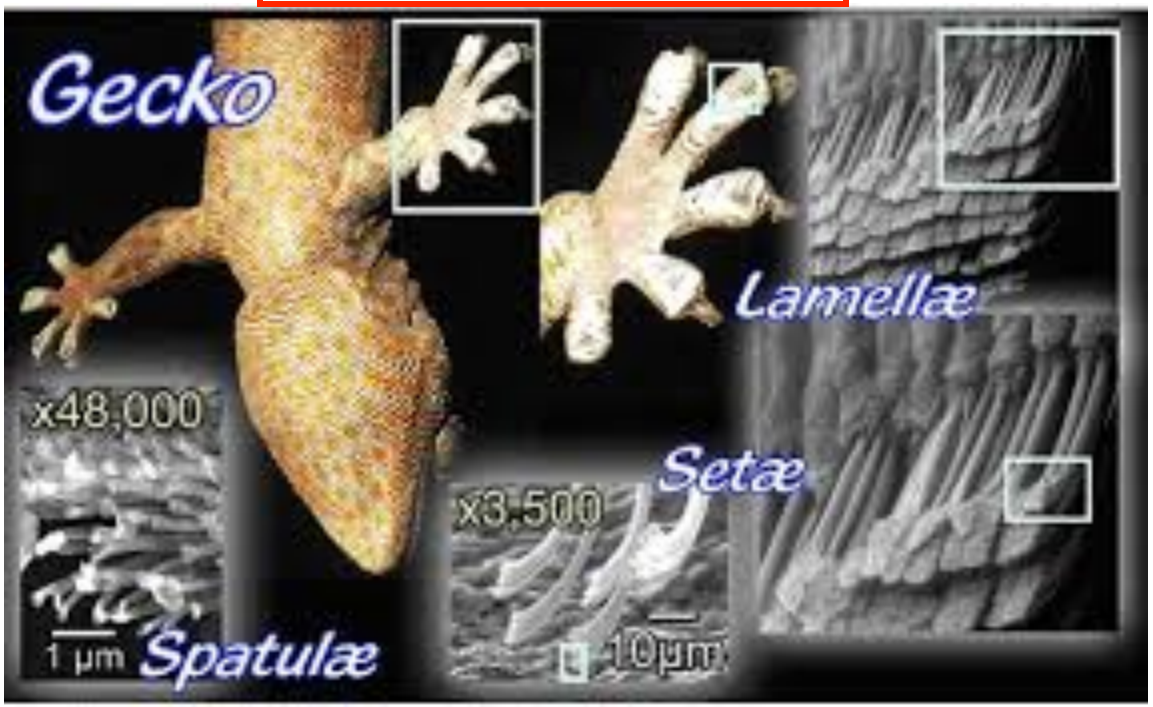
Constante de Hamaker

$$A_H = \pi^2 \beta_{12} \rho_1 \rho_2$$

$\sim 10^{-20}$ J

As forças de van der Waals existem!

Hierarquia estrutural



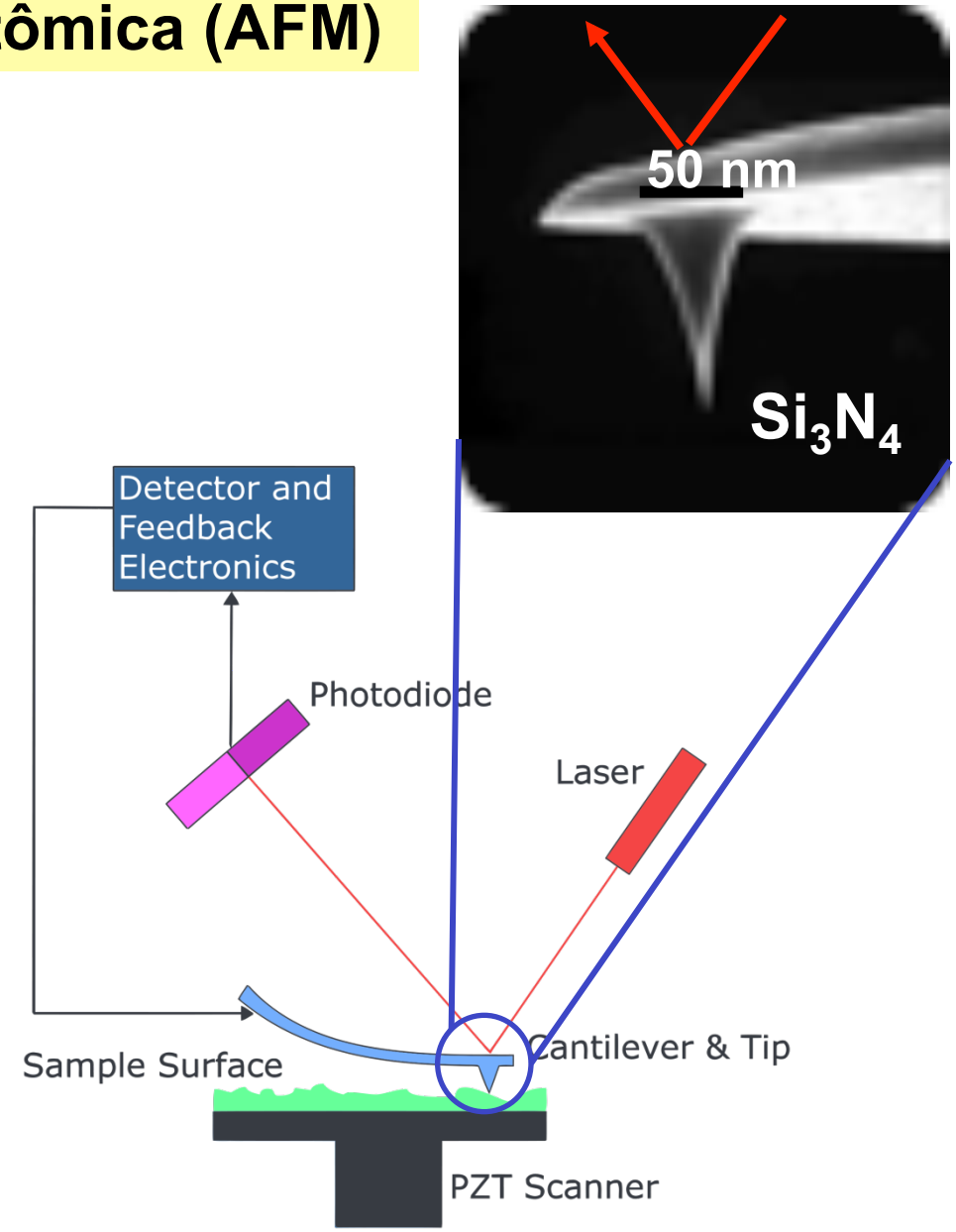
The adhesion force generated by a single setae is 40µN or more so that each spatula can exert 10nN of adhesive force

Each setae
110 µm in length
and 4.2 µm in diameter

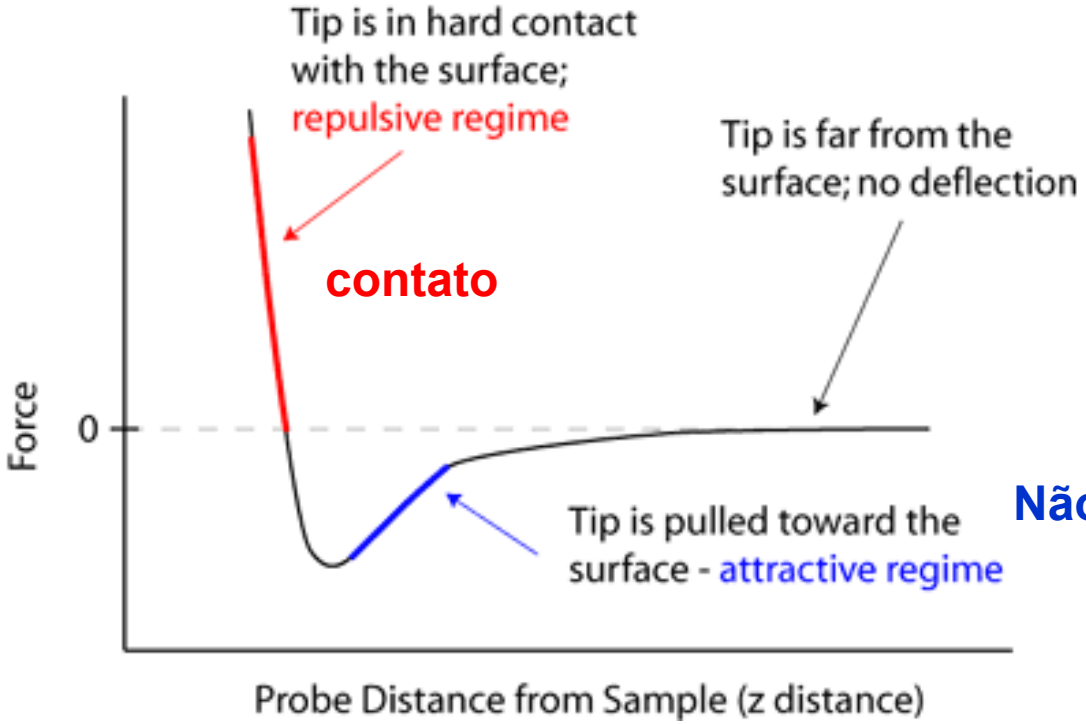
10^6 spatulae
each spatula ~200 nm diameter

Image: K. Autumn from http://www.oregon15.org/15sr02a.html

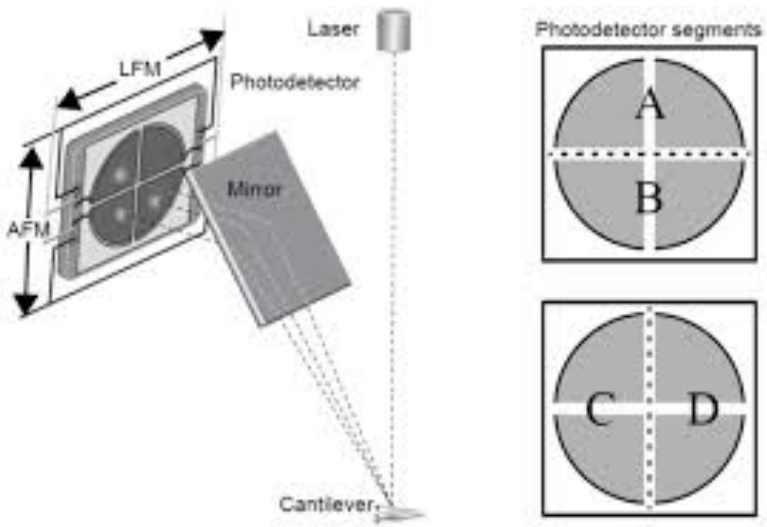
Microscopia de força atômica (AFM)

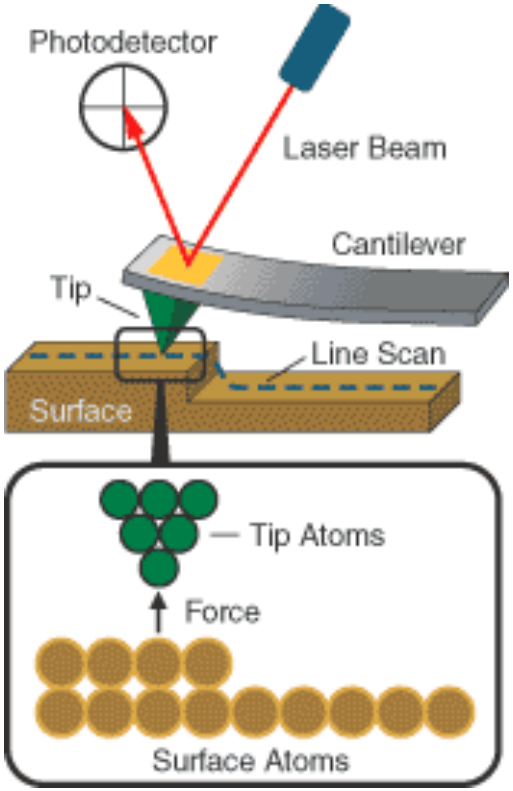


Binning, Quate, Gerber *Phys Rev Lett* 1986, 56, 930
Hermann et al. Polímeros: Ciência e Tecnologia - Out/Dez – 97, pag. 51-61

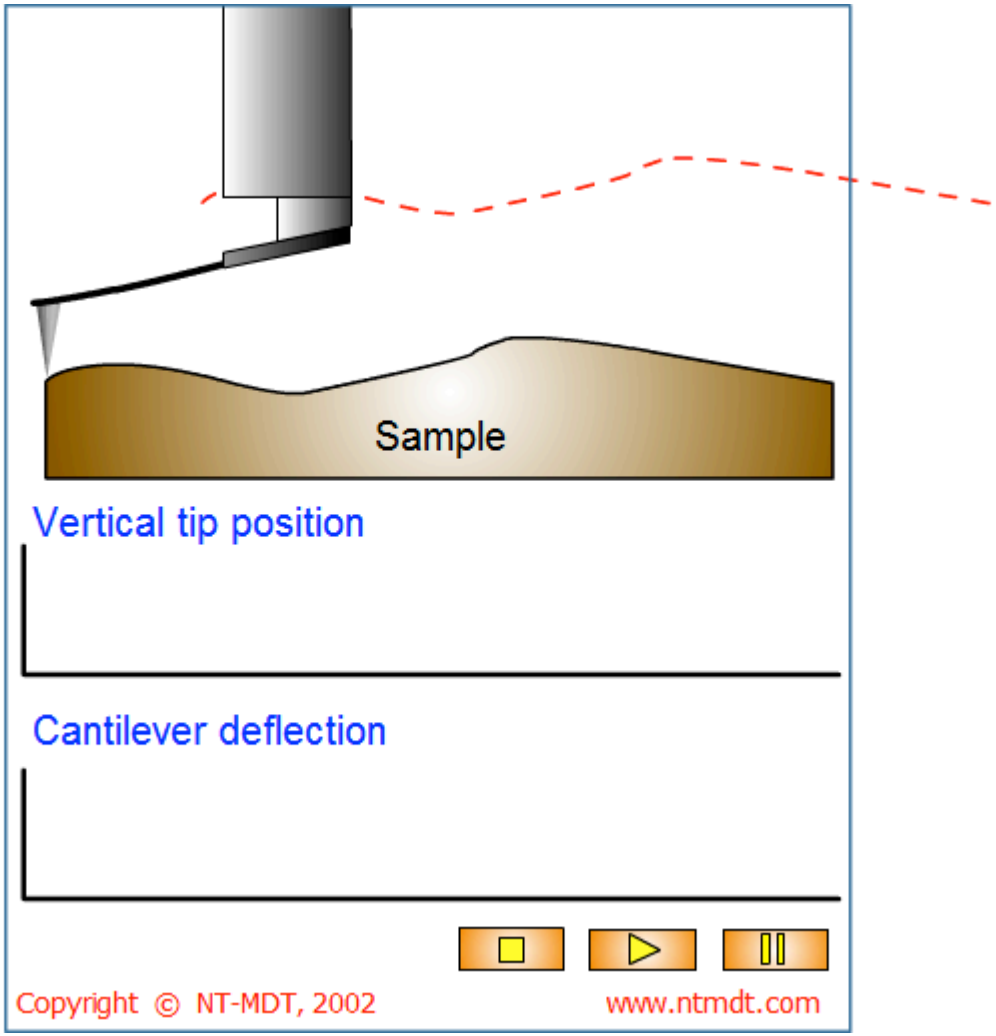

Tip is in hard contact with the surface; repulsive regime
Tip is far from the surface; no deflection
contato
Force
0
Tip is pulled toward the surface - attractive regime
Probe Distance from Sample (z distance)

Não-contato

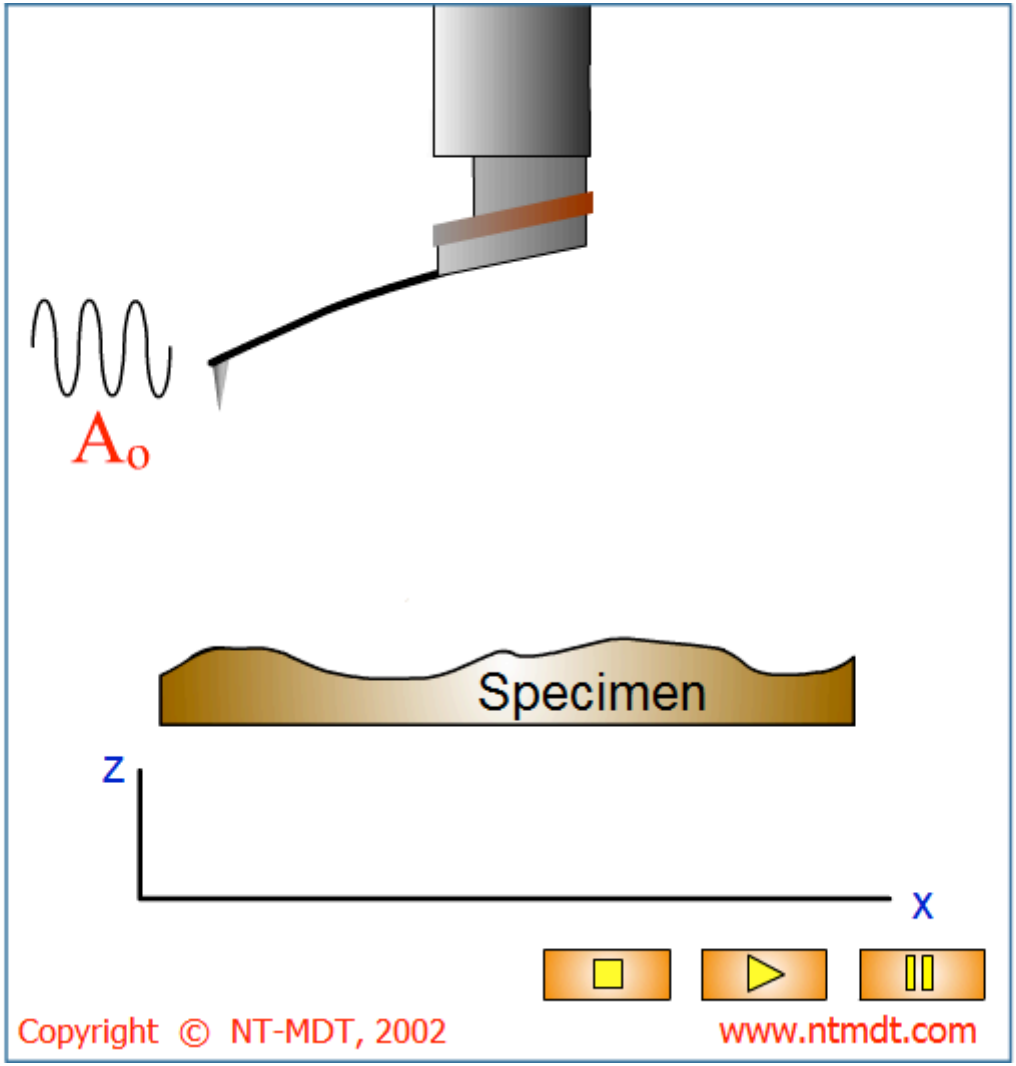

Laser
LFM
Photodetector
Mirror
AFM
Cantilever
Photodetector segments
A
B
C
D


Photodetector
Laser Beam
Cantilever
Tip
Line Scan
Surface
Tip Atoms
Force
Surface Atoms

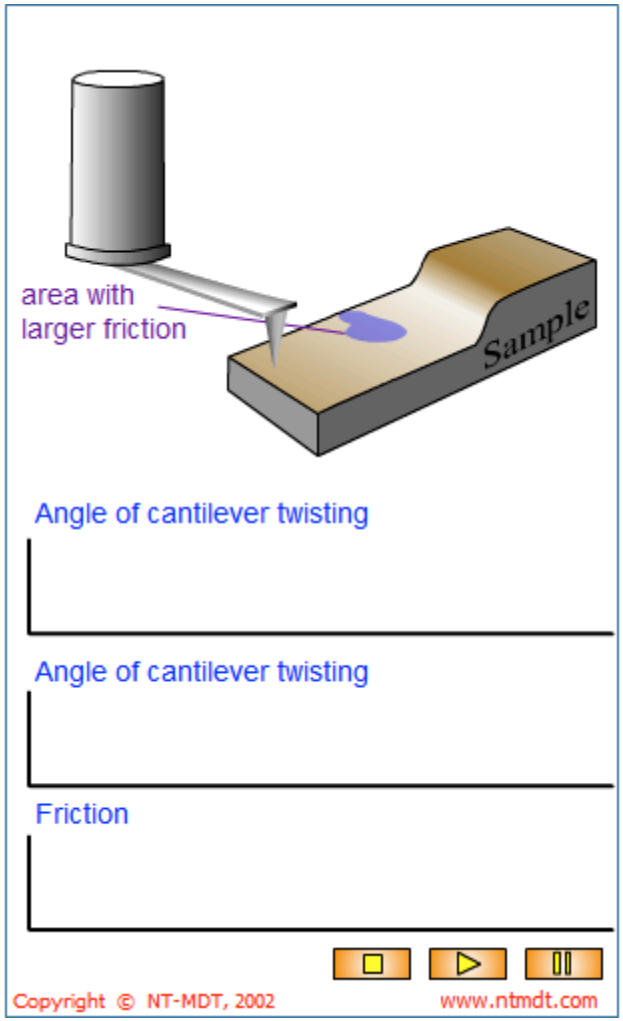
Modo Contato – Força Constante



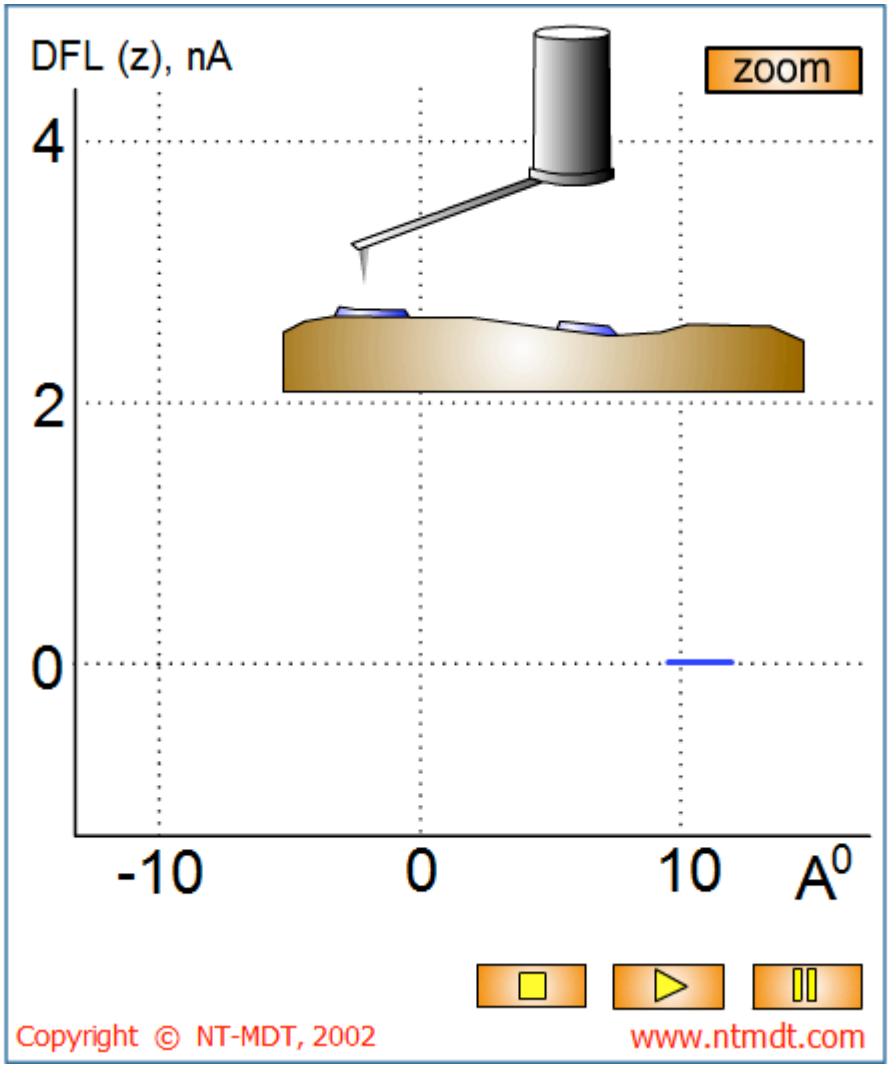
Modo Contato Intermitente – Topografia



Modo Contato – Lateral Force



Forças de Adesão



FEBS 17259

FEBS Letters 390 (1996) 161–164

In situ observation of streptavidin-biotin binding on an immunoassay well surface using an atomic force microscope

S. Allen[a,*], J. Davies[b], A.C. Dawkes[b], M.C. Davies[a], J.C. Edwards[b], M.C. Parker[a], C.J. Roberts[a], J. Sefton[b], S.J.B. Tendler[a], P.M. Williams[a]

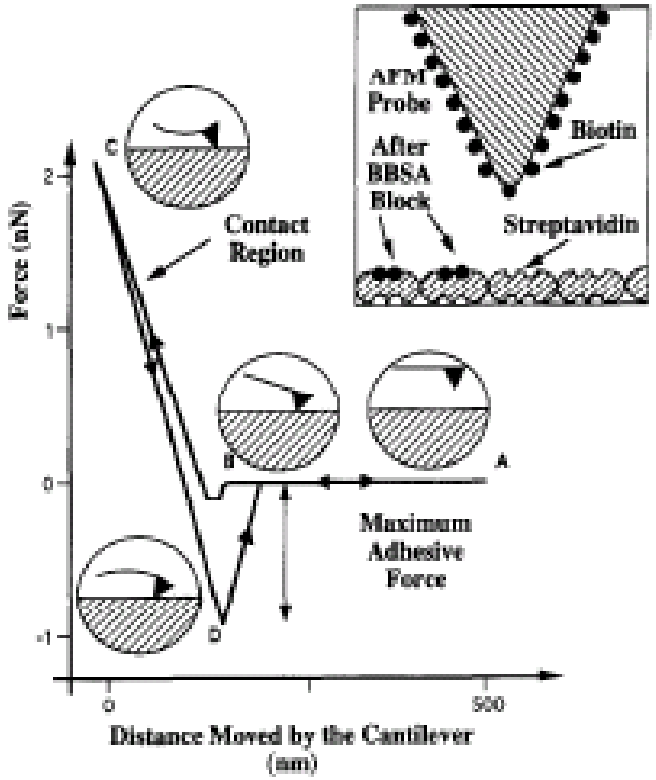
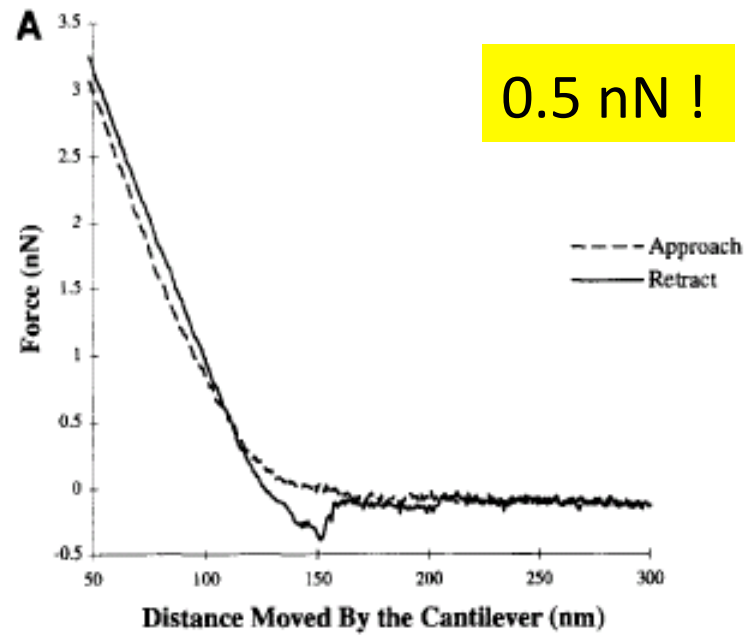
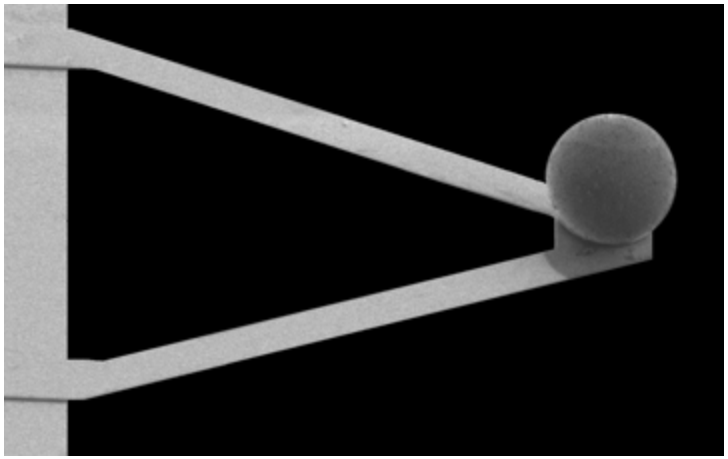


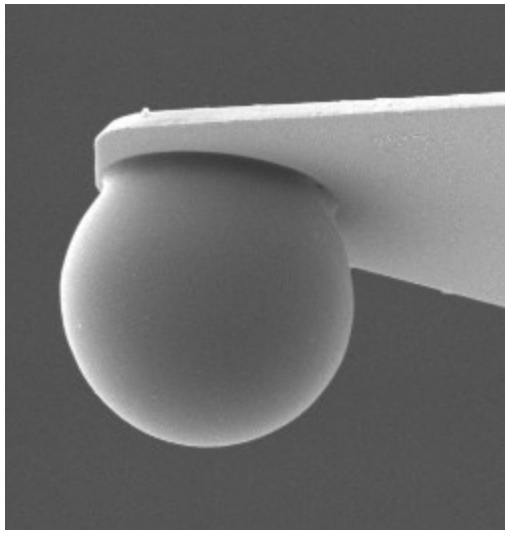
Fig. 1. (Main diagram) A schematic diagram of a typical force measurement cycle. The relative position of the AFM probe to the

S. Allen et al./FEBS Letters 390 (1996) 161–164



AFM - Colloidal probe technique for adhesion forces



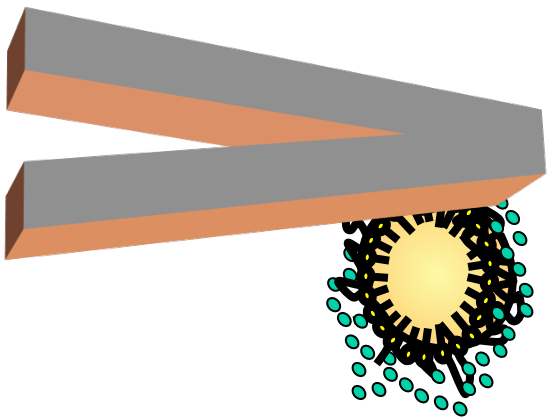


Micromanipulador para colar a esfera na extremidade do cantilever
Medidas feitas no fluido

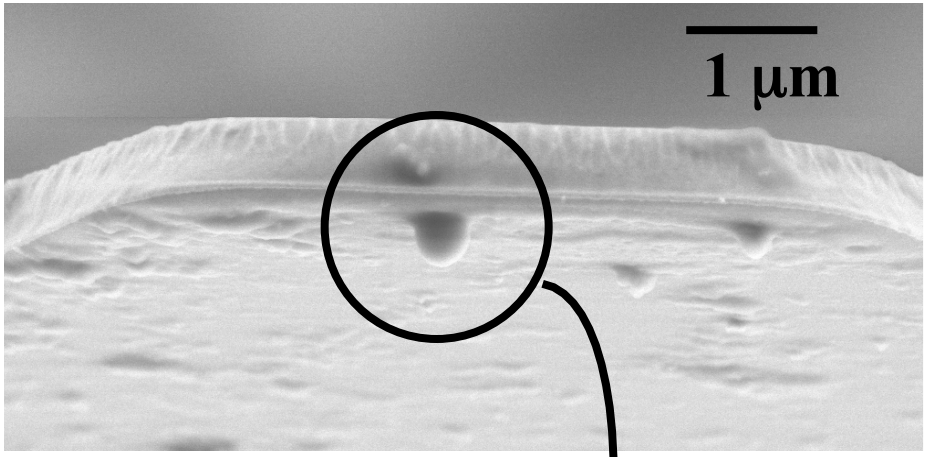
Kappl, M. and Butt, H.-J. (2002), The Colloidal Probe Technique and its Application to Adhesion Force Measurements. *Part. Part. Syst. Charact.*, **19**: 129–143.

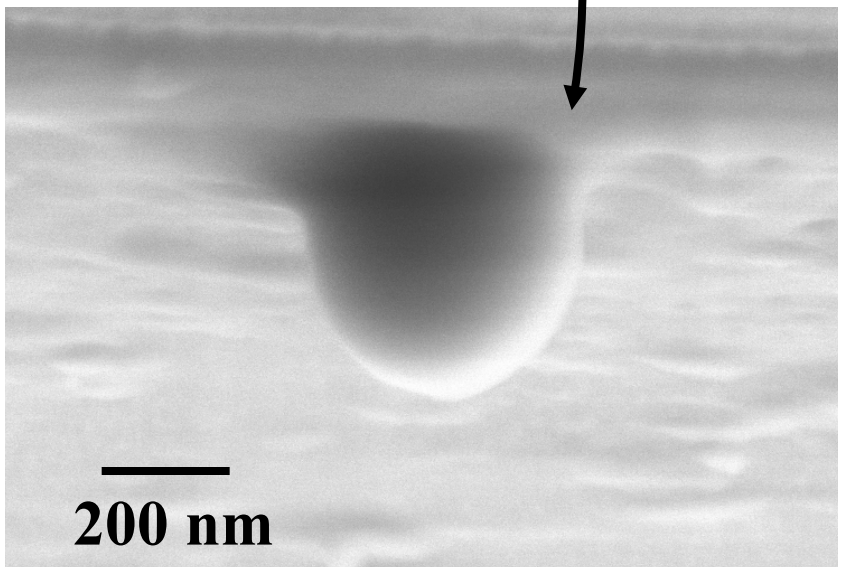
H. J. Butt, B. Cappella, M. Kappl (2005). *Surf. Sci. Rep*. **59**: 1-152

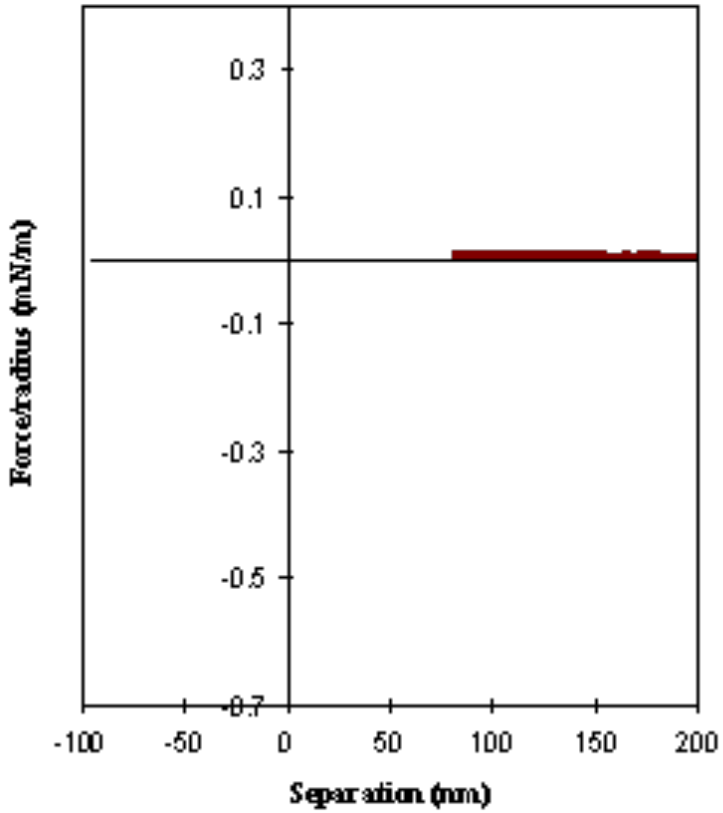
AFM - Colloidal probe technique for adhesion forces

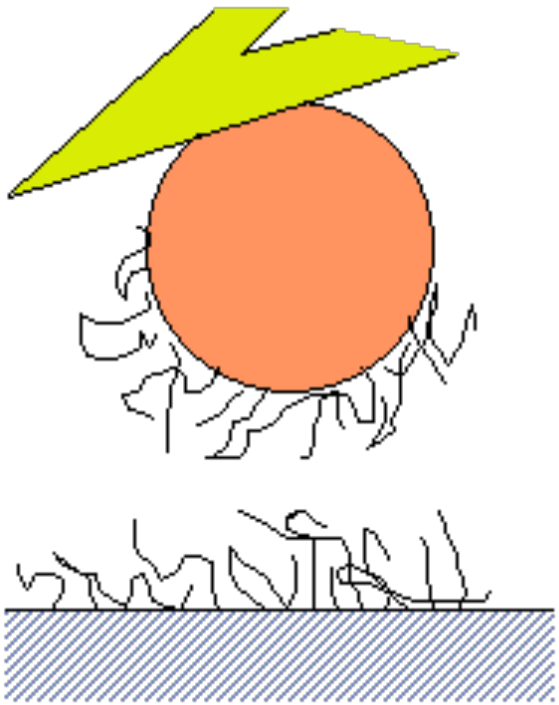


Core = poli(metacrilato de metila), PMMA
Shell = carboximetil celulose, CMC = polianion






0.3
0.1
-0.1
-0.3
-0.5
-0.7
-100
-50
0
50
100
150
200
Force/radius (mN/m)
Separation (nm)



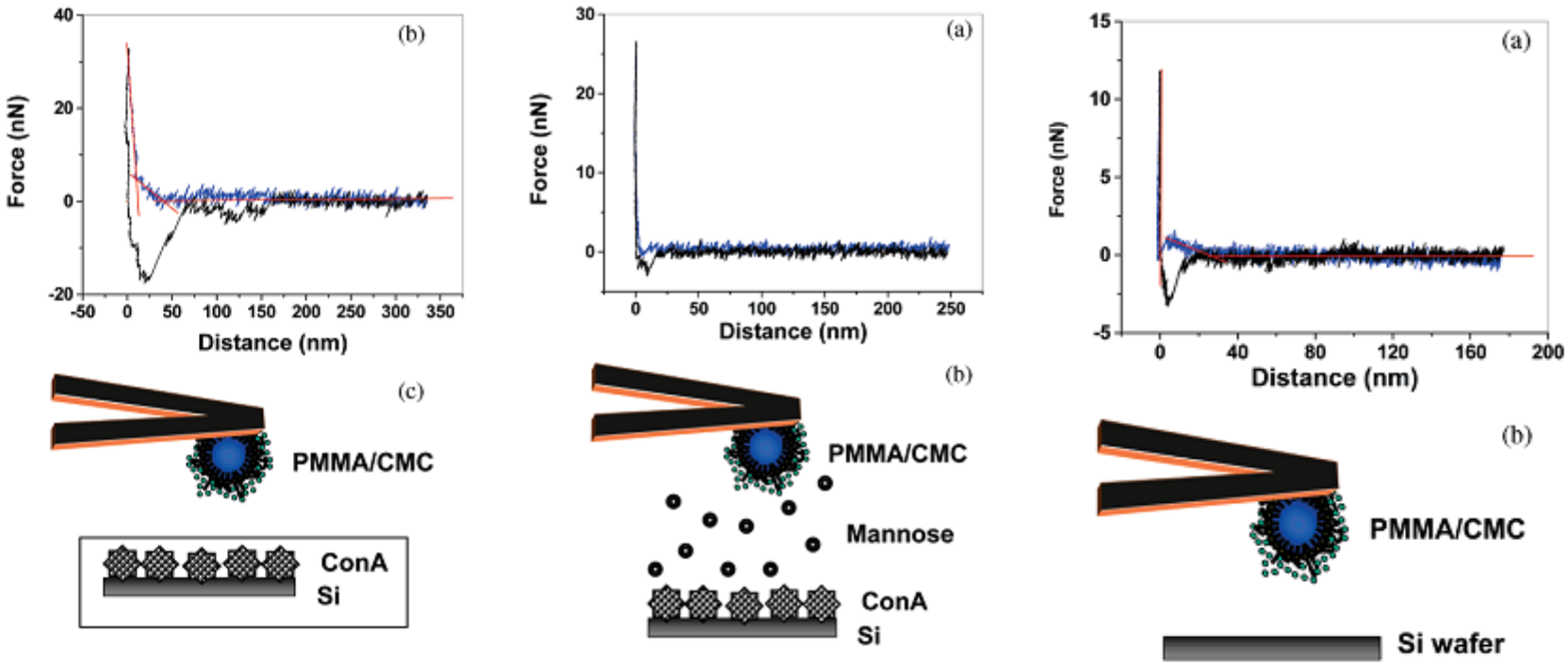
Langmuir **2006**, *22*, 3757−3762

Adhesion Forces between Hybrid Colloidal Particles and Concanavalin A

Lizandra B. R. Castro,† Michael Kappl,‡ and Denise F. S. Petri*,†

Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes 748, 05508-900 São Paulo, Brazil, and Max Planck Insitut for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

Concanavalina A: glicoproteína específica para glicose e manose
Carboximetil celulose (CMC): rica em glicose

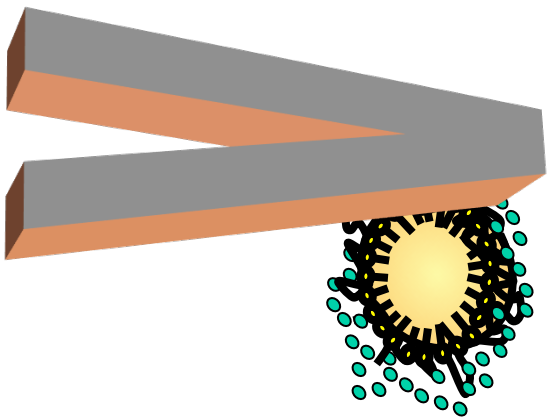


11 ± 7 nN
250 curvas

3 ± 1 nN
250 curvas

3 ± 1 nN
250 curvas

AFM - Colloidal probe technique to measure the thickness of polyelectrolyte layer



Core = poliestireno, PS
Shell = quitosana, polication

Qual a espessura da camada de quitosana sobre a esfera?
1)Em agua
2)Em NaCl 10 mM

Implicações sobre estabilidade coloidal

8520 *J. Phys. Chem. B* **2007**, *111*, 8520−8526

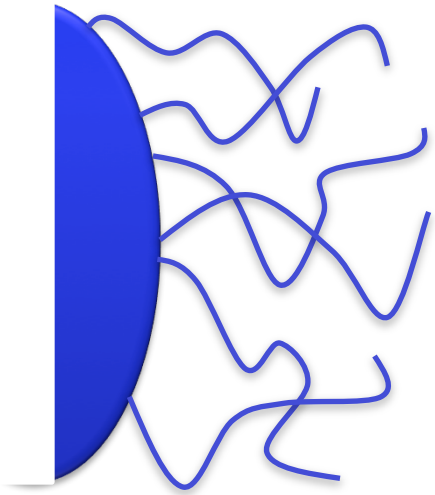
Immobilization of Hexokinase onto Chitosan Decorated Particles[†]

Lizandra B. R. Castro,[‡,§] Fabio F. Silva,[‡] Ana M. Carmona-Ribeiro,[‡] Michael Kappl,[‡] and Denise F. S. Petri*[,‡]

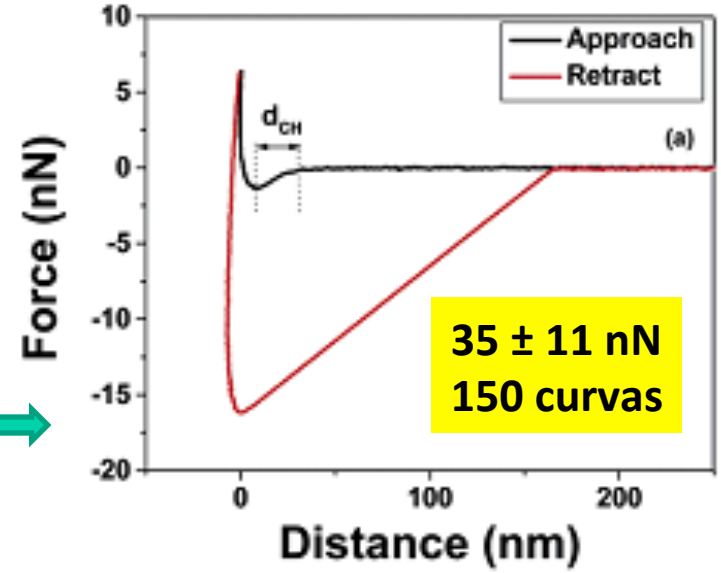
Instituto de Química, Universidade de São Paulo, P. O. Box 26077, São Paulo, SP, 05513-970, Brazil, and Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

Received: December 16, 2006; In Final Form: January 31, 2007

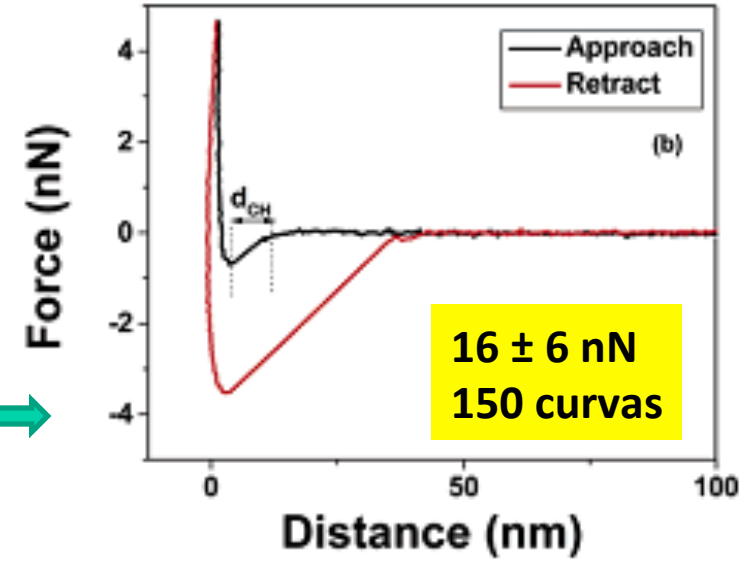
AFM - Colloidal probe technique to measure the thickness of polyelectrolyte layer



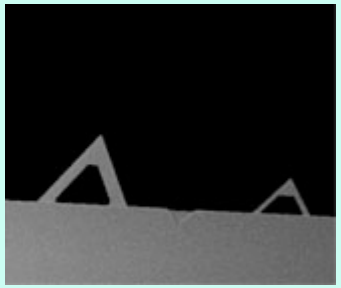
agua



NaCl 10 mM

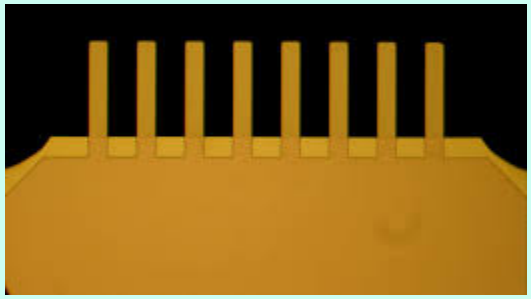


Tipos de cantileveres comuns



AFM contato
AFM não contato
AFM colloidal probe

AFM contato
intermitente



AFM colloidal probe
Sensores
nanomecânicos

Sergei Magonov
Digital Instruments,
Santa Barbara, USA
~ 1992

Problema com as superficies moles:
o modo contato danificava a estrutura

Solução do problema:
DI patenteia o "tapping mode"

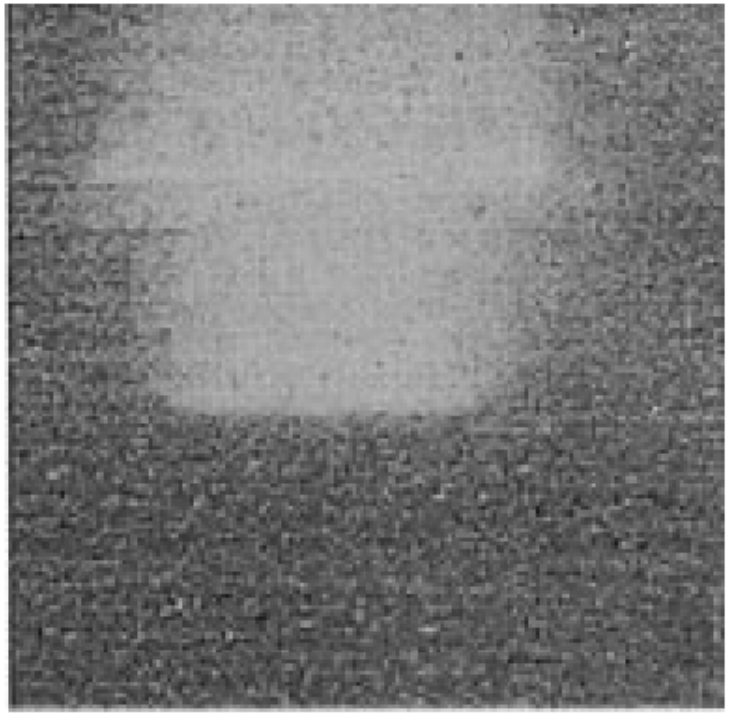


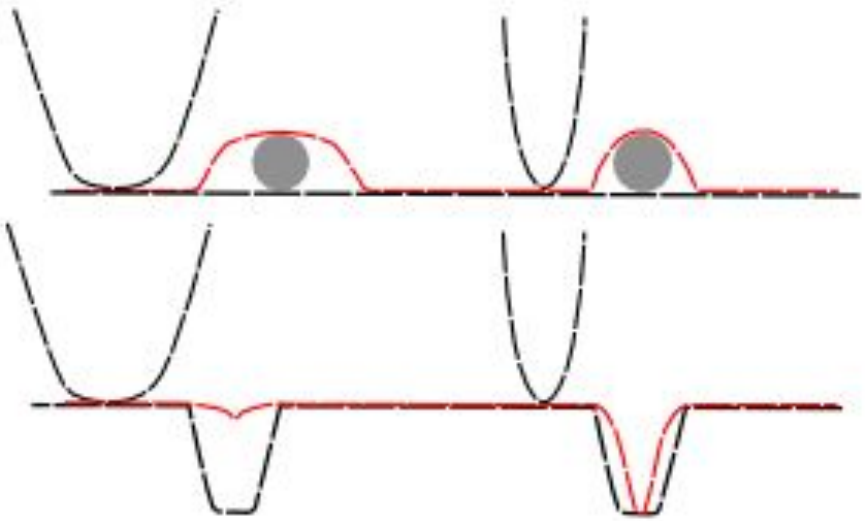
Figure 3. Frictional (LFM) image of freshly prepared APS monolayers on silicon wafer after five image scans.

Langmuir **1999**, *15*, 4520–4523

An Improved Method for the Assembly of Amino-Terminated Monolayers on SiO_2 and the Vapor Deposition of Gold Layers

Denise F. Siqueira Petri,*,†,‡ Gerhard Wenz,† Peter Schunk,§ and Thomas Schimmel§

Efeitos de convolução da ponta na largura dos objetos



<u>A altura ou profundidade são informações confiáveis, a largura não</u>

Para corrigir precisa conhecer o raio da ponta

Nobelpriset i kemi 2015
The Nobel Prize in Chemistry 2015
Nobelpriset i kemi 2015
KUNGL. VETENSKAPS AKADEMIEN
Tomas Lindahl
Francis Crick Institute and Clare Hall Laboratory, Hertfordshire, UK
Paul Modrich
Howard Hughes Medical Institute and Duke University School of Medicine, Durham, NC, USA
Aziz Sancar
University of North Carolina, Chapel Hill, NC, USA
"för mekanistiska studier av DNA-reparation"
"for mechanistic studies of DNA repair"
© Kungl. Vetenska

Dano e reparo de DNA

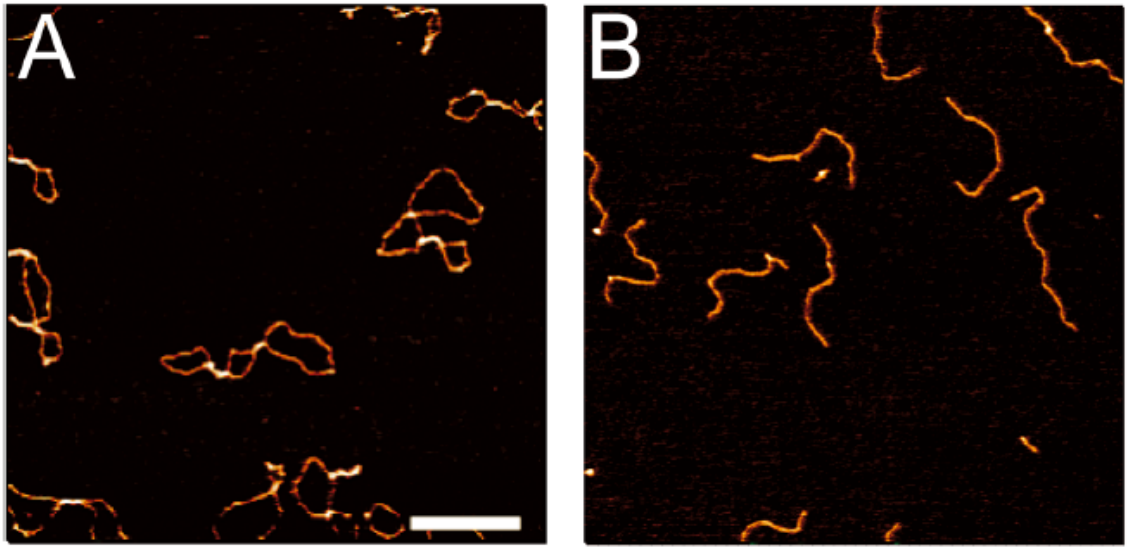
Review

Received: 17 June 2013, Revised: 14 August 2013, Accepted: 15 August 2013, Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jmr.2311

DNA substrate preparation for atomic force microscopy studies of protein–DNA interactions

Claudia N. Buechner and Ingrid Tessmer*



Plasmídeos de DNA circulares e lineares

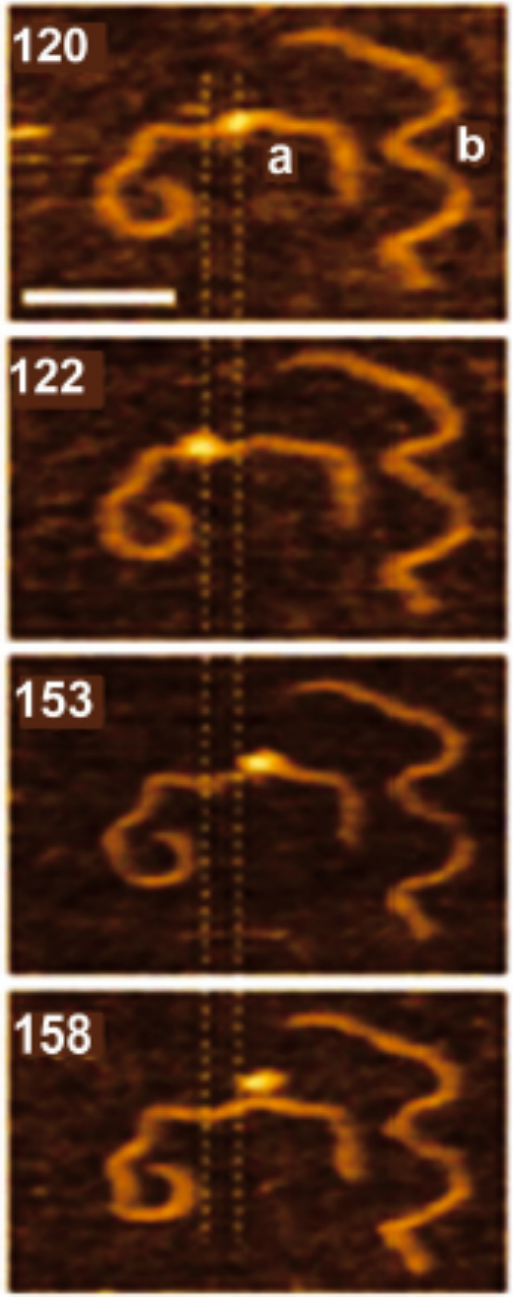
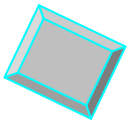


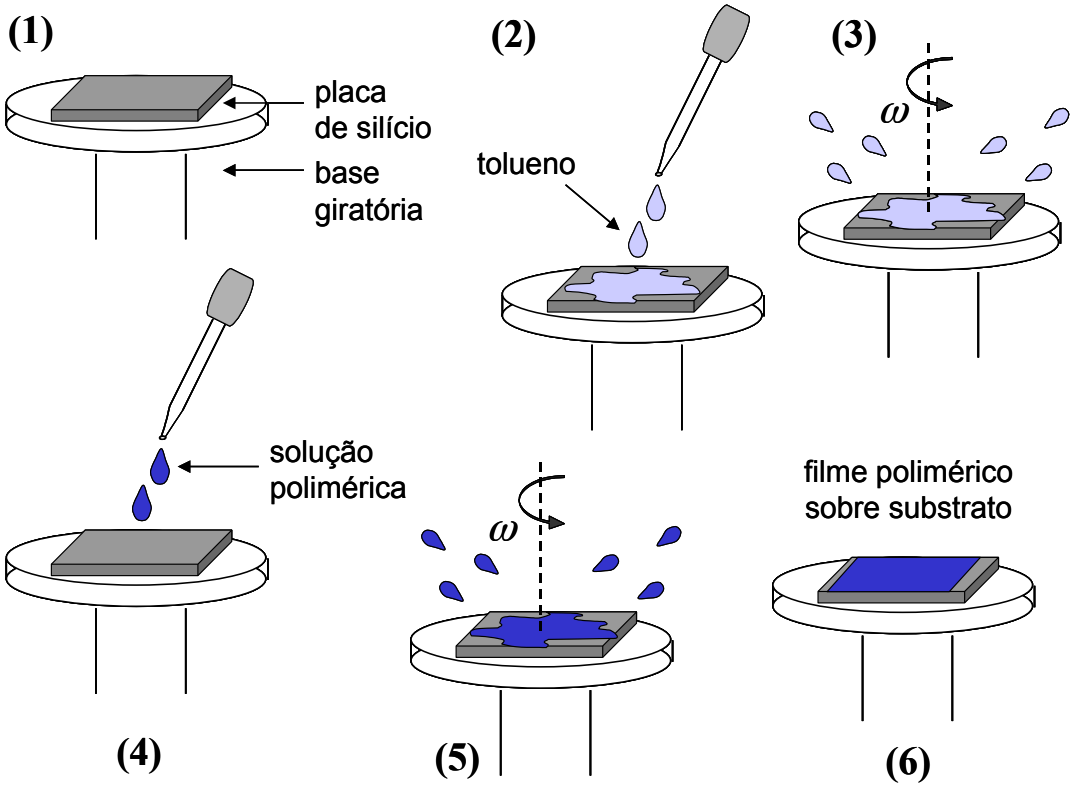
Figure 4. Atomic force microscopy time lapse series showing two DNA fragments, (a) and (b) and a single molecule of cytosine deaminase sliding on a ssDNA gap region within one of the dsDNA fragments (Shlyakhtenko *et al.*, 2012a). The location of the gap in the DNA is indicated by the dashed lines. The protein dissociates upon reaching the single stranded/double stranded DNA junction. The numbers in the images indicate the frame number of the time series, collected with a time resolution of 720 ms/frame.

Formação de filmes poliméricos

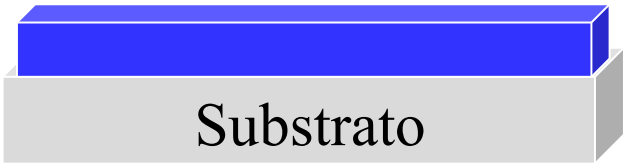
Spin-coating (não-equilíbrio)



Wafer de Si/SiO_2



Como medir a espessura do filme?

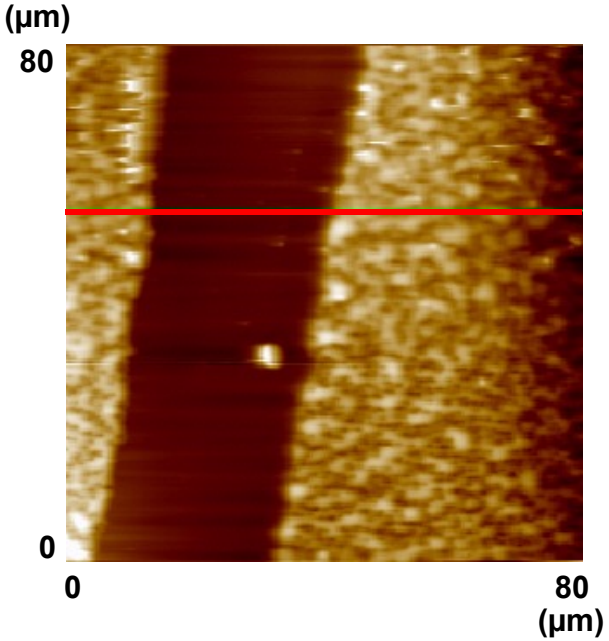


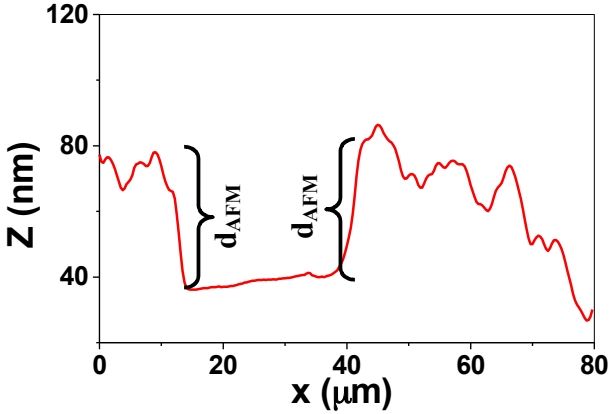
Spin-coating (não-equilíbrio)

filme

$$D = \sqrt[3]{\frac{3\eta\phi}{2\rho^2\omega^2}}$$

Meyerhofer, D. *Appl. Phys.* 1978, *49*, 3993

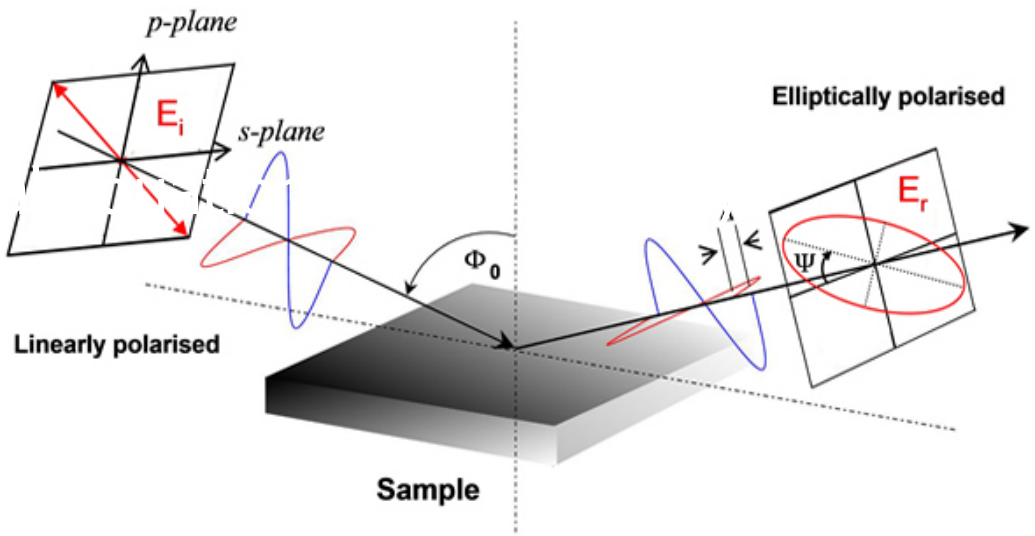


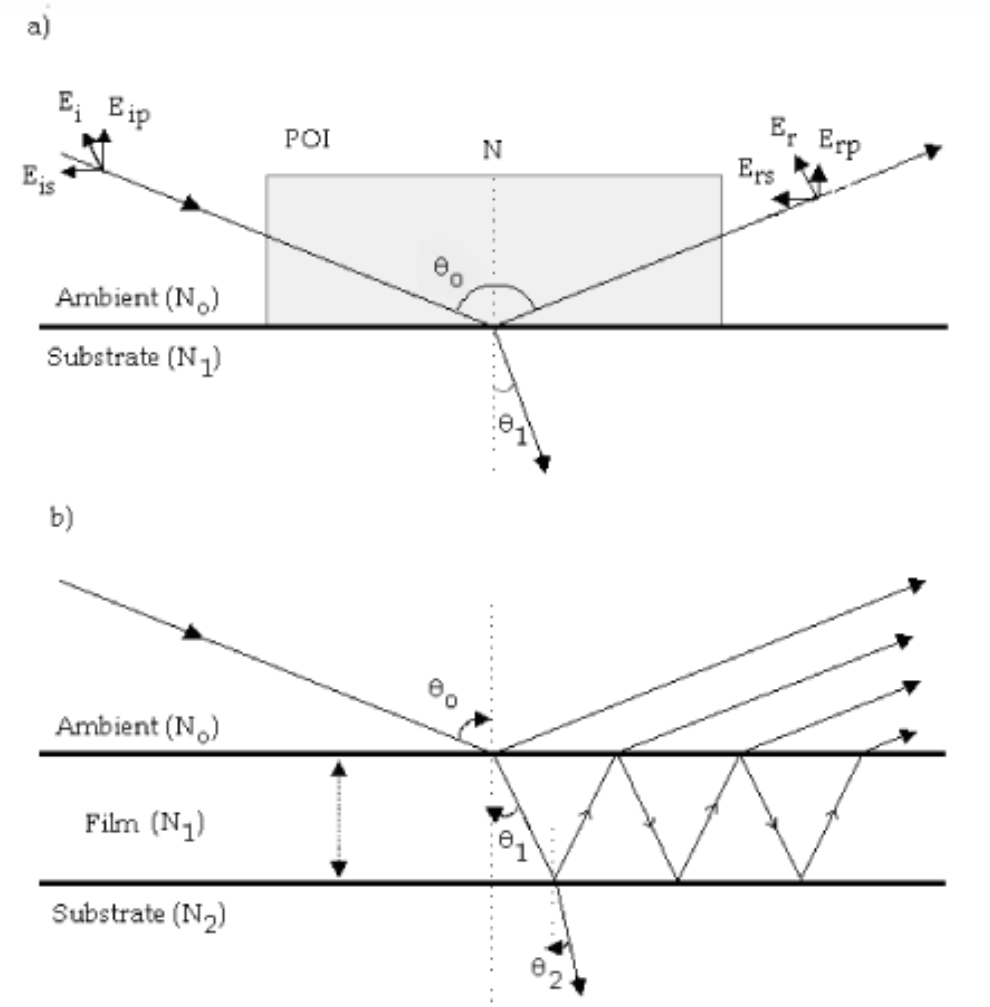


Acetato propionato de celulose (CAP) em AE (5 g/L)

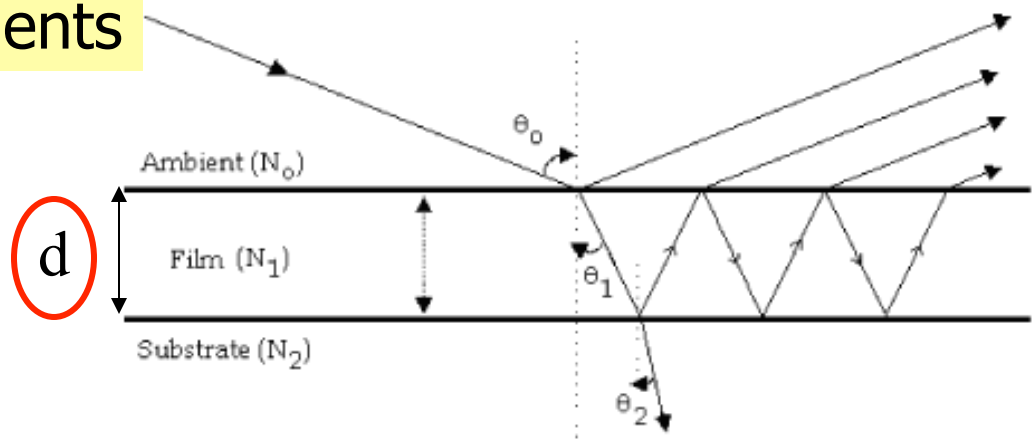
AMIN Jr, KOSAKA, PETRI *Cellulose* 2008, *15*, 527

Elipsometria mede a mudança do estado de polarização da luz após a reflexão a partir de uma dada superfície




a)
E_i
E_{ip}
E_{is}
POI
N
E_r
E_{rp}
E_{rs}
θ_o
Ambient (N_o)
Substrate (N_1)
θ_1
b)
θ_o
Ambient (N_o)
Film (N_1)
θ_1
Substrate (N_2)
θ_2

Fresnel reflection coefficients



$$R^p = \frac{\mathbf{r}^p_{01} + \mathbf{r}^p_{12} \exp(-j2\beta)}{1 + \mathbf{r}^p_{01}\, \mathbf{r}^p_{12} \exp(-j2\beta)}$$

$$R^s = \frac{\mathbf{r}^s_{01} + \mathbf{r}^s_{12} \exp(-j2\beta)}{1 + \mathbf{r}^s_{01}\, \mathbf{r}^s_{12} \exp(-j2\beta)}$$

$$\beta = 2\pi(d/\lambda)N_1 \cos\theta_1$$

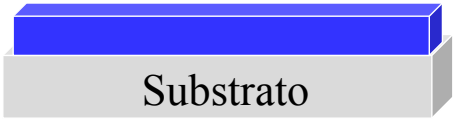
$$\mathbf{r}^s_{12} = \frac{n_1 \cos\phi_1 - n_2 \cos\phi_2}{n_1 \cos\phi_1 + n_2 \cos\phi_2}$$

$$\mathbf{r}^p_{12} = \frac{-n_1 \cos\phi_2 + n_2 \cos\phi_1}{n_1 \cos\phi_2 + n_2 \cos\phi_1}$$

Eq. fundamental da elipsometria:

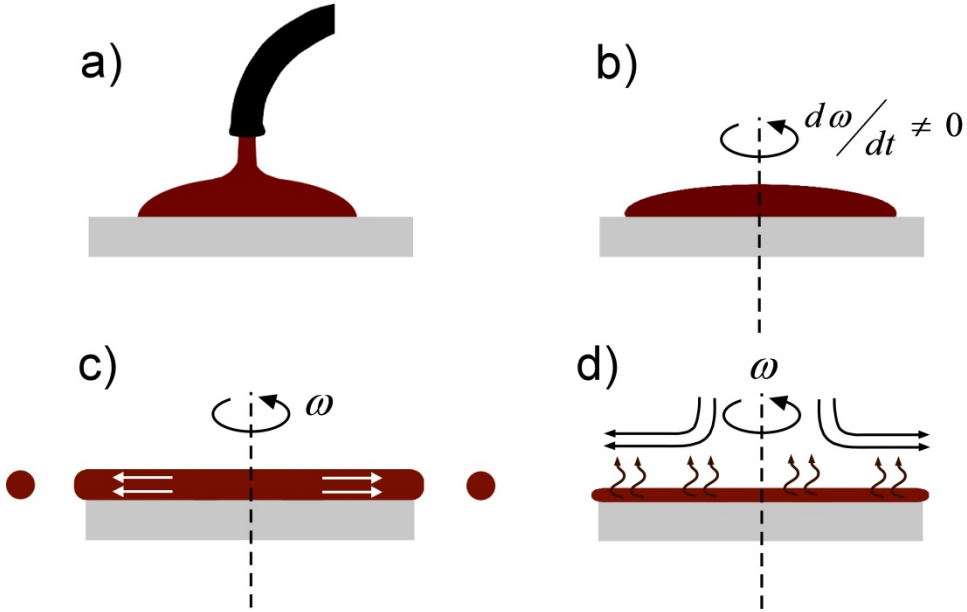
$$\tan \Psi \, e^{j\Delta} = \frac{|R^p|}{|R^s|} = f\,(n_k, d_k, \lambda, \phi)$$

Modelo considera a camada homogênea e isotrópica



Realidade?
Nem sempre ...

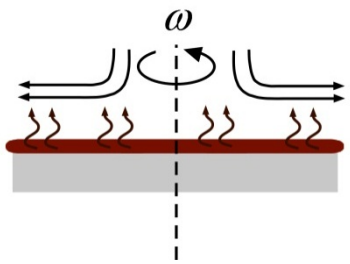
Efeito da umidade relativa (UR)



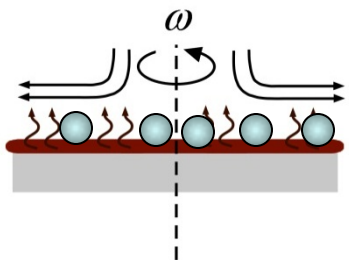
alta UR

"breath figures"

resfriamento

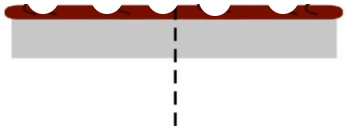


condensação de gotículas de água



$z_0 = (\gamma w - \gamma w/S) / \gamma S$

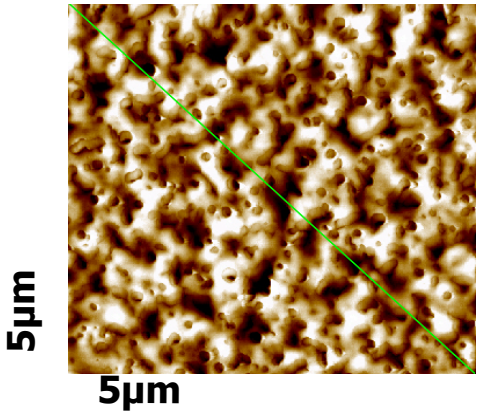
$z_0 > 1$

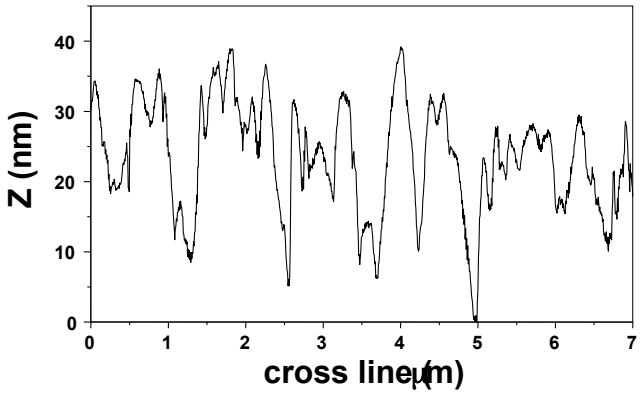


Widawski G, Rawiso M, François B (1994) *Nature* 369, p. 387

Efeito da umidade relativa (UR)

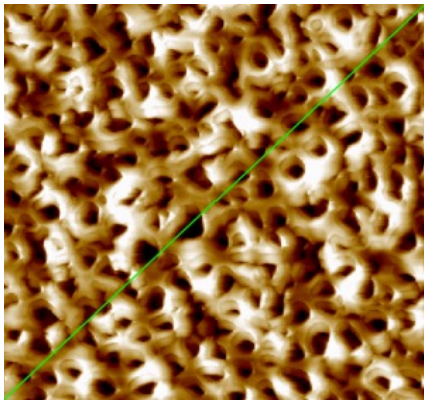
Acetato de celulose (CA) a partir de soluções em THF, 10 g/L

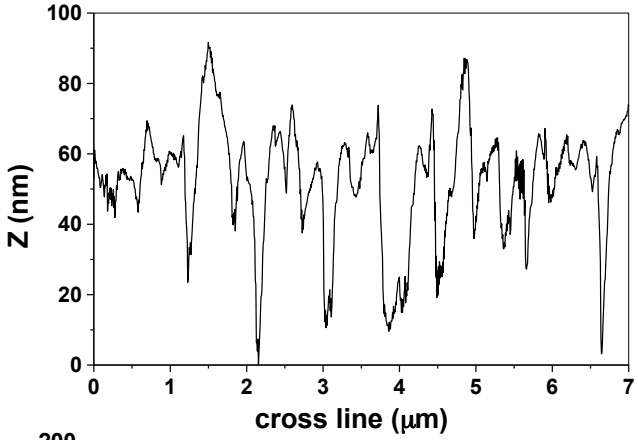




UR (35 ± 5)%

Antireflex ~ 19 %

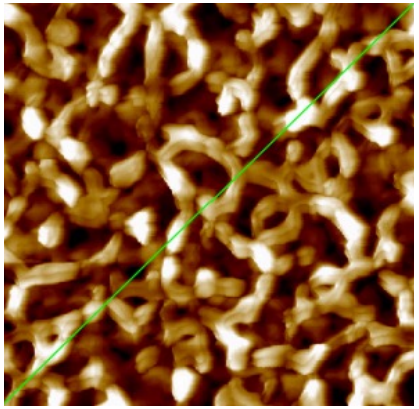


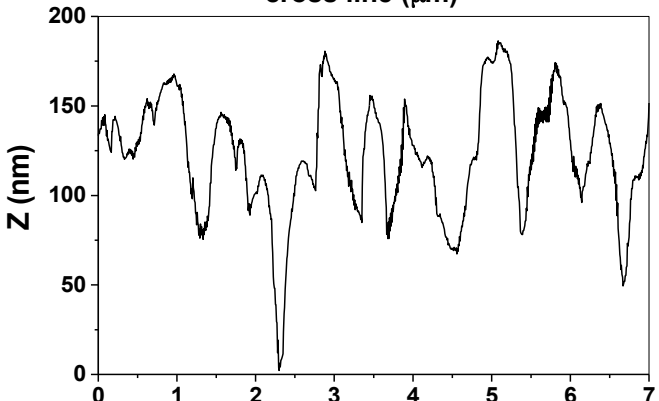


UR (55 ± 5)%

Antireflex ~ 40%

Antireflex ~ 70%





UR (75 ± 5)%

$z_0 = (\gamma w - \gamma w/S)/\gamma S$

$z_0 = 2{,}8$

Blachechen, Souza, Petri
Cellulose **2012**, 19, 443

AFM com controle de temperatura para acompanhar transições de fases

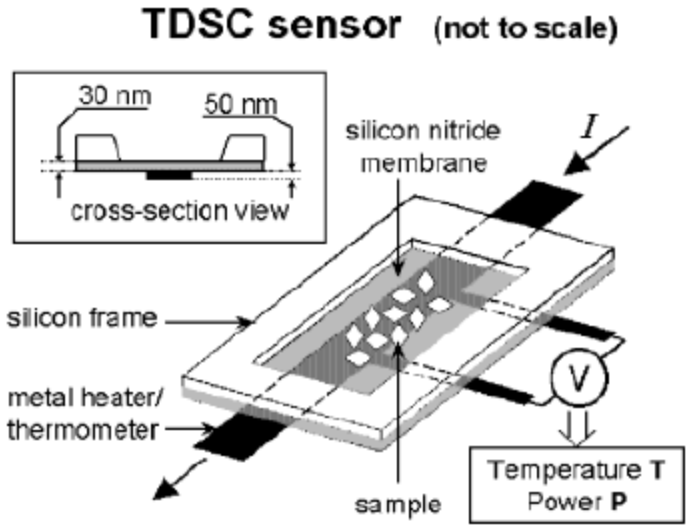


Figure 1. Schematic of the thin-film differential scanning calorimeter. A sample of PE single crystals is deposited on top of the heater area. Included is a cross-section view of the sensor. Relative dimensions have been exaggerated for clarity.

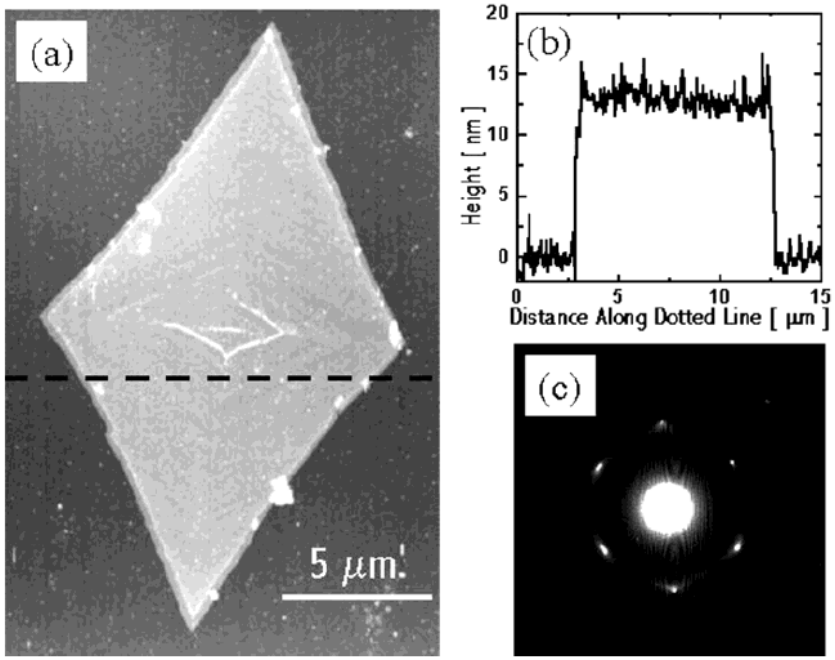


Figure 2. (a) AFM micrograph of a typical 20 μm × 13 μm × 12 nm single crystal. The pleats near the center of the crystal are a result of the original tent-shaped crystal collapsing on the flat silicon nitride surface. (b) The section profile of the crystal. The x axis of the profile corresponds to the dotted line in part A. (c) A diffraction pattern of a similar crystal obtained by TEM.

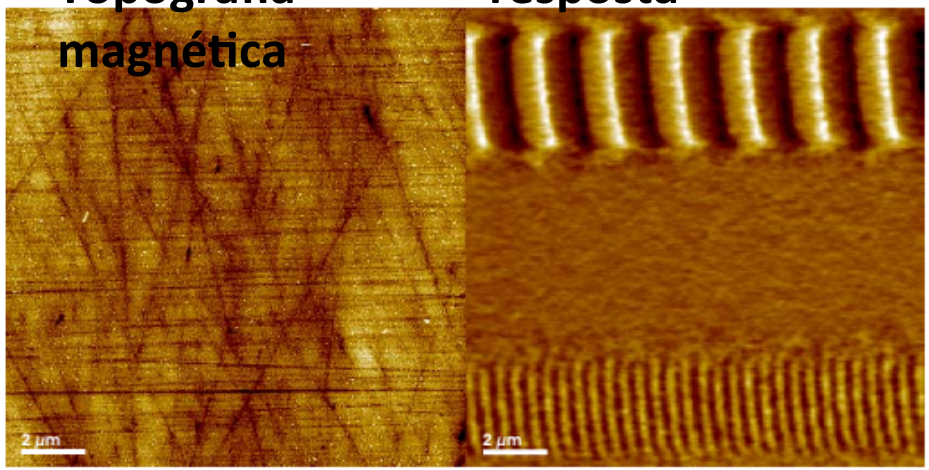
Kwan et al.

Journal of Polymer Science: Part B: Polymer Physics, Vol. 39, 1237–1245 (2001)

Magnetic Force Microscopy (MFM), 1987

Disco rígido (IBM)

Topografia **resposta magnética**



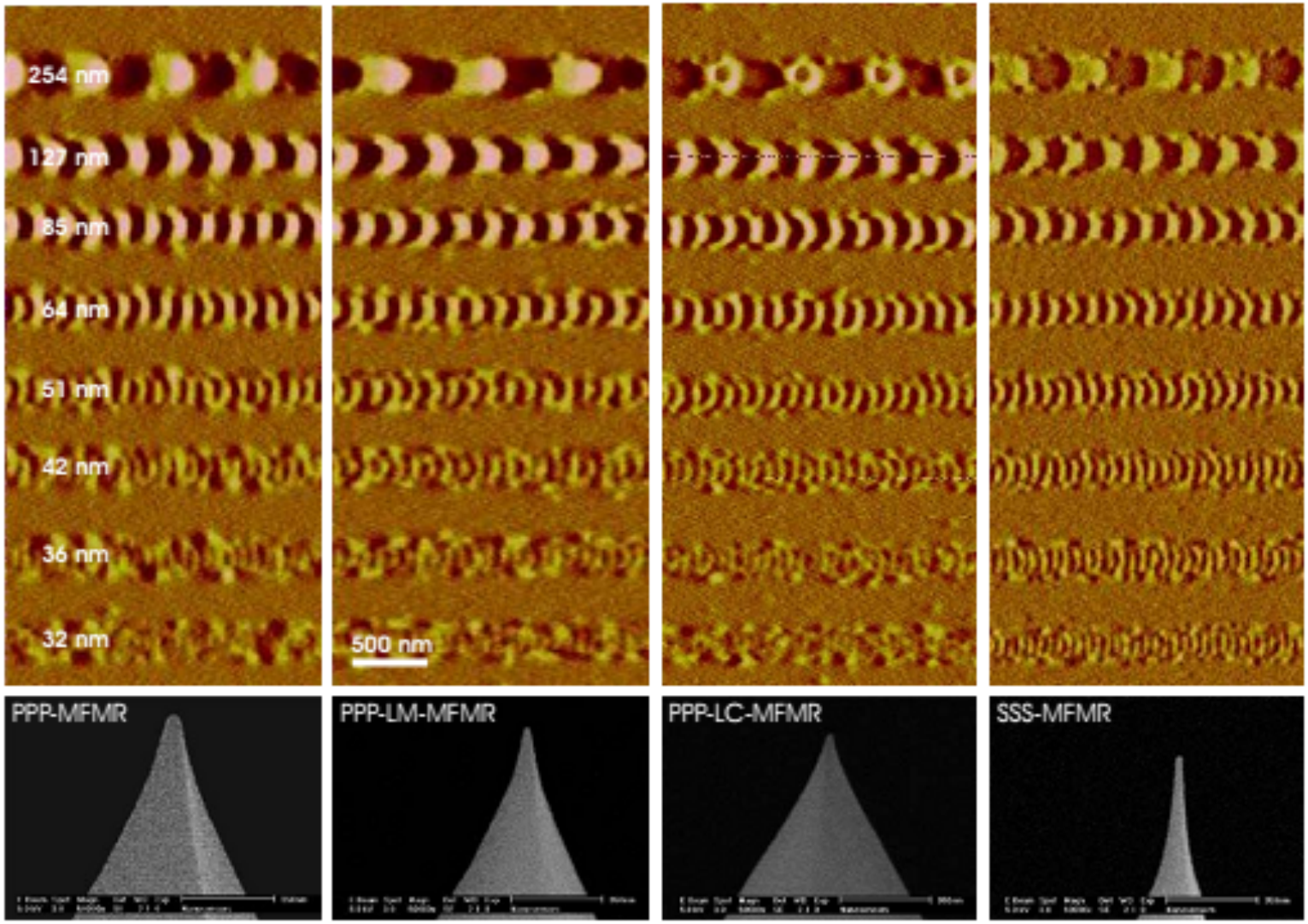
Topography (left) and magnetic frequency shift image (right) of an experimental hard disk (courtesy of IBM) measured with a PPP-MFMR probe (z-range topography: 72nm, magnetic image scale: 22.5Hz).

Ponta recoberta com liga de Co

NANOSENSORS™ The World Leader in Scanning Probes

NANO WORLD INNOVATIVE TECHNOLOGIES

Comparison of Lateral Resolution



Magnetic images (phase shift) of an experimental hard disk with varied bit length (courtesy of Maxtor).

Electrostatic Force Microscopy (EFM)

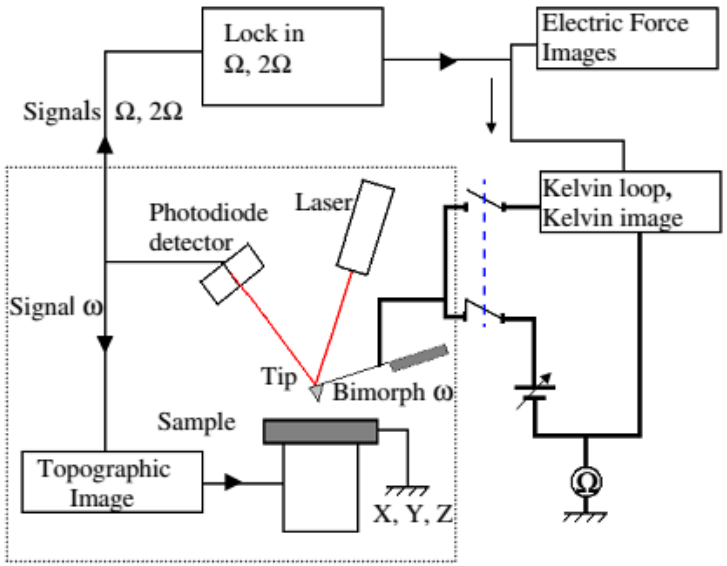
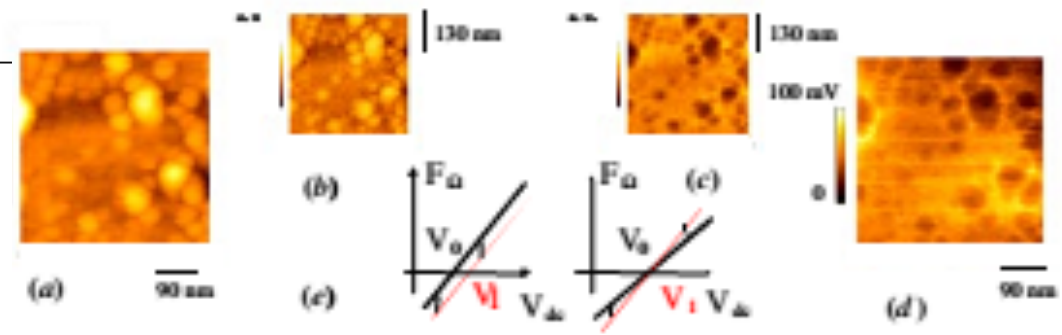
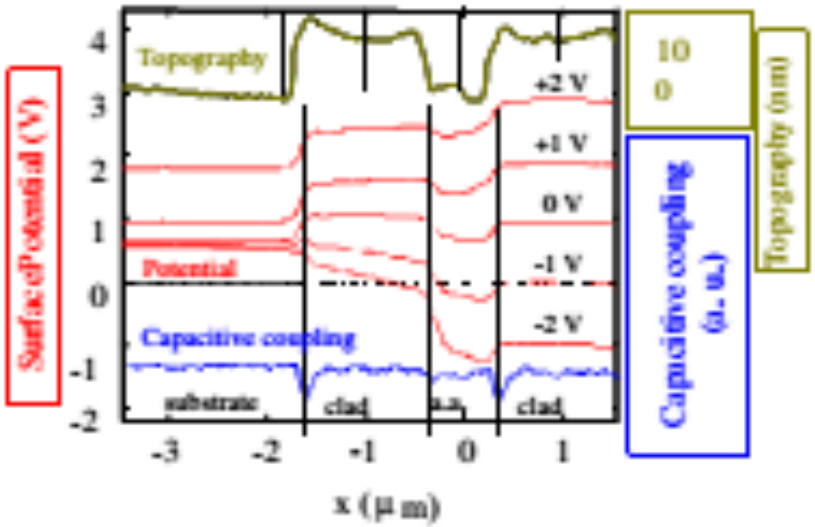


Figure 2. Schematic diagram of the electrostatic force microscope generally based on a commercial AFM (inside dotted box). Two lock-ins allow the detection of the Ω and 2Ω signals and a closed loop brings the surface voltage.





INSTITUTE OF PHYSICS PUBLISHING NANOTECHNOLOGY
Nanotechnology **12** (2001) 485–490 PII: S0957-4484(01)26780-4

Electrostatic force microscopy: principles and some applications to semiconductors

Paul Girard

AFM-IR

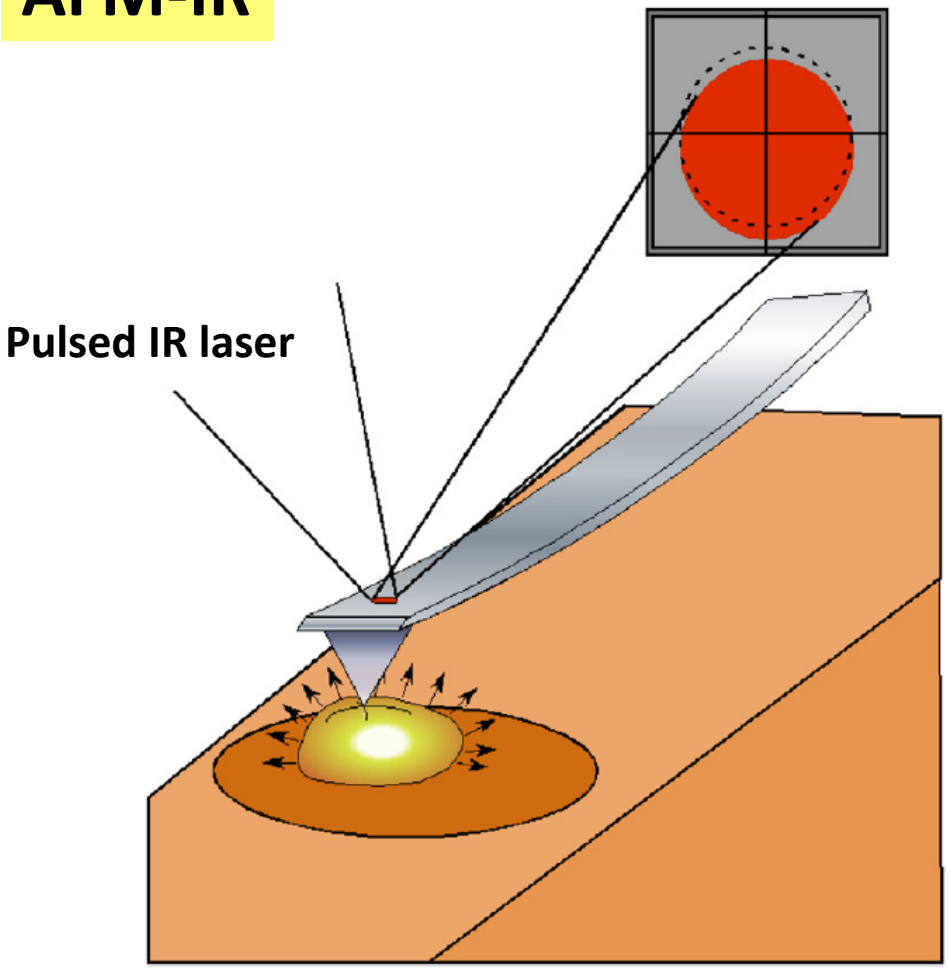


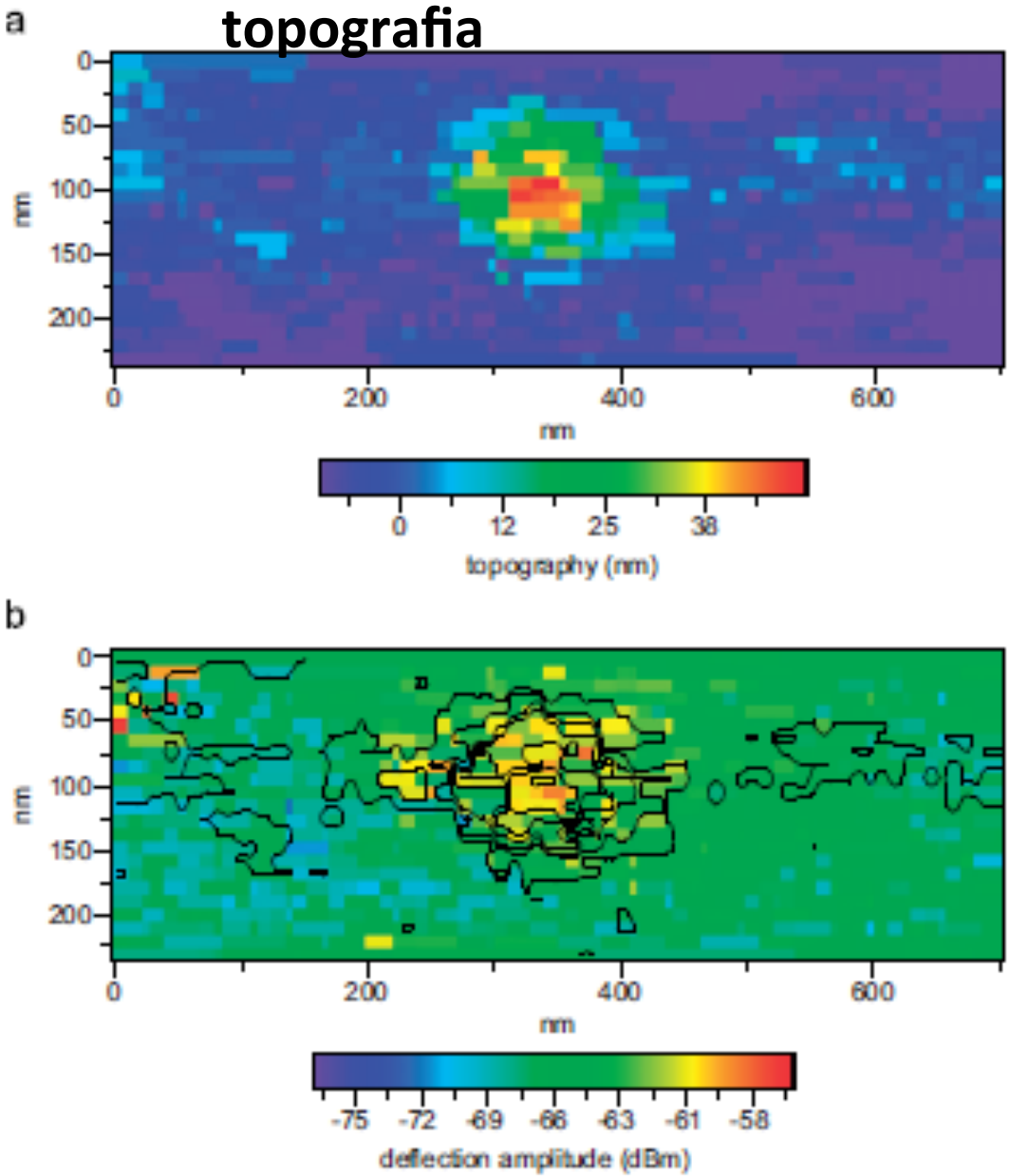
Fig. 1. Experimental set-up. The thermal expansion of a sample induces a displacement of the tip of an AFM cantilever, which is monitored by an HeNe laser and a 4-quadrant detector.

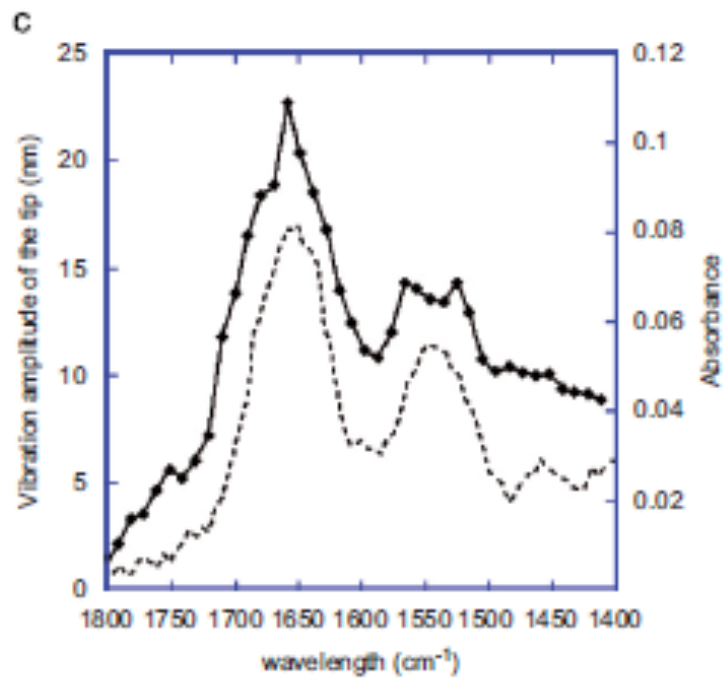
When a sample is illuminated by the laser at the wavelength corresponding to one of its absorption bands, it absorbs one part of the incident energy and is heated almost instantaneously compared to the AFM response time (typically < 1μs).

The increase of temperature creates a fast expansion of the object that displaces the AFM tip .

The AFM cantilever starts then to oscillate. By measuring the amplitude of oscillation we get a signal, which is proportional to the absorption. By recording this signal as function of wavelength of the laser, we are able to make ultralocal infrared spectroscopy

A. Dazzi et al. / Ultramicroscopy 108 (2008) 635–641



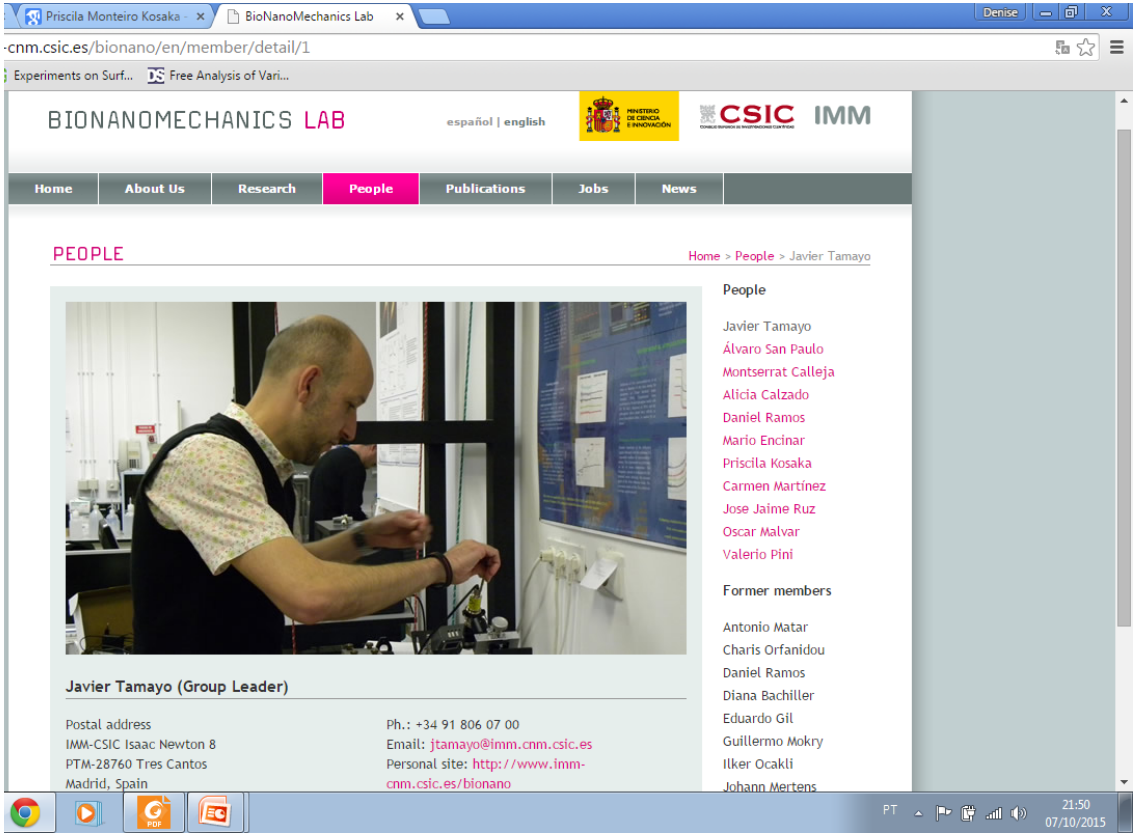


Mapeamento químico para banda da amida 1650 cm $^{-1}$

AFM + espectroscopia Raman (Renishaw, Bruker) ou IR

Nanonics Imaging Ltd, Bruker Nano Surfaces, JPK, Park and NT-MDT

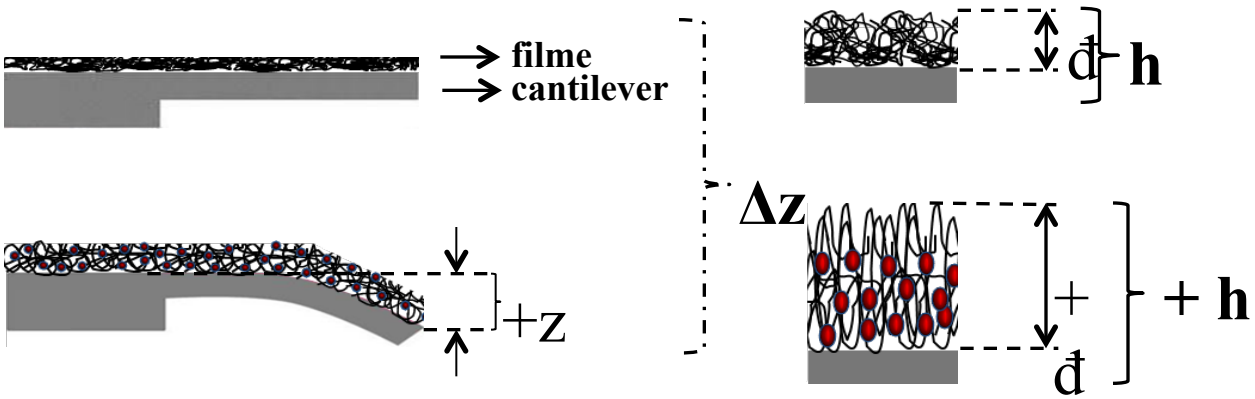
Sensores Nanomecânicos



Mulheres na Ciência
mulhernaciencia@

Biosensors based on nanomechanical systems
J Tamayo, PM Kosaka, JJ Ruz, Á San Paulo, M Calleja
Chemical Society Reviews 42 (2013), 1287-1311

Sensores Nanomecânicos



$$\Delta\sigma = \frac{Eh^2}{3(1-\nu)L^2}\Delta z$$

equação de Stoney

$\Delta\sigma$: Diferença no estress de superfície.

Δz: Deflexão do cantilever

h: Espessura

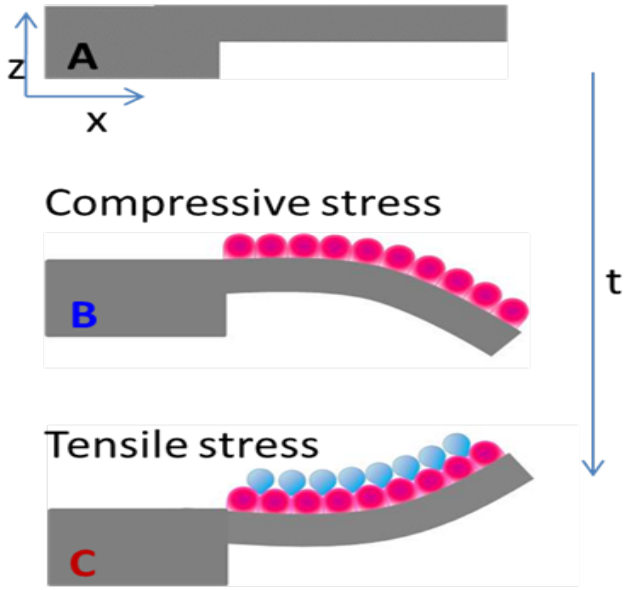
ν: Coeficiente de Poisson

E: Módulo de Young

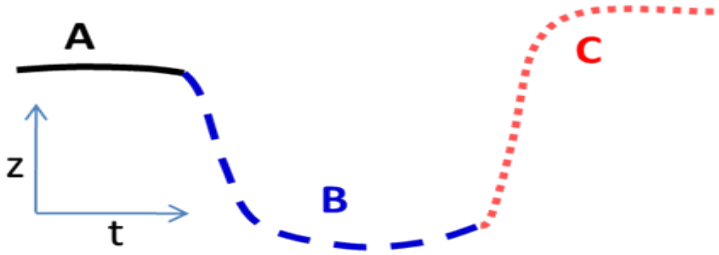
L: Comprimento do cantilever

$$\Delta\sigma = \frac{Eh^2}{3(1-\nu)L^2}\Delta z$$

(a) Static mode

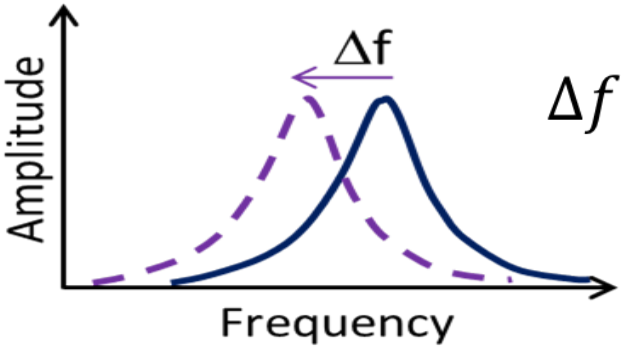


Cantilever deflection – Real time



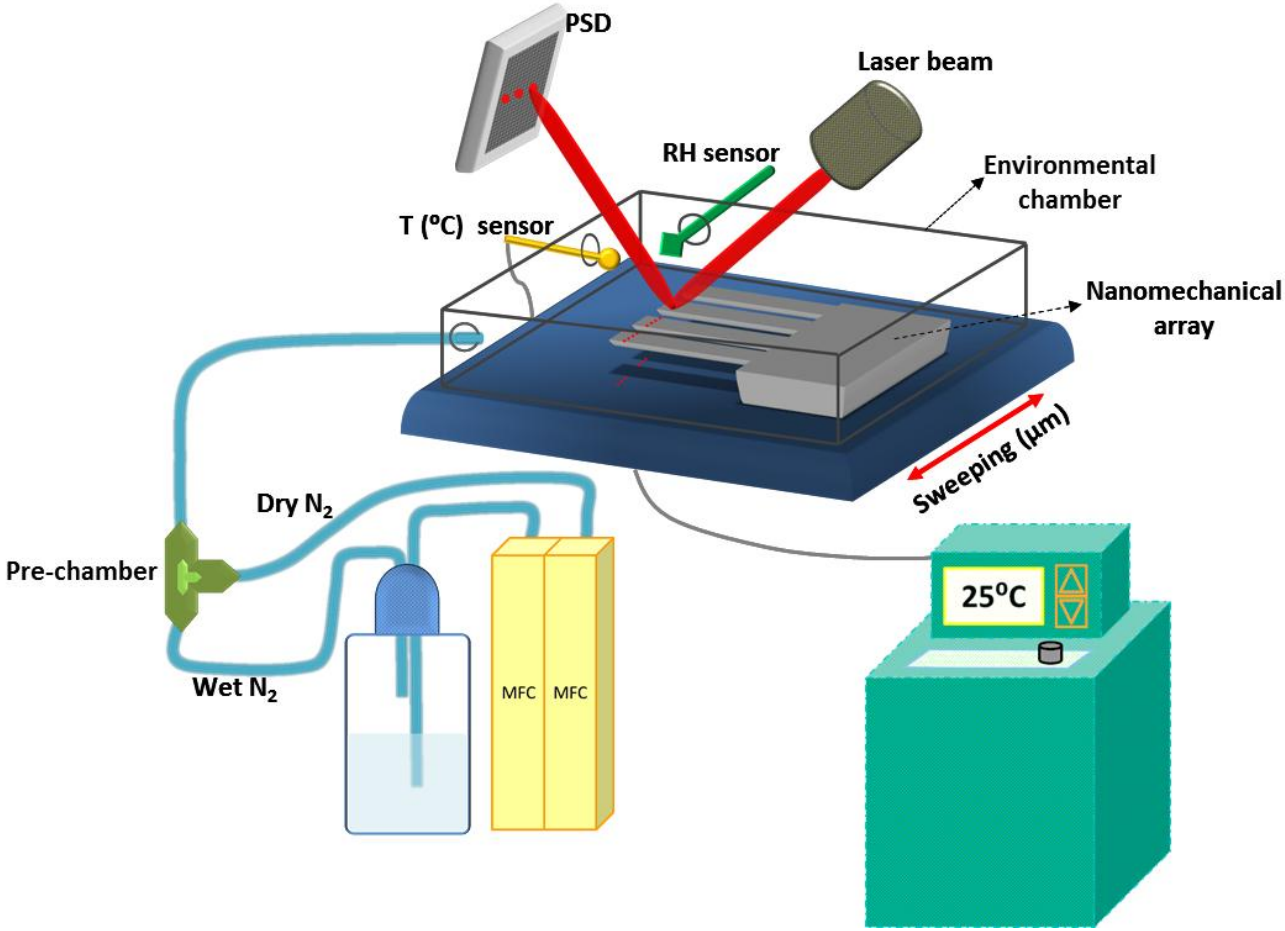
(b) Dynamic mode

Added mass



$$\Delta f = \frac{1}{2\pi}\sqrt{\frac{k}{\Delta m}}$$

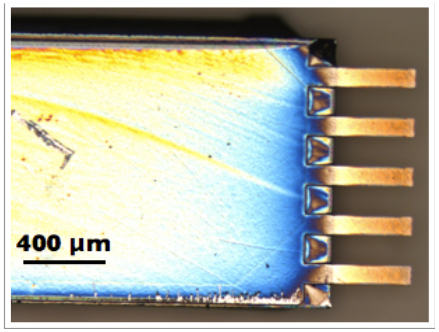
Sensores Nanomecânicos para umidade

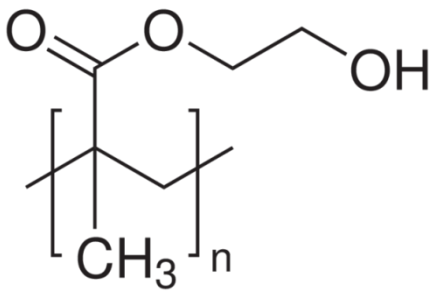


M del Rey, RA da Silva, D Meneses, DFS Petri, J Tamayo, M Calleja, P M Kosaka
Sensors and Actuators B: Chemical 204, 602-610 (**2014**)

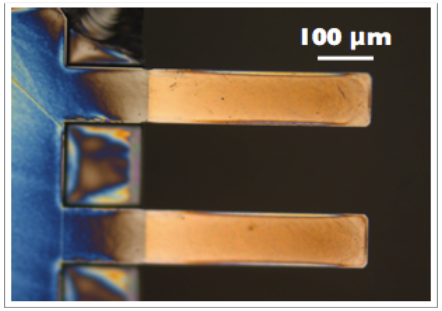
Materiais

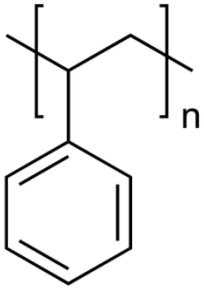
PHEMA, 30 g/L em methanol





PS, 30 g/L em Tolueno





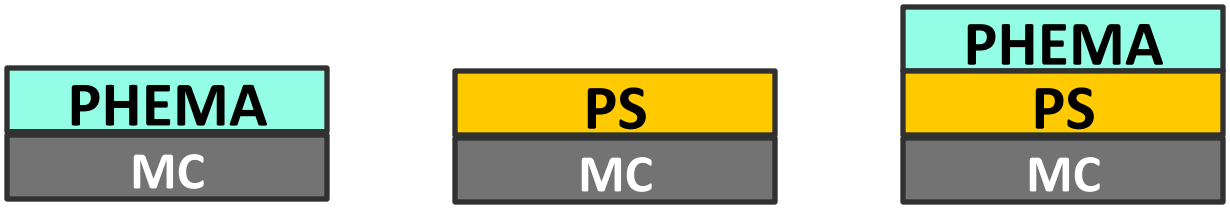
Imagens de campo claro dos microcantileveres após spin coating.

Polímero	Massa molar ($g.mol^{-1}$)
Poliestireno (PS)	M_w = 350.000
PHEMA - 20K	Mv = 20.000
PHEMA – 300K	Mv = 300.000
PHEMA – 1000K	Mv = 1000.000

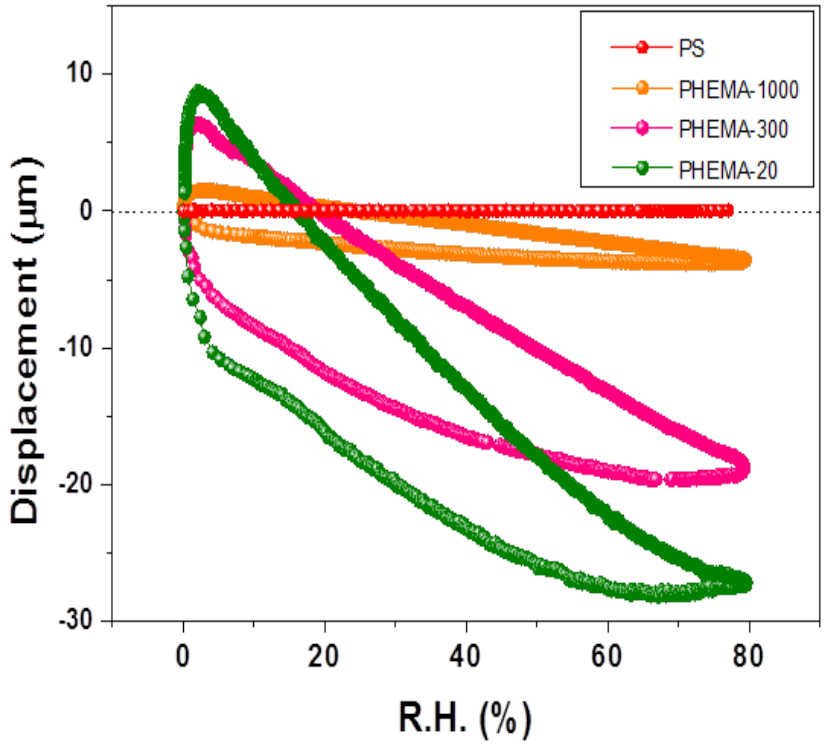
Dimensões do MC

- 400µm de comprimento
- 100 µm de largura
- 1 µm de espessura

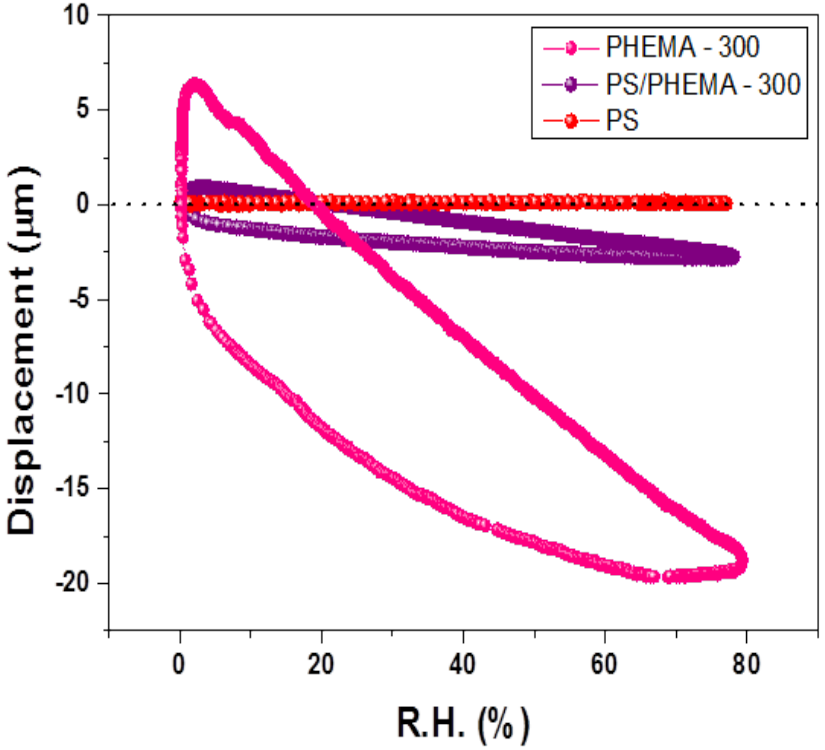
PHEMA ou PS foi removido do lado oposto dos microcantileveres por plasma (80 W, $7.5.10^{-9}$ torr, O_2 em 20 cm^3/min, Instrumento RIE Oxford Plasmalab 80 PLUS.)

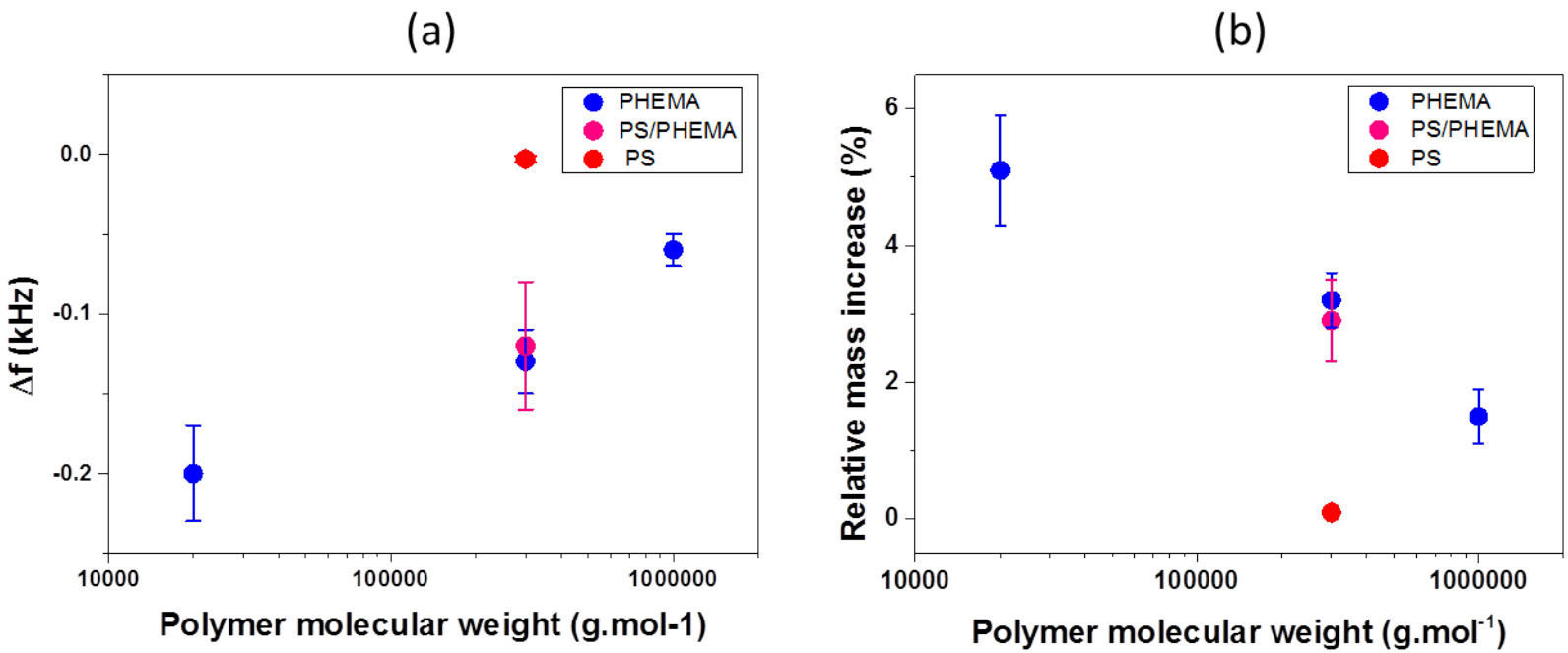

PHEMA
MC
PS
MC
PHEMA
PS
MC

(a)


PS
PHEMA-1000
PHEMA-300
PHEMA-20
Displacement (µm)
R.H. (%)
10
0
-10
-20
-30
0
20
40
60
80

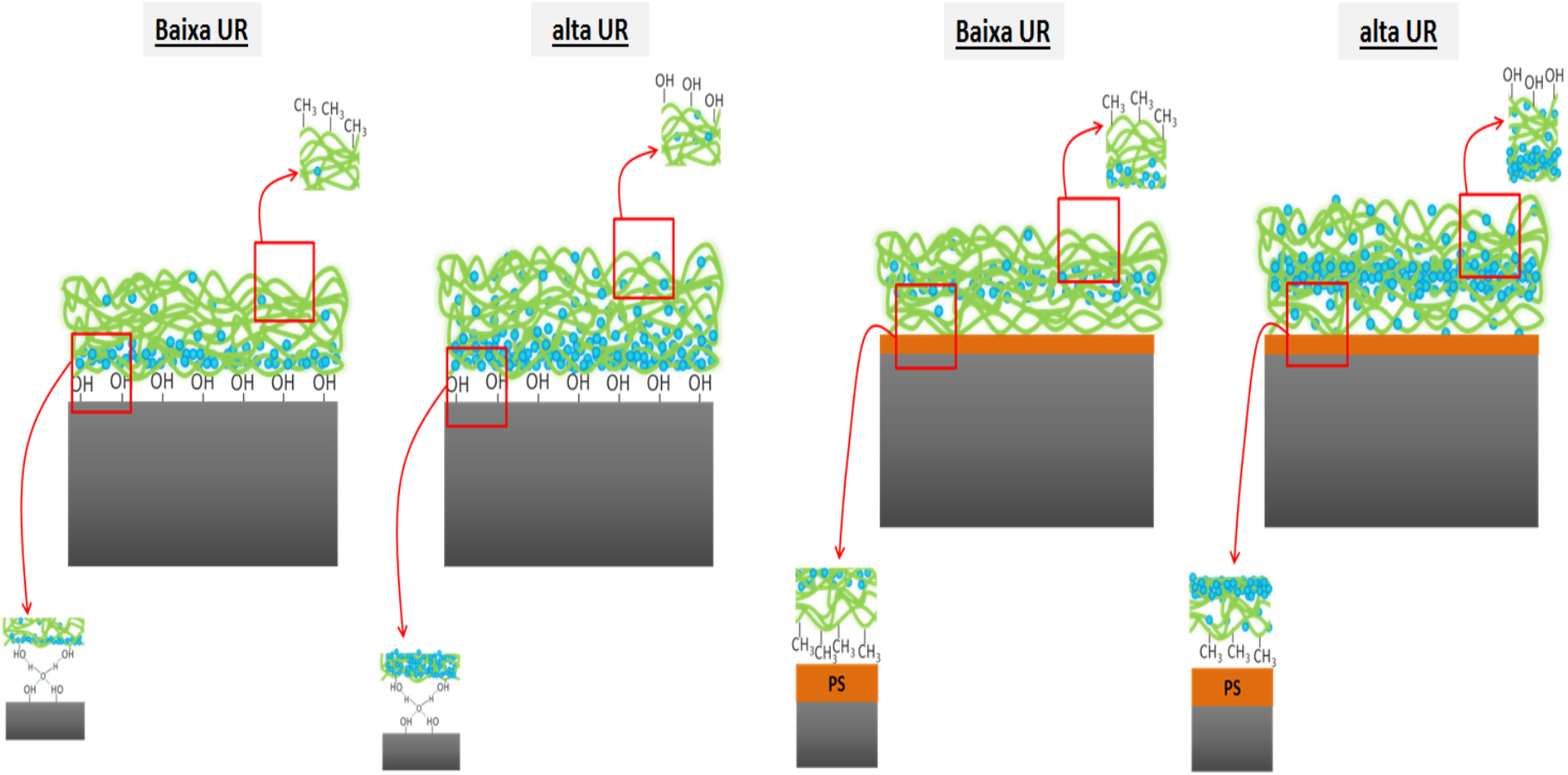
(b)


PHEMA - 300
PS/PHEMA - 300
PS
Displacement (µm)
R.H. (%)
10
5
0
-5
-10
-15
-20
0
20
40
60
80

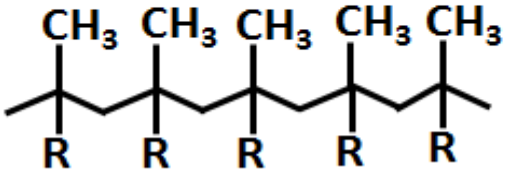


$$\Delta s_{mix} = - k\,(n_1 \ln \varphi_1 + n_2 \ln \varphi_2)$$

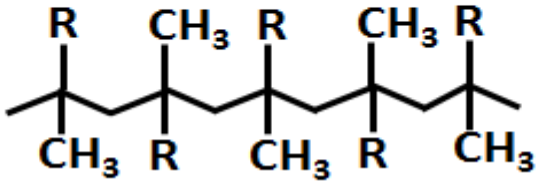
Coating	$\Delta\sigma_{max}$ (N/m)*	Δf (kHz)	Relative mass increase (%)	Thickness (nm)
PHEMA-20	10 ± 1	- (0.20 ± 0.03)	5.1 ± 0.8	106 ± 4
PHEMA-300	7 ± 1	- (0.13 ± 0.02)	3.2 ± 0.4	220 ± 14
PS/PHEMA-300	0.9 ± 0.3	- (0.12 ± 0.04)	2.9 ± 0.6	207 ± 10
PHEMA-1000	1.4 ± 0.3	- (0.06 ± 0.01)	1.5 ± 0.4	233 ± 17
PS	0.004 ± 0.003	- (0.003 ± 0.002)	0.09 ± 0.06	223 ± 15

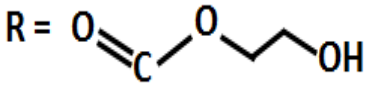


Baixa UR

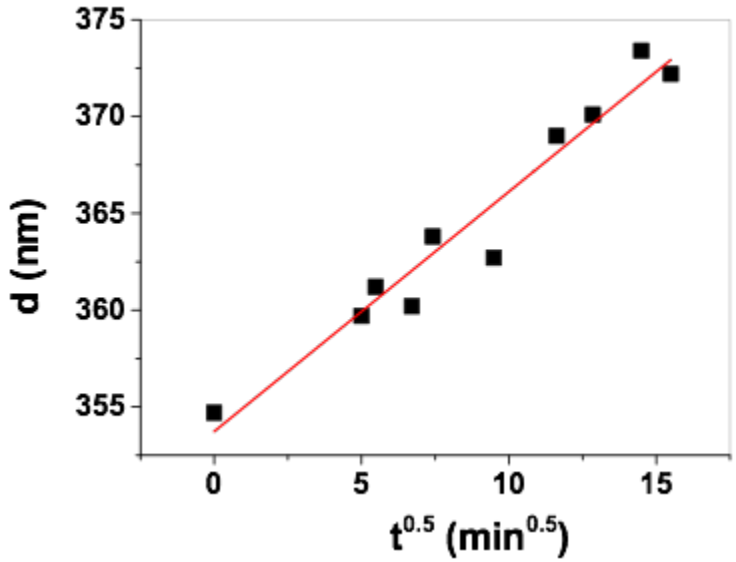


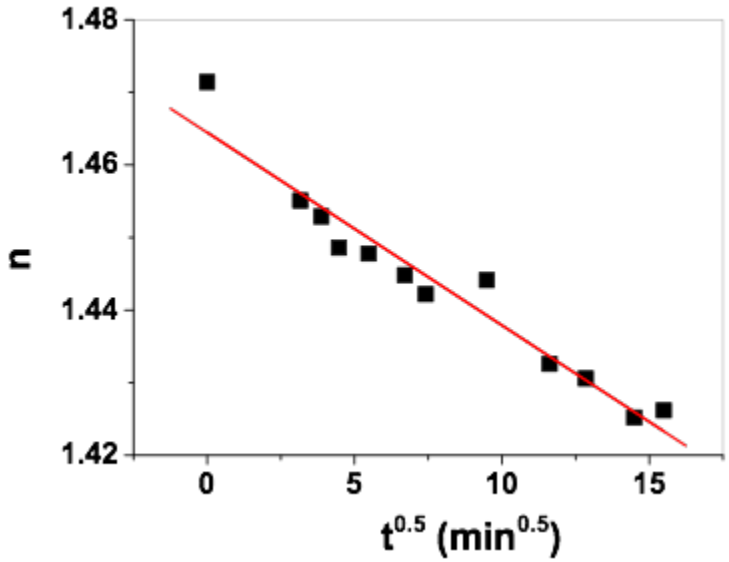
Alta UR





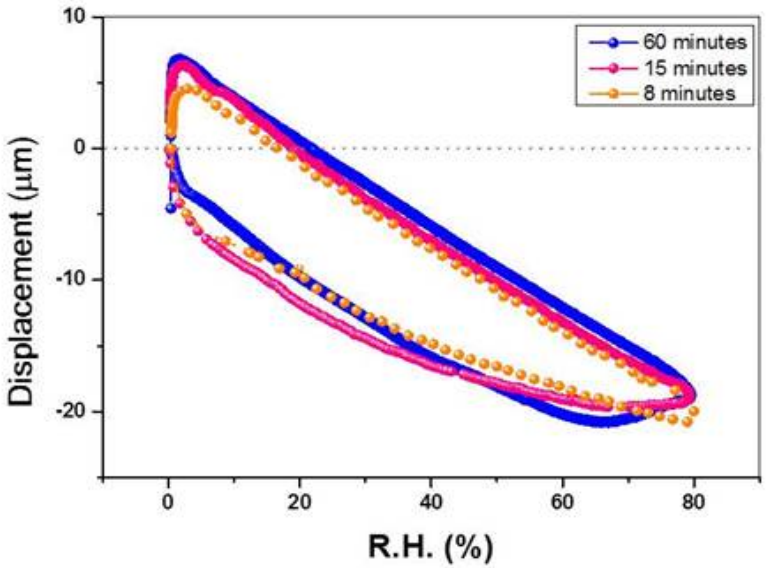
Elipsometria somente a 80% UR





Deflexão do MC

Sensibilidade muito superior



Sensor Nanomecânico / dosagem sérica de biomarcador

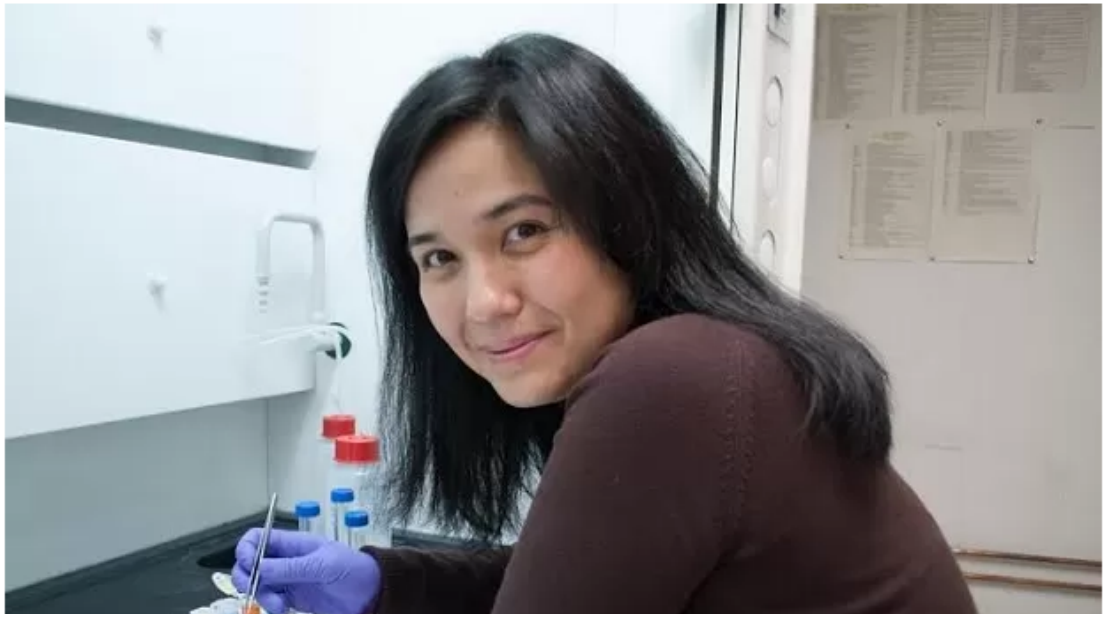
07/04/2015 07h25 - Atualizado em 07/04/2015 07h25

Cientista de Brasília cria sensor que acha câncer antes de sintoma surgir

Priscila Kosaka desenvolve projeto há seis anos em laboratório espanhol. Técnica é 10 milhões de vezes mais sensível do que as atuais disponíveis.

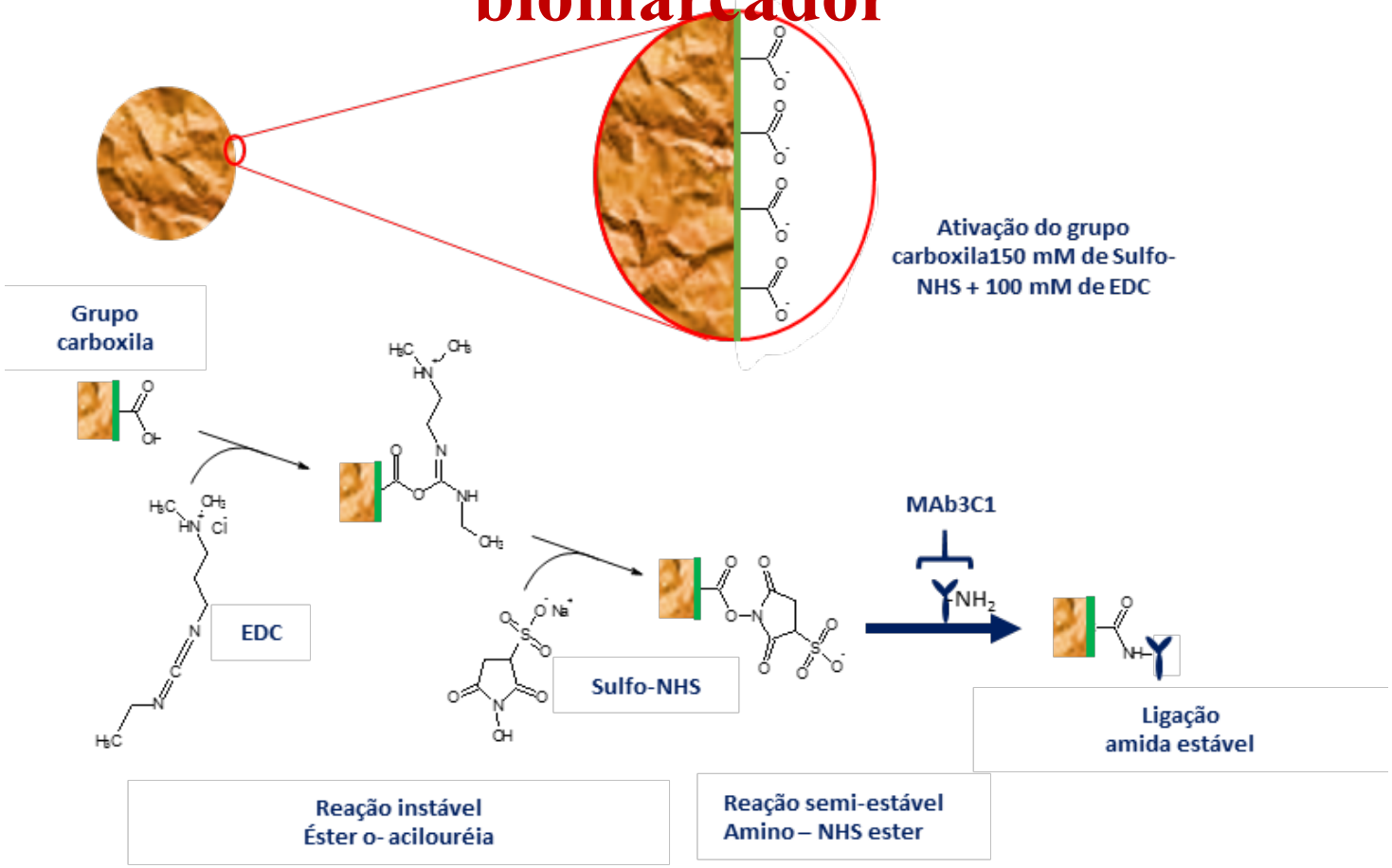
Raquel Morais
Do G1 DF

FACEBOOK



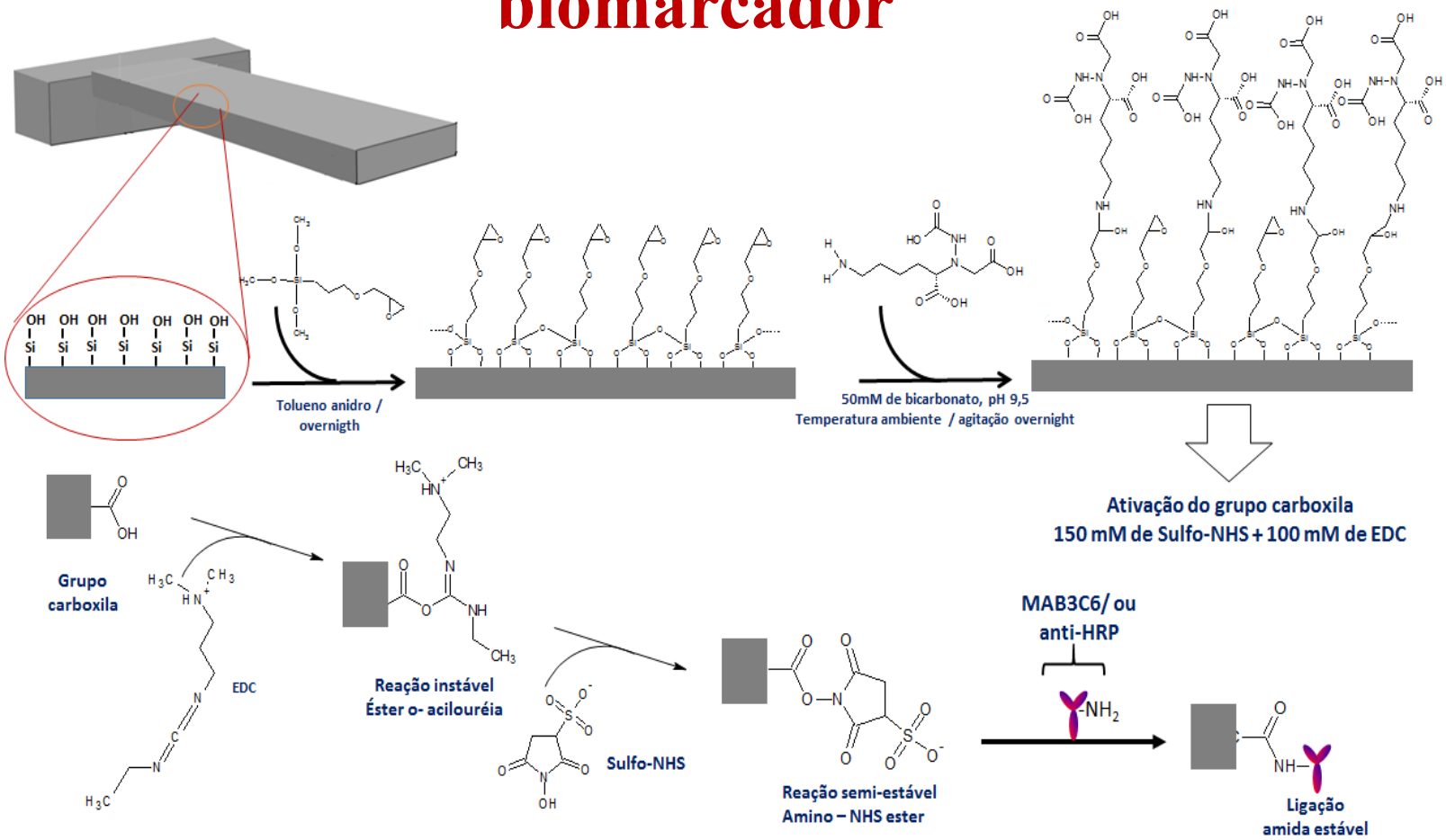
Priscila M Kosaka, Valerio Pini, José J Ruz, RA da Silva, MU González, D Ramos, Montserrat Calleja, Javier Tamayo *Nature Nanotechnology* 9, 1047–1053 (**2014**)

Sensor Nanomecânico / dosagem sérica de biomarcador

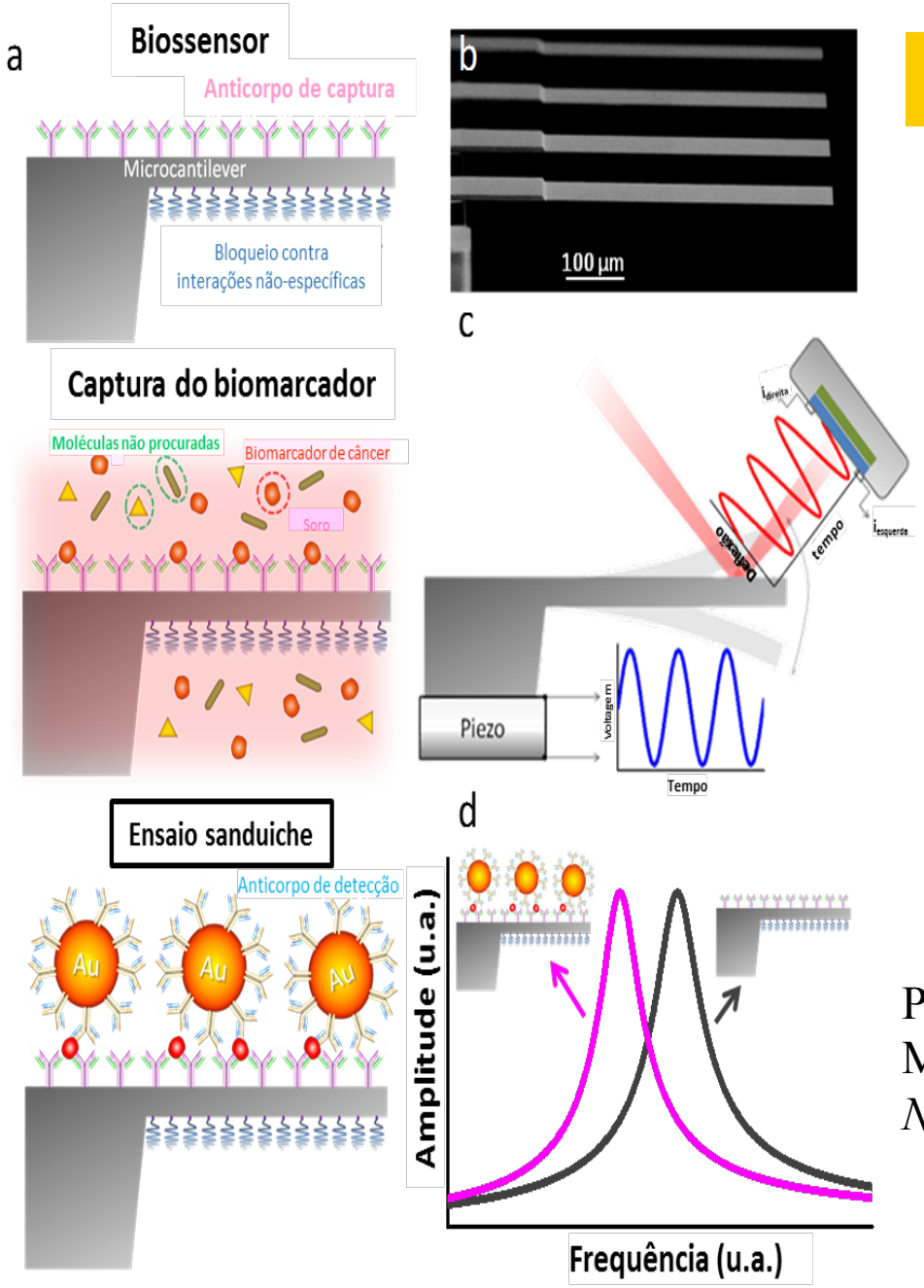


Esquema de funcionalização das nanopartículas de ouro com 100 nm de diâmetro revestidos com carboxi-polímero e posterior imobilização covalente dos anticorpos de detecção (MAb3C1)

Sensor Nanomecânico / dosagem sérica de biomarcador

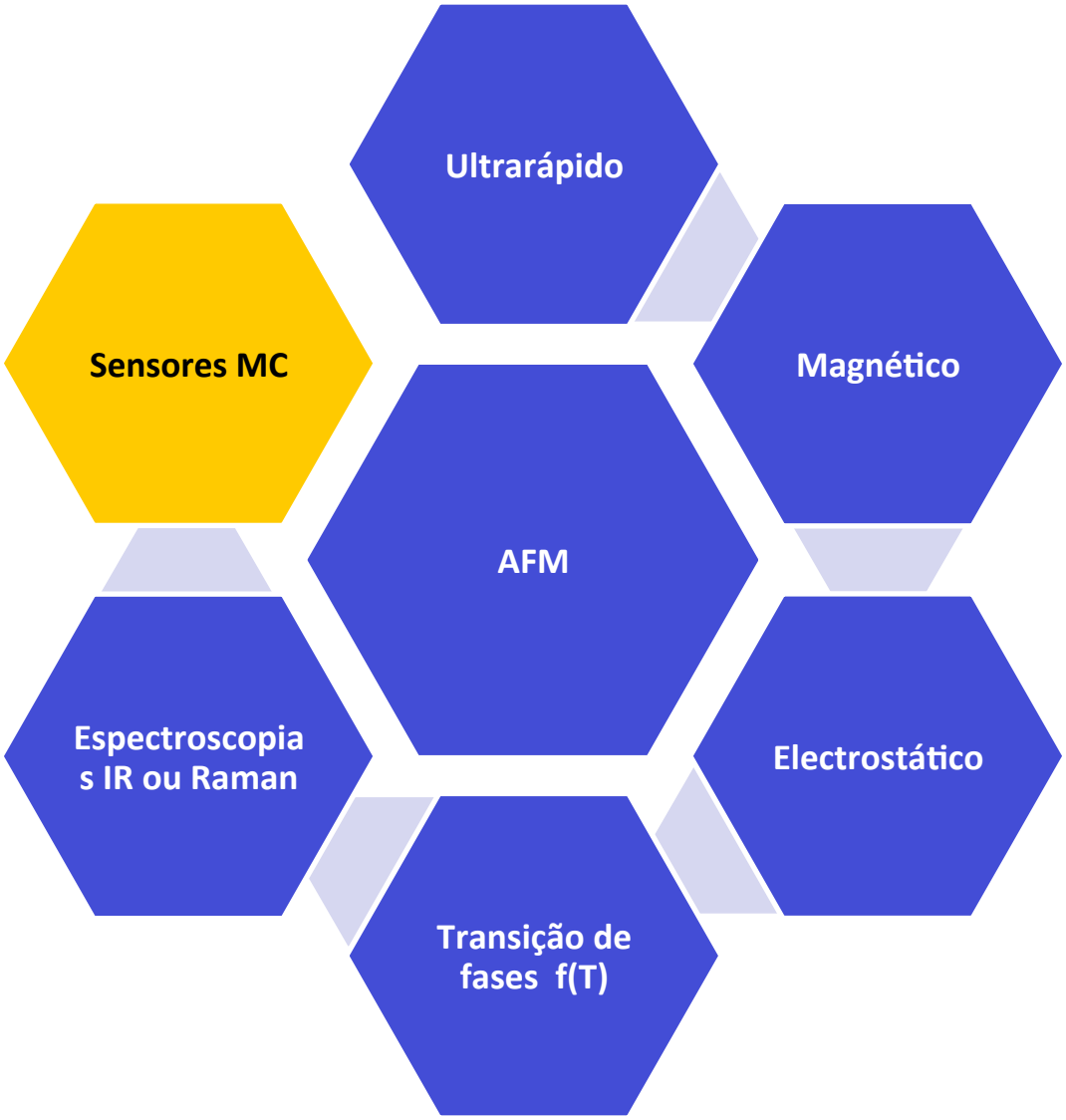


Esquema de funcionalização do cantilever por meio da silanização com (3-glicidiloxipropil) trimetoxisilano e posteriores reações de ativação de grupos funcionais e imobilização do anticorpo de captura (MAb3C6)



Detecção na faixa de 10^{-16} g/L

PM Kosaka, V Pini, JJ Ruz, RA da Silva, MU González, D Ramos, J Tamayo
Nature Nanotechnology **2014**


Ultrarápido
Sensores MC
Magnético
AFM
Espectroscopia
s IR ou Raman
Electrostático
Transição de
fases f(T)