

Nanomechanical mapping of soft materials with the atomic force microscope: Methods, theory and applications

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R. Garcia, Chem. Soc. Rev. 49, 5850-5884 (2020)

Introduction

Classification Nanomechanical methods

Theory of nanomechanical AFM

Force-distance curve methods

FV family

Torsional harmonics

Parametric methods

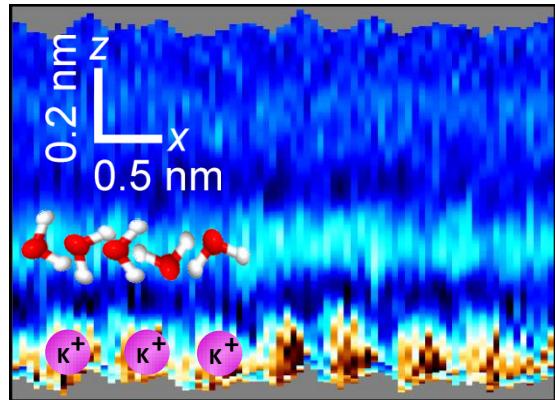
Bimodal AFM

Contact resonance AFM

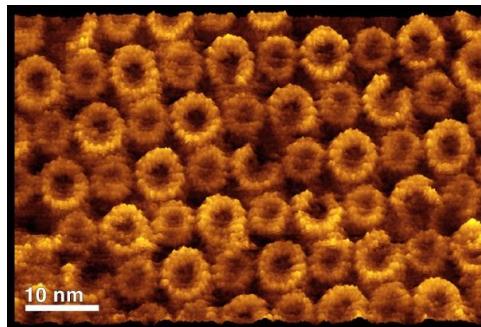
AFM phase-imaging

Spatial resolution and quantitative accuracy

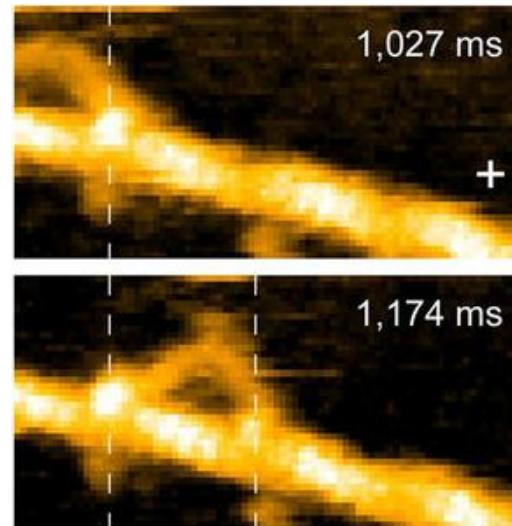
Summary



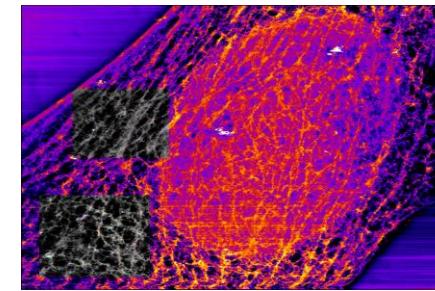
Solid-liquid interfaces (2016)



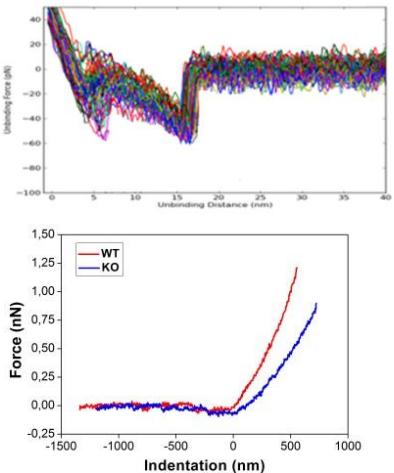
Molecular motors, H Seelertet, A. Engel, D.J. Muller (2000)



Polymers, R. Magerle (2004)

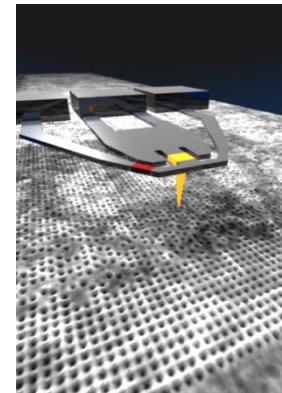


Living cells (2019)

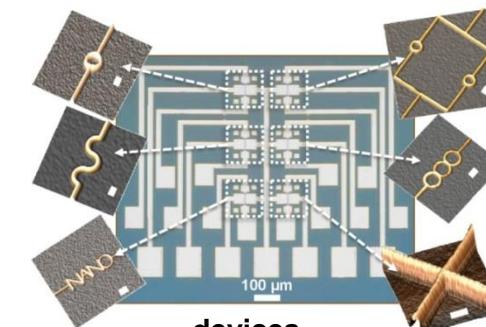


Nanomechanical sensors
J. Tamayo (2007)

High-spatial resolution imaging
+
force spectroscopy = nanomechanical mapping



Scanning Probe Lithography



devices

Why Nanomechanics ?

Food Processing, packaging, Smart materials, Medicine, Microscopy

Biomedicine



146, 148-163 (2011)

Biomechanical Remodeling of the
Microenvironment by Stromal Caveolin-1
Fa



Food Processing

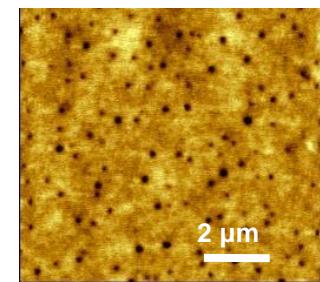


AFM

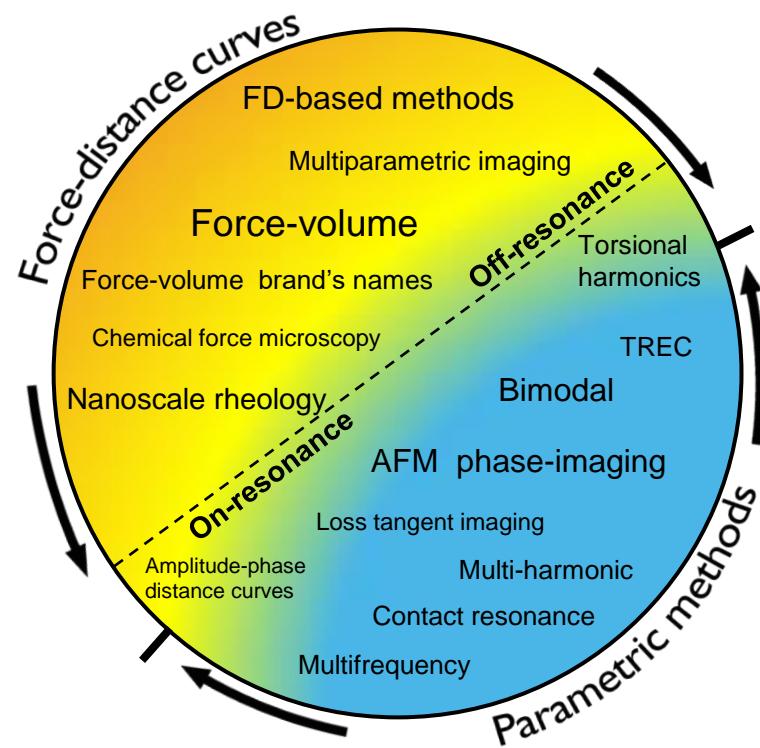


Goals in Nanomechanical spectroscopy

- Simultaneous topography & nanomechanical & electrical...mapping
- Fast
- Efficient (few data points per pixel)
- Quantitative accuracy
- Highest spatial resolution (atomic, molecular...)
- Wide range of Nanomechanical properties (Store, loss modulus, adhesion...)
- Robust

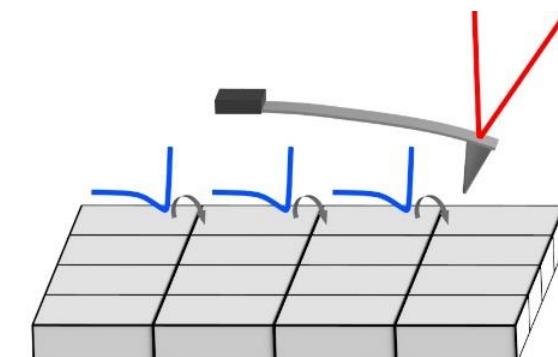


Classification nanomechanical AFM methods

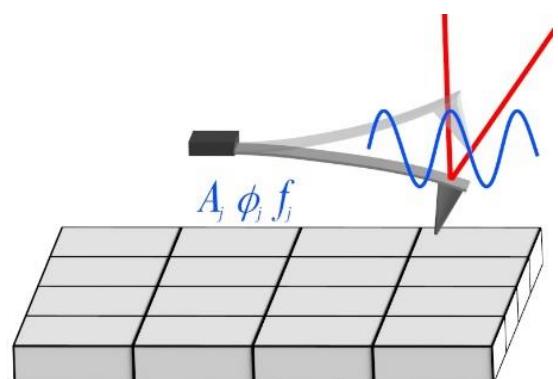


R. Garcia, Chem. Soc. Rev. 49, 5850-5884 (2020)

Force-distance curve vs parametric

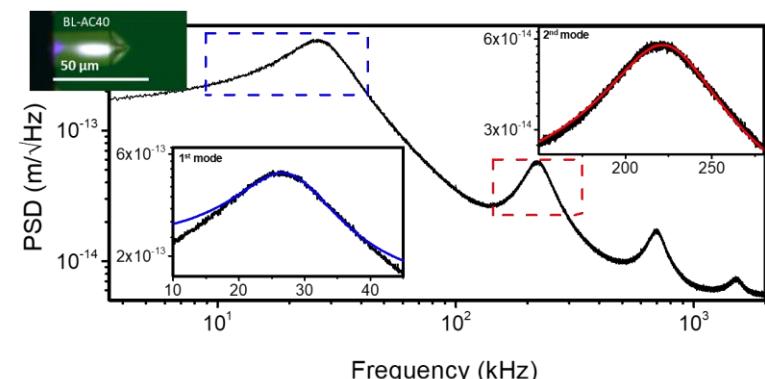


On each pixel a FDC is measured

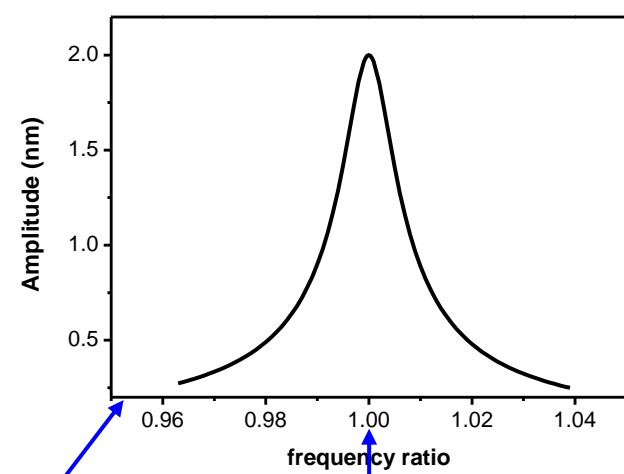


Parametric: observables are directly related to mechanical properties

On and off-resonance methods



S. Benaglia et al. Nat. Protocols 13, 2890 (2018)



Off-resonance:
modulation frequency <<
1st resonant frequency

On-resonance:
modulation frequency
coincides (or very close)
with frequency of a mode

Theory of Nanomechanical force spectroscopy

1 Equation of motion of the tip
(to determine the force)

2 Interaction force in terms of mechanical properties
(models)

3 Mechanical parameters in terms of AFM observables

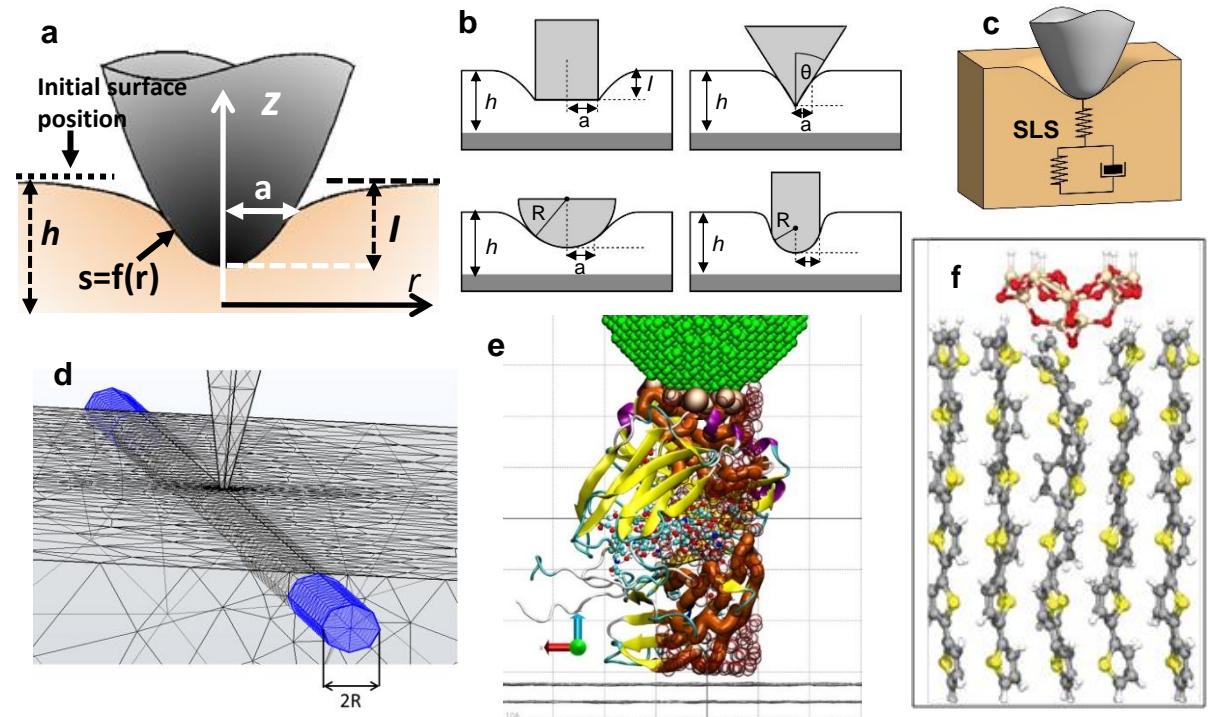
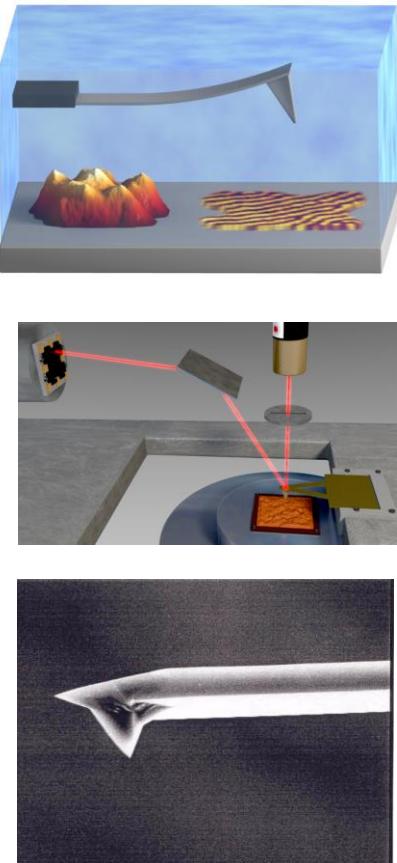


Fig. 4. Models and simulations of tip-soft material interfaces. (a) Definition of the main interfacial parameters; a is the radius of the projected contact area; h is the sample thickness; r is the radial coordinate; $f(r)$ is the shape of the contact. (b) Axisymmetric tip geometries. From left to right and top to bottom, cylinder, cone, sphere and nanowire. (c) Scheme of a tip-linear viscoelastic material (SLS) interface. (d) FEM simulation of the deformation of a fiber embedded in a soft matrix. Reprinted from ref. 67. (e) MD simulation of the deformation of an IgG antibody domain by a carbon nanotube tip. Image by R. Perez and J.G. Vilhena. (f) *ab initio* MD simulations of the deformation induced on a sextiophene chain by a silica tip. The force produces the bending of the head molecular groups and its transmission through the whole chain. Color code for atoms: oxygen (red), sulphur (yellow), carbon (grey), hydrogen (white) and silicon (light brown). Reprinted from ref. 78.



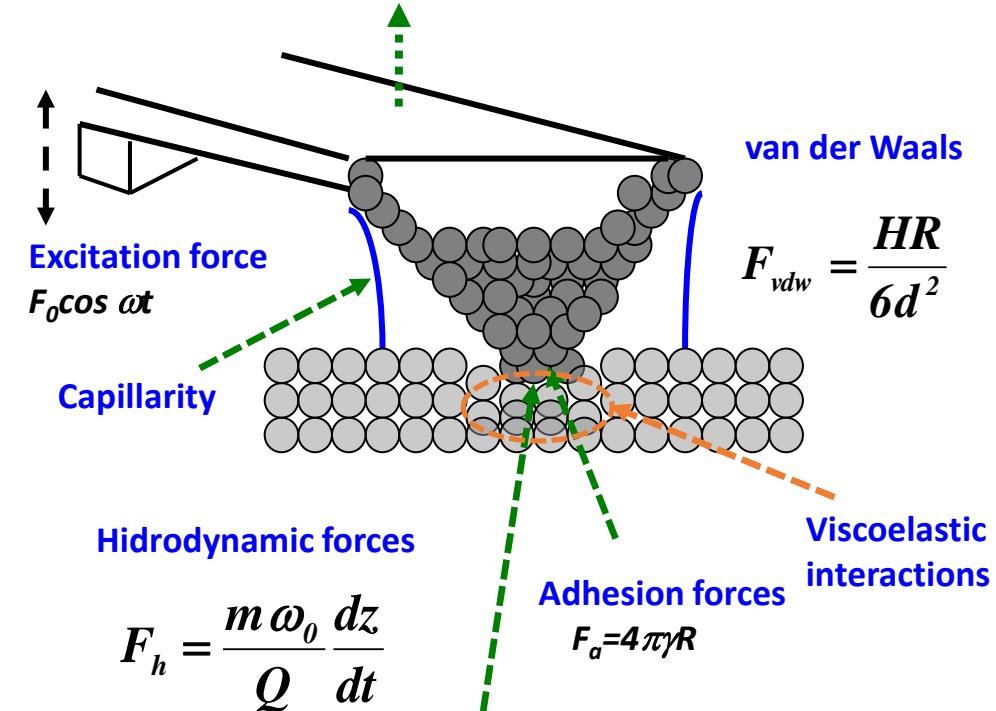
Common quantities in nanomechanical mapping		
Material property	Symbol	Definition
Young's/elastic modulus	E	Elastic quantity. Proportional factor between the stress (force per unit of area) and the strain (change of length per unit of length) in an uniaxial deformation.
Shear/ torsional modulus	G	Elastic quantity. Proportional factor between the shear stress and shear strain.
Poisson's ratio	ν	Elastic quantity. Ratio between lateral and longitudinal deformations.
Relationship between elastic quantities		$E=2G(1+\nu)$
Complex modulus	$E^*=E'+iE''$	Viscoelastic quantity.
Storage modulus	E'	Viscoelastic quantity. Real component of the complex modulus. It is proportional to the average energy stored per unit of volume of the material during a cycle of deformation.
Loss modulus	E''	Viscoelastic quantity. Imaginary component of the complex modulus. It is proportional to the energy dissipated per unit of volume of the material during a cycle of deformation.
Loss tangent	$\tan \phi$	Ratio between the loss and storage moduli.
Viscosity coefficient	η	Proportional factor between the shear stress and the velocity of shear.

Seudo material properties	Symbol	
Interfacial stiffness	k_s	Elastic quantity. Slope of a force-distance curve. It is a pseudo material property because it depends on the geometry.
Adhesion force		It depends on the contact area
Deformation	δ	It depends on the applied force
Energy dissipation	E_{ts} or E_{dis}	It depends on the contact area

Forces in AFM

Restoring force cantilever

$$F_c = -kz$$



Adhesion forces
i). Unspecific
ii). specific

R. Garcia, Amplitude modulation AFM, Wiley (2010)

Equation of motion

On-resonance

$$F_{ts}(d(t)) = kz + \frac{m\omega_0}{Q}\dot{z} + m\ddot{z} - F_0 \cos \omega t$$

$$z = z_0 + A \cos(\omega t - \phi)$$

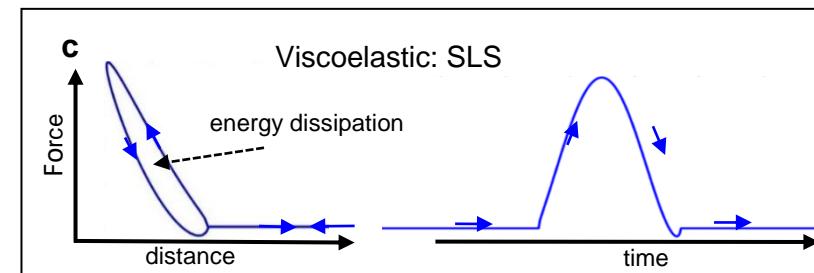
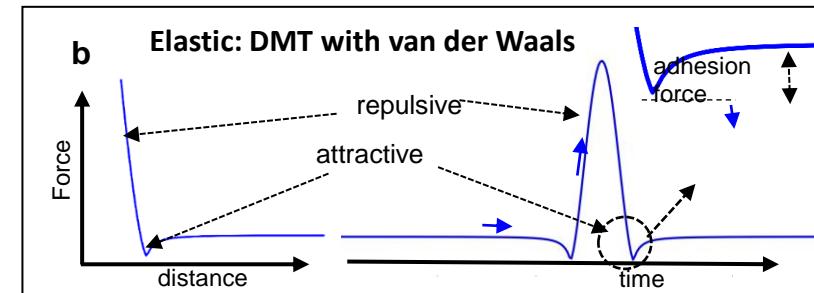
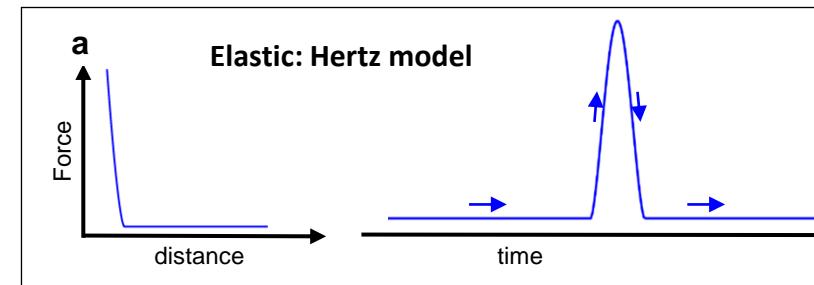
Off-resonance

$$F_{ts}(d(t), \dot{d}(t)) = kz + \frac{m\omega_0}{Q}\dot{z} + m\ddot{z}$$

$$d(t) = z_c + z(t) + A_m g(\omega_m t)$$

Main features of force-distance curves

$$F_{ts} = F_{con} + F_{dis}$$



Forces

Elastic deformations

Constitutive equations. It relates stress and strain

$$\sigma_{ij}(t) = \frac{E}{3(1-2\nu)} u_{ll}(t)\delta_{ij} + \frac{E}{1+\nu} \left(u_{ij}(t) - \frac{1}{3} u_{ll}(t)\delta_{ij} \right)$$

$$F_{ts}(I) = \alpha EI^\beta$$

axisymmetric probe

I.N. Sneddon, *Int. J. Eng. Sci.* **3**, 47-57 (1965)

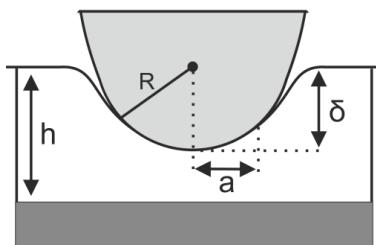
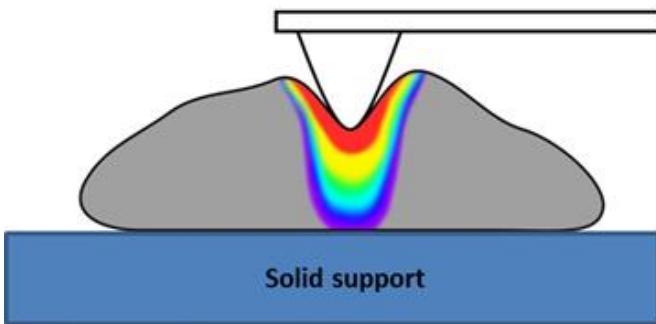
$$\frac{1}{E_{eff}} = \frac{1-\nu^2}{E} + \frac{1-\nu_t^2}{E_t} \approx \frac{1-\nu^2}{E}$$

coefficients to determine the force

Tip's geometry	α	β
Cylinder	$2a$	1
	$\frac{2a}{(1-\nu^2)}$	
Cone	$\frac{2 \tan \theta}{\pi(1-\nu^2)}$	2
Half-sphere	$\frac{4\sqrt{a}}{3(1-\nu^2)}$	1.5

R. Garcia, Chem. Soc. Rev. 49, 5850-5884 (2020)

Elastic models for soft matter as finite layers (bottom-effect elastic correction theories)

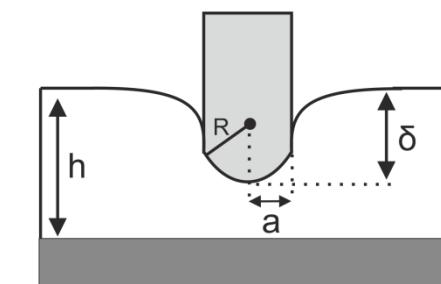
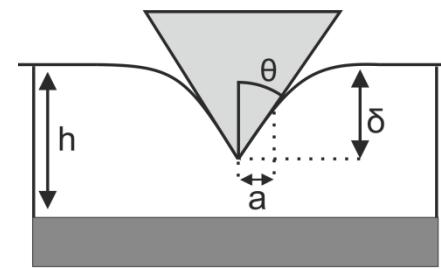
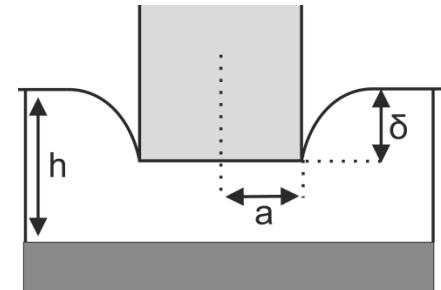


$$\textbf{Force } (I) = \mathbf{F}_{\text{Sneddon}} + \mathbf{F}(a, h)$$

$$\text{Force } (I) = \sum_{j=0}^N \alpha_j EI^{\beta_j} = \sum_{j=0}^N F_j$$

j	F_j (sphere)
0	$\frac{16}{9} \sqrt{RI}[EI]$ semi-infinite
1	$1.133 \frac{16}{9} \sqrt{RI}[EI] \frac{\sqrt{RI}}{h}$
2	$1.497 \frac{16}{9} \sqrt{RI}[EI] \left(\frac{\sqrt{RI}}{h}\right)^2$
3	$1.469 \frac{16}{9} \sqrt{RI}[EI] \left(\frac{\sqrt{RI}}{h}\right)^3$
4	$0.755 \frac{16}{9} \sqrt{RI}[EI] \left(\frac{\sqrt{RI}}{h}\right)^4$

Expressions for any axisymmetric tip (paraboloid, cone, cylinder, needle)
P.D. Garcia, R. Garcia, Biophys. J. 114, 2923 (2018)

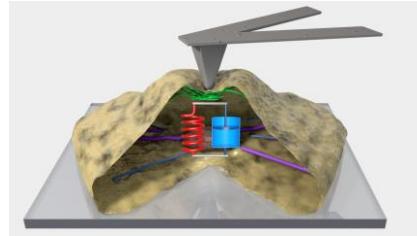


Forces

Viscoelastic deformations

Equivalence elastic-viscoelastic principle

E. H. Lee and J. R. M. Radok, *J. Appl. Mech.* 1960, **27**, 438-444



$$F(I(t), t) = \alpha \int_0^t \psi_E(t-t') \frac{d}{dt'} (I(t')^\beta) dt'$$

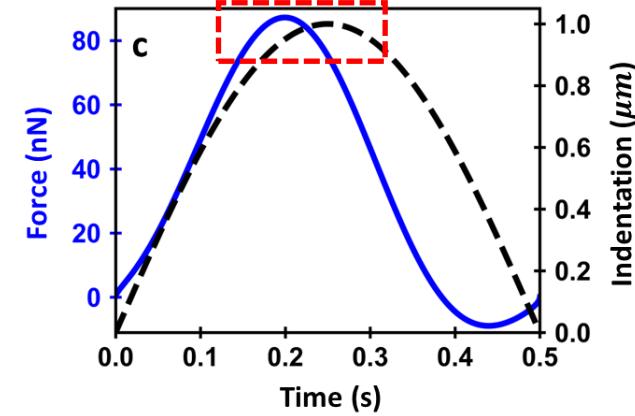
$$\psi_{KV}(t) = E + 3\eta I(t)$$

$$F(I, t) = \alpha I(t)^{\beta-1} [EI(t) + 3\beta\eta \dot{I}(t)]$$

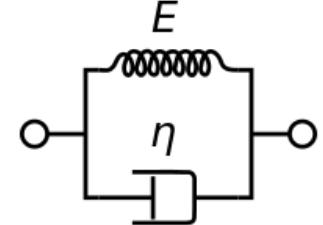
$$F_{ts}(I, dI/dt) = \frac{4}{3} \frac{E}{1-\nu^2} \sqrt{R} I^{3/2} + \frac{6}{1-\nu^2} \sqrt{RI} \eta \dot{I}$$

P.D. Garcia, R. Garcia, *Nanoscale* 10, 19799 (2018)

Viscoelasticity: Lag between force and deformation

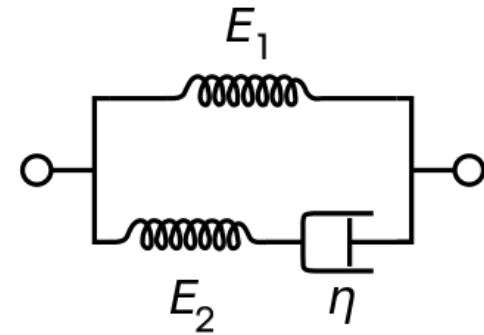


Kelvin-Voigt



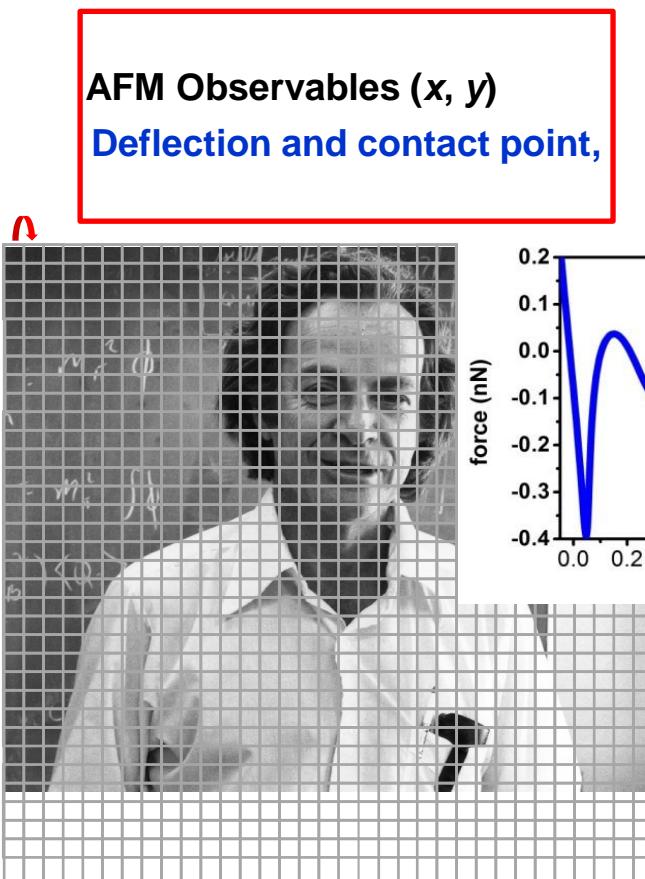
$$\sigma(t) = E\varepsilon(t) + \eta\dot{\varepsilon}(t)$$

SLS model



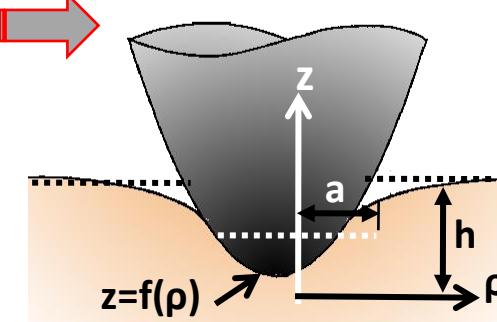
$$\dot{\varepsilon} = \frac{\dot{\sigma} + \frac{E_2}{\eta}\sigma - \frac{E_1 E_2}{\eta}\varepsilon}{E_1 + E_2}$$

Force-volume approaches



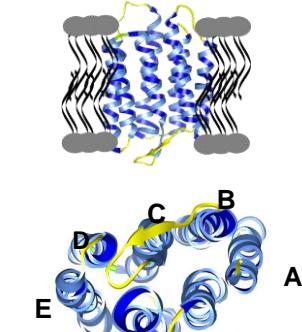
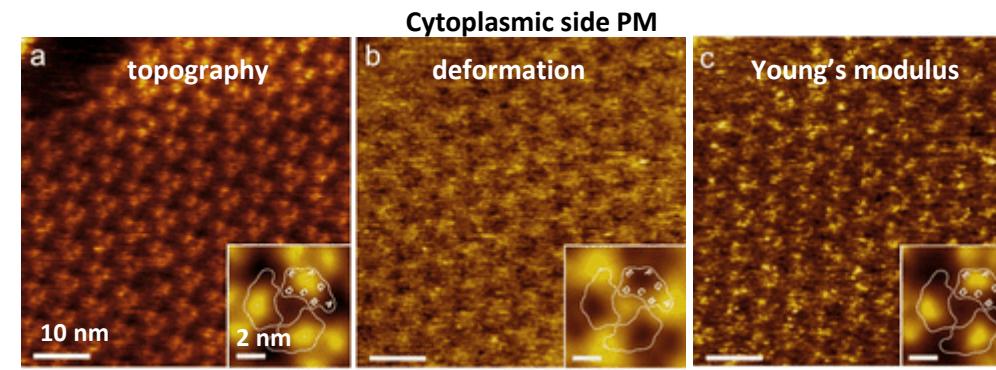
Slope of FDC,
adhesion &
hysteresis

Contact mechanics



Material properties

Young's modulus,
energy adhesion hysteresis,
viscoelastic parameters



I. Medalsy, U. Hensen and D. J. Muller, *Angew. Chem. Int. Ed.* 2011, **50**, 12103-12108.

References:

Pioneer: M. Radmacher, ..., P. Hansma, *Biophys. J.* 66, 2159 (1994)

Theory: C.A. Amo and R. Garcia, *ACS Nano* 10, 7117 (2016)

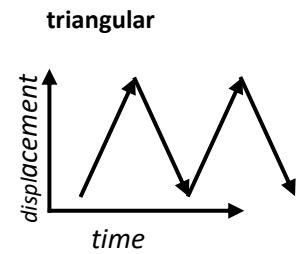
Reviews : W. F. Heinz and J.H. Hoh, *Trends Biotechnol.* 17, 143–150 (1999);

H.J. Butt et al. *Surf. Sci. Rep.* **59**, 1–152 (2005); R. Garcia, *Chem. Soc. Rev.* (2020)

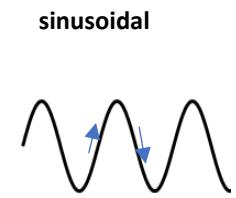
FV names (comercial software): PeakForce QNM, FastForceMapping, PinPoint Nanomechanical mode or Quantitative Imaging QI.

Related methods: Pulse-force mode

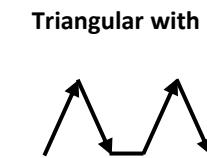
FV waveforms



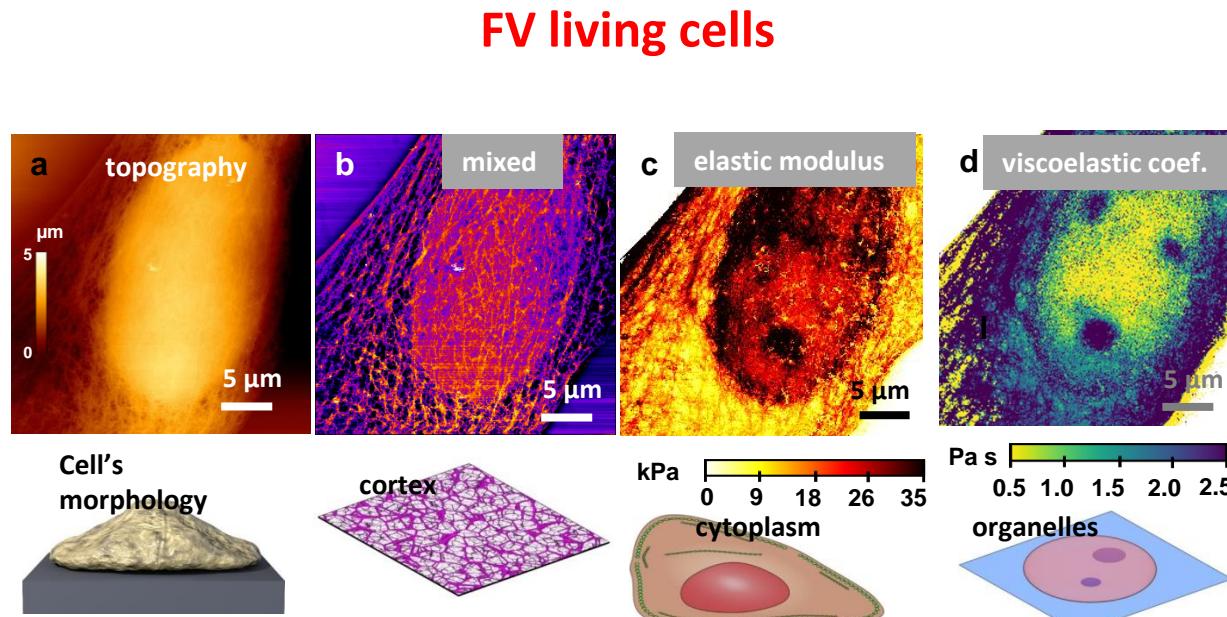
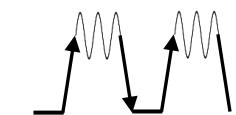
constant velocity
change of speed at the turn around point
Many harmonics



Variable velocity
Analytical solutions
Single harmonic



Loading and dwell steps

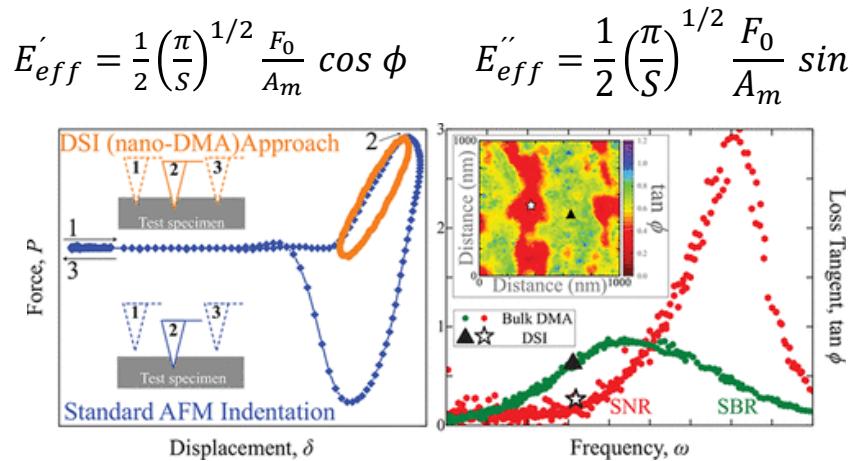


C.R. Guerrero, P.D. Garcia, R. Garcia, ACS Nano **13**, 969 (2020)

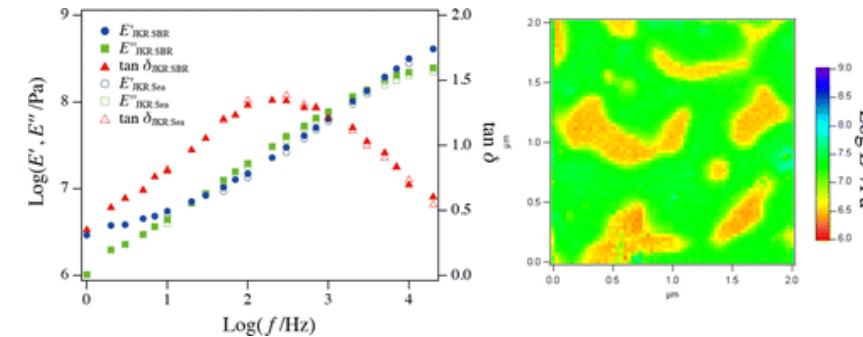
Nanoscale rheology

Definition: spatially-resolved maps of time-dependent properties. Currently based on FDCs

Nanoscale rheology with sinusoidal waveforms

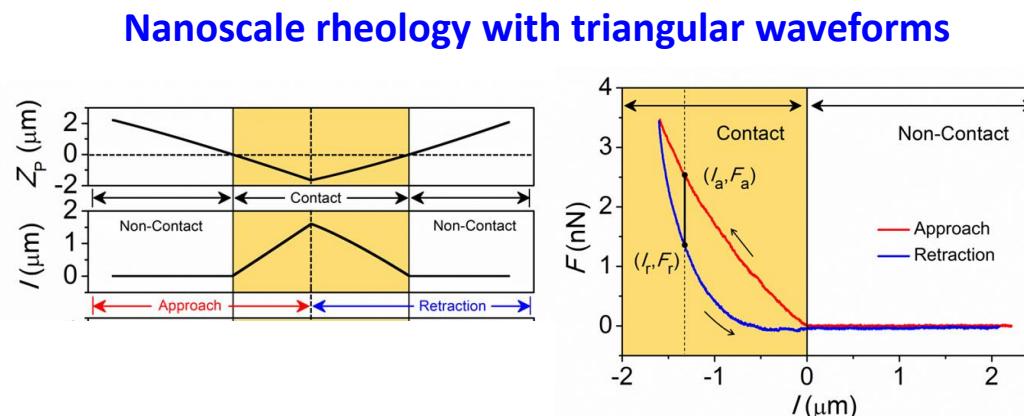


P.V. Kolluru,..., L.C. Brinson, *Macromolecules* 51, 8964 (2018).

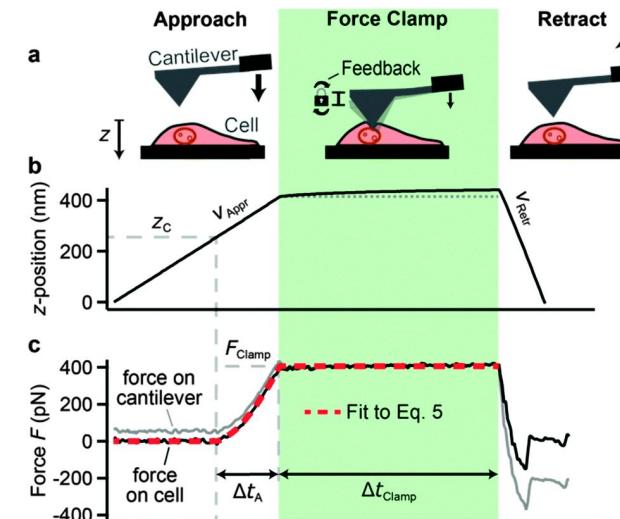


T. Igarashi, ..., K. Nakajima, *Macromolecules* 2013, **46**, 1916-1922.

Nanoscale rheology with mixed waveforms



P.D. Garcia, C.R. Guerrero, R. Garcia, *Nanoscale* 9, 12051 (2017)



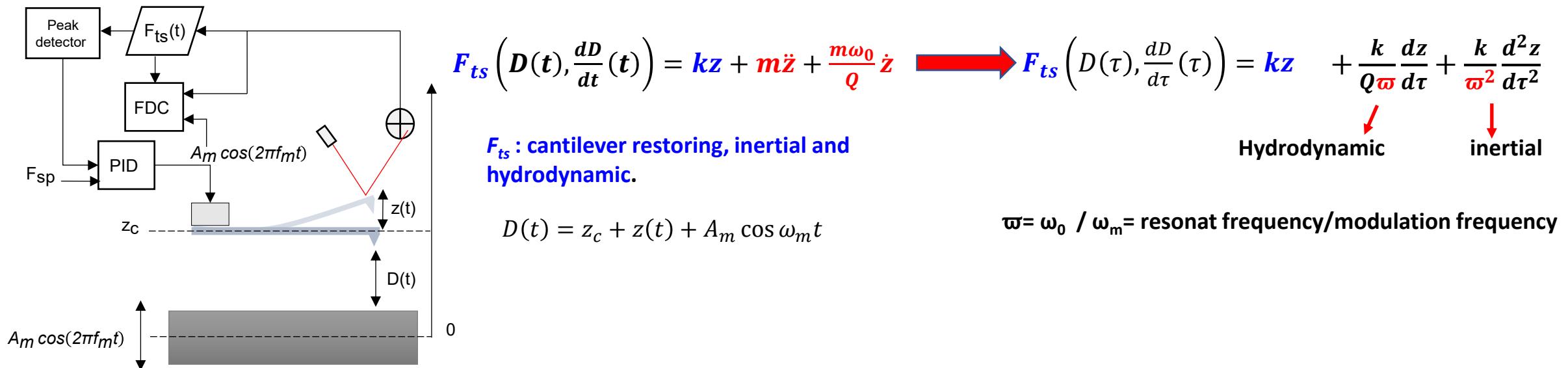
F.M. Hecht, ..., B. Fabry and T.E. Schäffer, *Soft Matter*, 2015, **11**, 4584.

The postulate of force spectroscopy

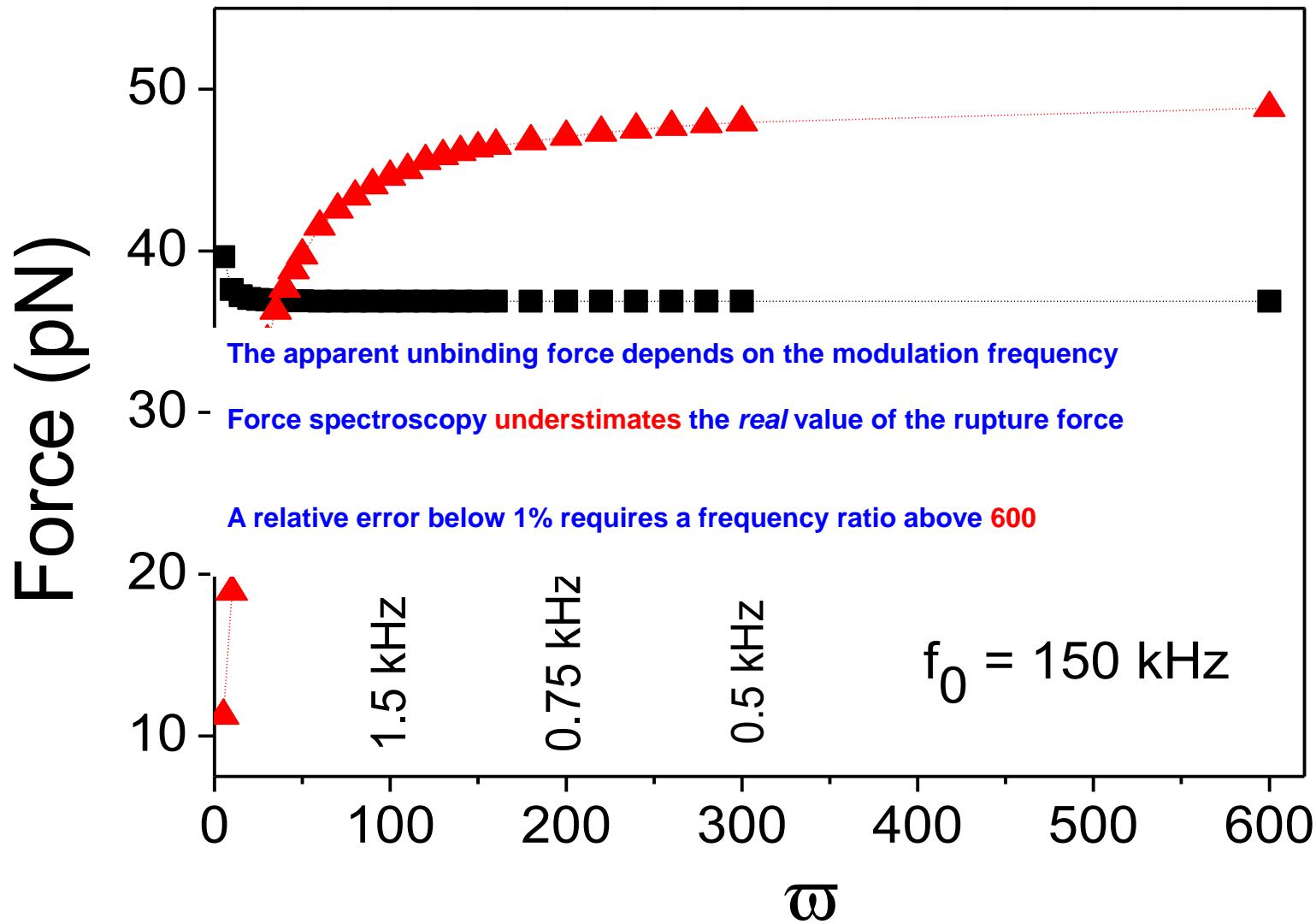
Force-volume and AFM-based single-molecule force spectroscopy measurements are based on Hooke's law. Hooke's law establishes that the interaction force coincides with the cantilever deflection times its force constant.

$$F_{ts}(t) = kz(t)$$

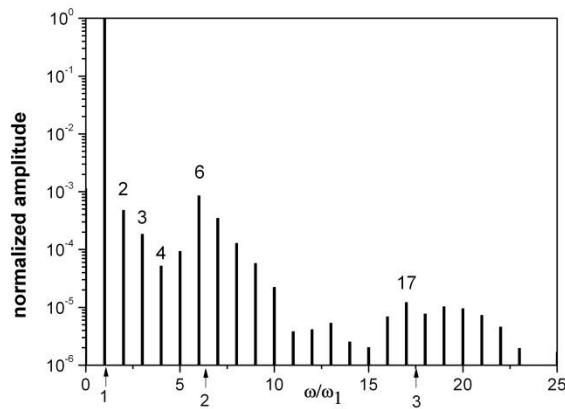
Strictly speaking, Hooke's law is only valid for a static deflection, nonetheless.



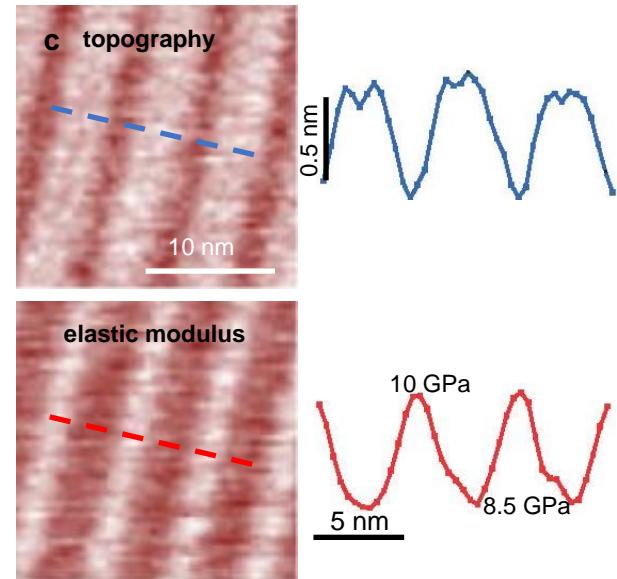
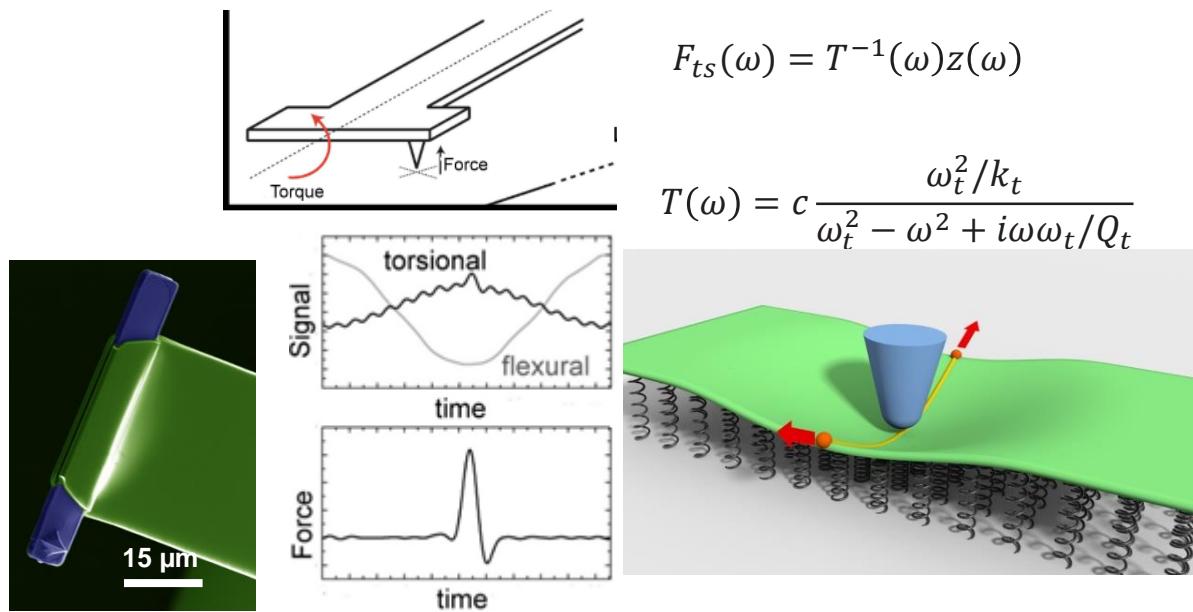
C.A. Amo, R. Garcia, ACS Nano **10**, 7117 (2016)



Time-resolved forces by measuring
the harmonic components



Torsional harmonics: Force-distance curves from on-resonance methods



L. Liu...M. Dong, Adv. Sci. 3, 1500369 (2016)

$F_{ts}(t)$ is obtained by performing the inverse Fourier transform of $F_{ts}(\omega)$. T is the transfer function of the torsional signal;

References:

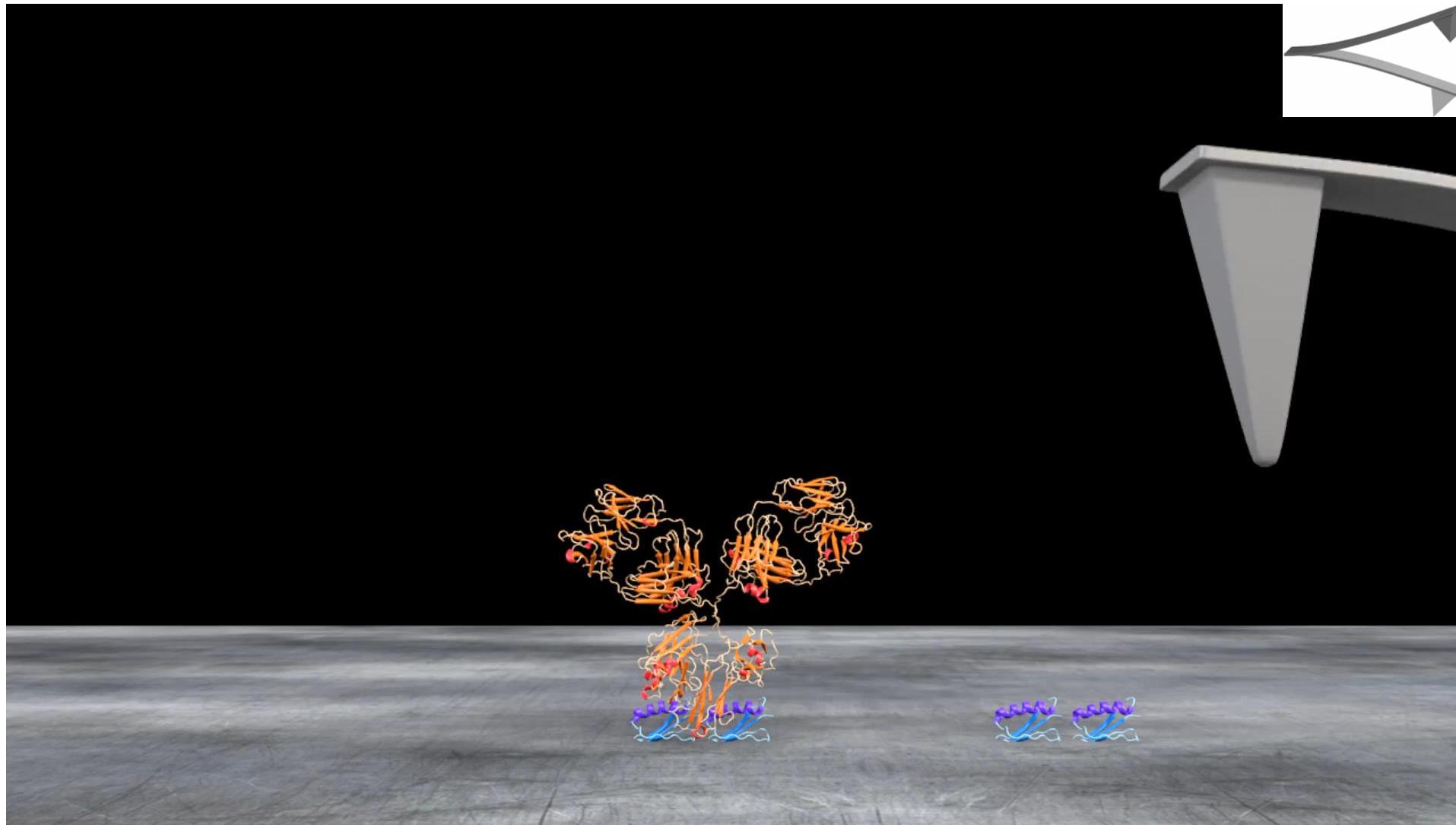
- Pioneer: M. Stark et al. *Proc. Natl. Acad. Sci.* 2002, **99**, 8473–8478;
O. Sahin et al. *Nat. Nanotechnol.* **2**, 507 (2007)
Reviews : S. Zhang,...,M. Dong, *Chem. Soc. Rev.* 2014, **43**, 7412–7429

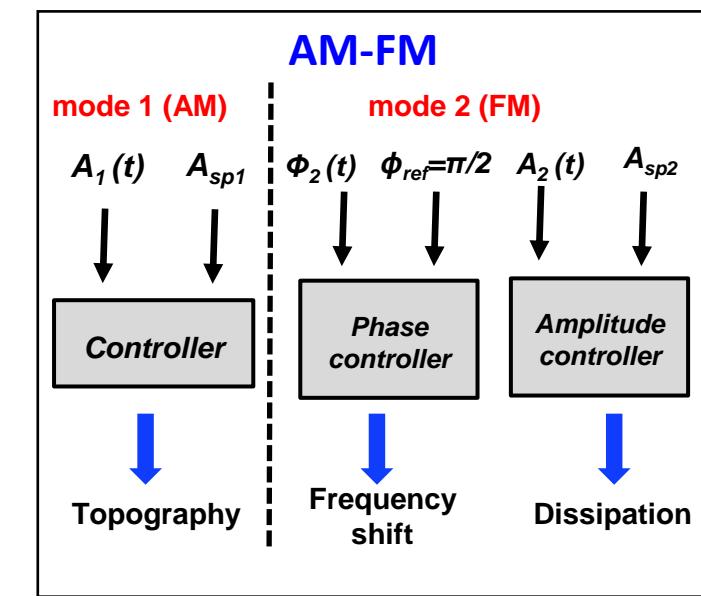
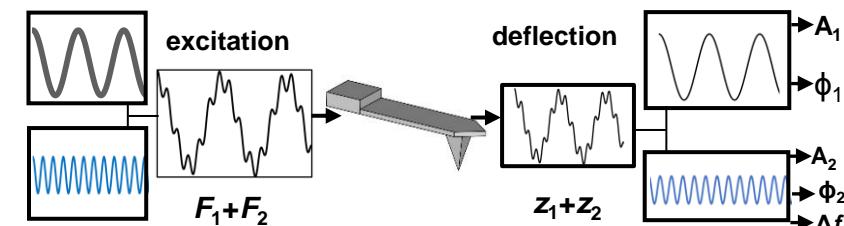
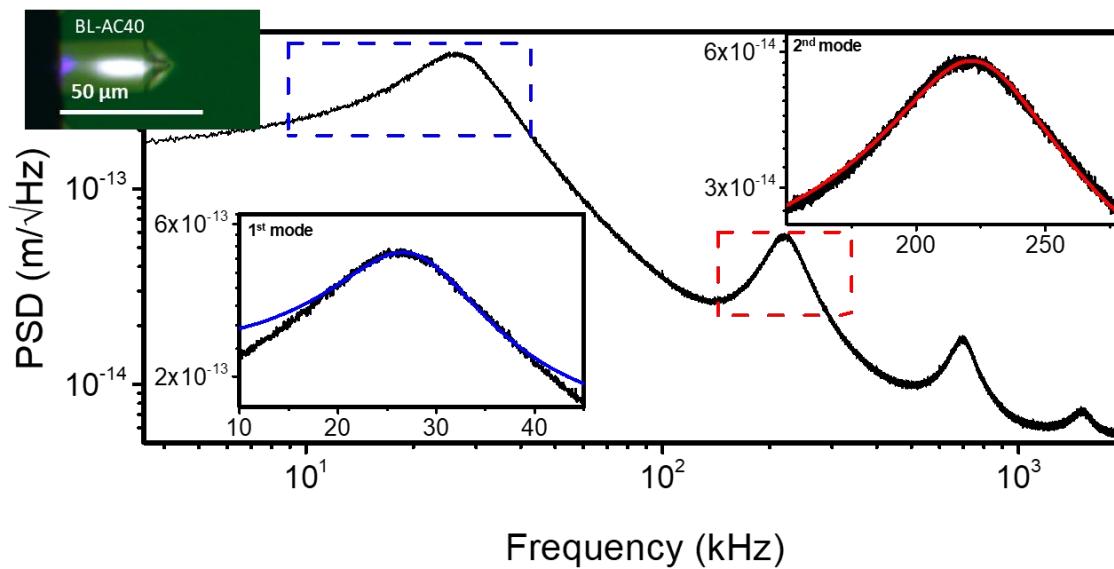
Cellular nanoscale stiffness patterns governed by intracellular forces

Nicola Mandriota^①, Claudia Friedsam², John A. Jones-Molina^①, Kathleen V. Tatem^{①,3},
Donald E. Ingber^{②,4,5} and Ozgur Sahin^{③,6*}

nature
materials LETTERS
<https://doi.org/10.1038/s41563-019-0391-7>

Nanomechanical mapping by Bimodal AFM

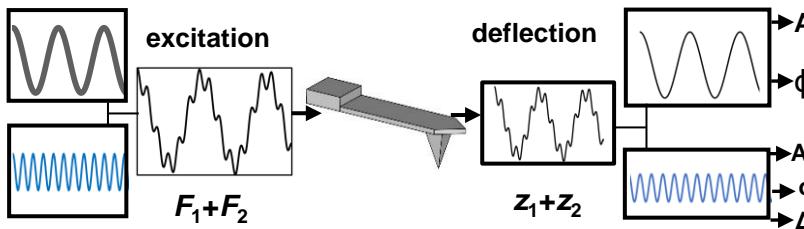




References:

- Pioneer: T.R. Rodriguez, R. Garcia, APL 84, 449 (2004);
 Theory: J.R. Lozano, R. Garcia, Phys. Rev. Lett. 100, 076102 (2008);
 A Labuda,..., R. Proksch, Beilstein J. Nanotechnol. 7, 970-982 (2016).
 Reviews : R.Garcia, R. Proksch Eur. Polym. J. 49, 1897 (2013);
 R. Garcia. Chem. Soc. Rev.49, 5850 (2020)

Theory Bimodal AFM: (AM-FM)



3D Kelvin-Voigt
(paraboloid)

Virial and energy balance theorems to the excited modes

J.R. Lozano and R. Garcia, Phys. Rev. B 79, 014110 (2009)

First and second mode observables

$$V_1 = -\frac{k_1 A_{01}}{2 Q_1} A_1 \cos \phi_1$$

$$V_2 = -\frac{A_2^2 k_2 \Delta f_2}{f_2}$$

$$E_{dis1} = \frac{\pi k_1 A_1}{Q_1} (A_1 - A_{01} \sin \phi_1)$$

C. A. Amo et al. ACS Nano, 11, 8650 (2017)

S. Benaglia, C.A. Amo, R. Garcia, Nanoscale (11, 15289) 2019

M. Kocun et al. ACS Nano 11, 10097 (2017)

$$F = 4/3 E_{eff} \sqrt{R_t I^3} - 2\eta_{com} \sqrt{R_t I} \dot{I}$$

Young's Modulus

$$E_{eff} = \sqrt{\frac{8A_{01}}{R_{tip}} \frac{V_2^2}{V_1} \frac{A_1}{A_2^4}}$$

viscosity

$$\eta_{com} = (2\pi\omega_1)^{-1} E_{eff} \frac{E_{dis1}}{V_1}$$

Observables

Contact Mechanics

Material properties

Analytical expressions:
observables & material properties

Indentation

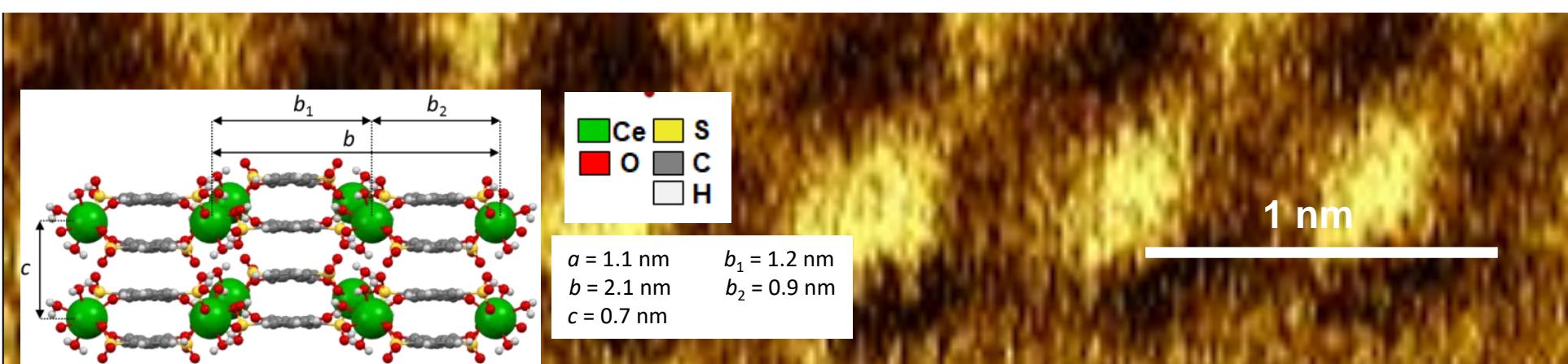
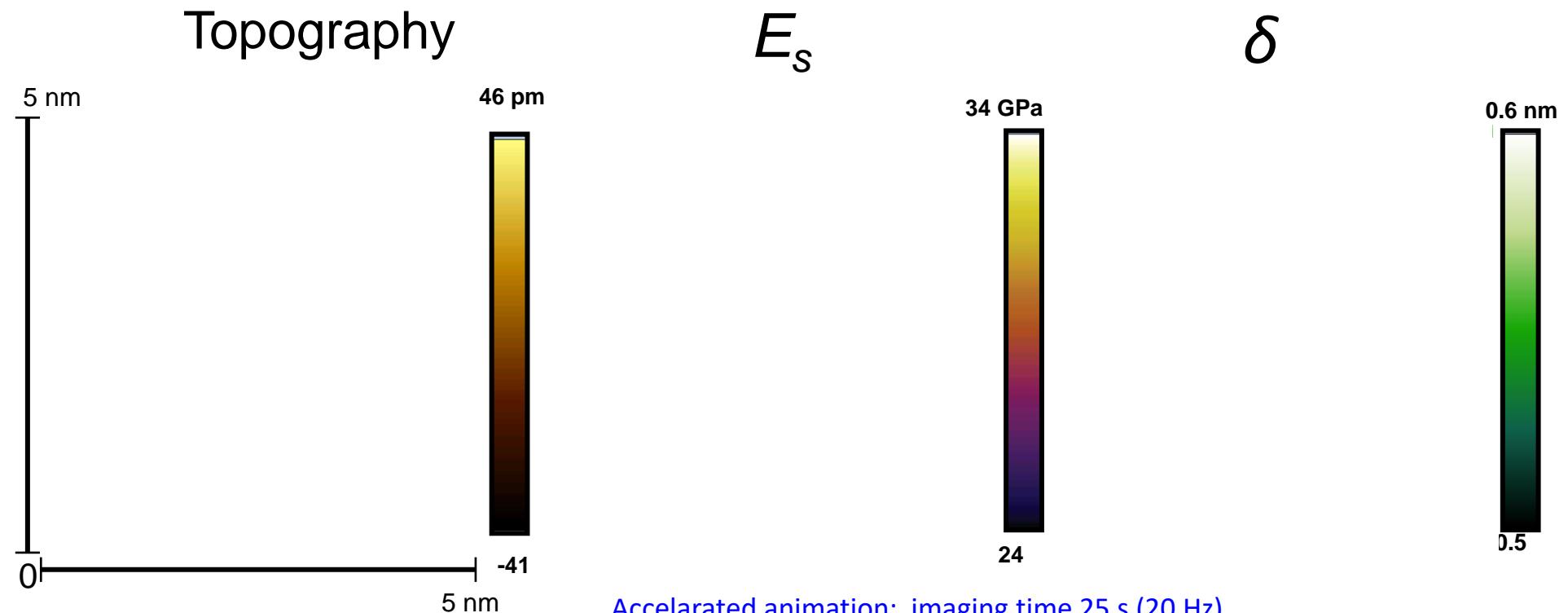
$$\delta = \frac{V_1}{V_2} \frac{A_2^2}{A_1}$$

Loss tangent

$$\tan \delta = \frac{\sin \phi_1 - A_1/A_{01}}{\cos \phi_1} = \omega_1 \frac{\eta_{com}}{E_{eff}} = \omega_1 \tau$$



Metal-organic-framework



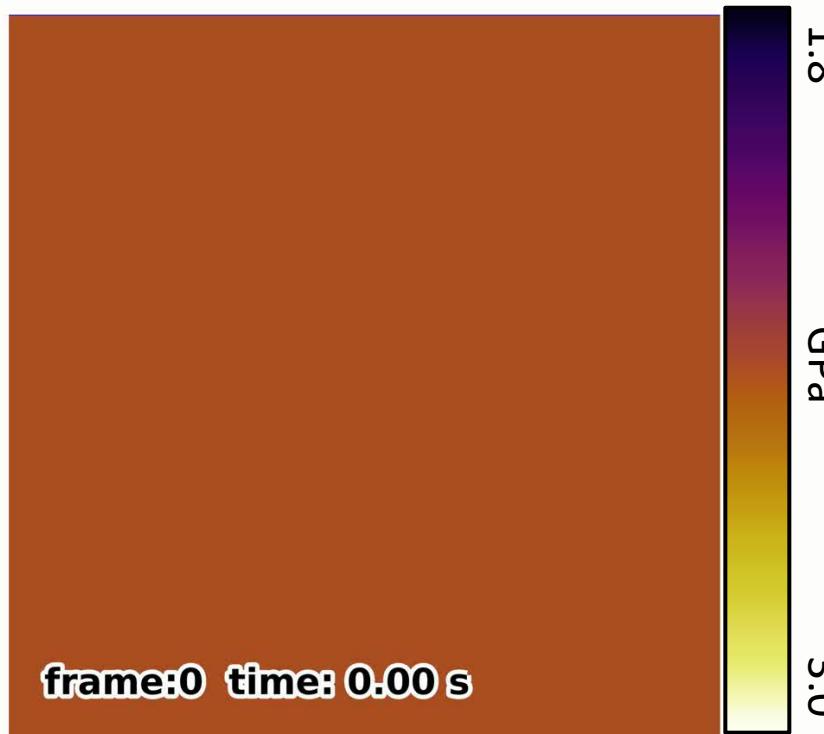
$a = 1.1 \text{ nm}$ $b_1 = 1.2 \text{ nm}$
 $b = 2.1 \text{ nm}$ $b_2 = 0.9 \text{ nm}$
 $c = 0.7 \text{ nm}$

1 nm

Bimodal AM-FM: towards video-rate imaging

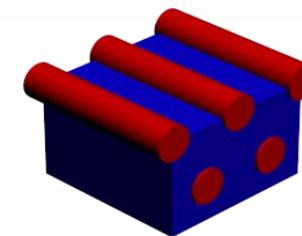
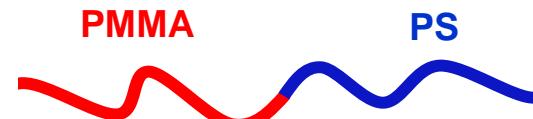
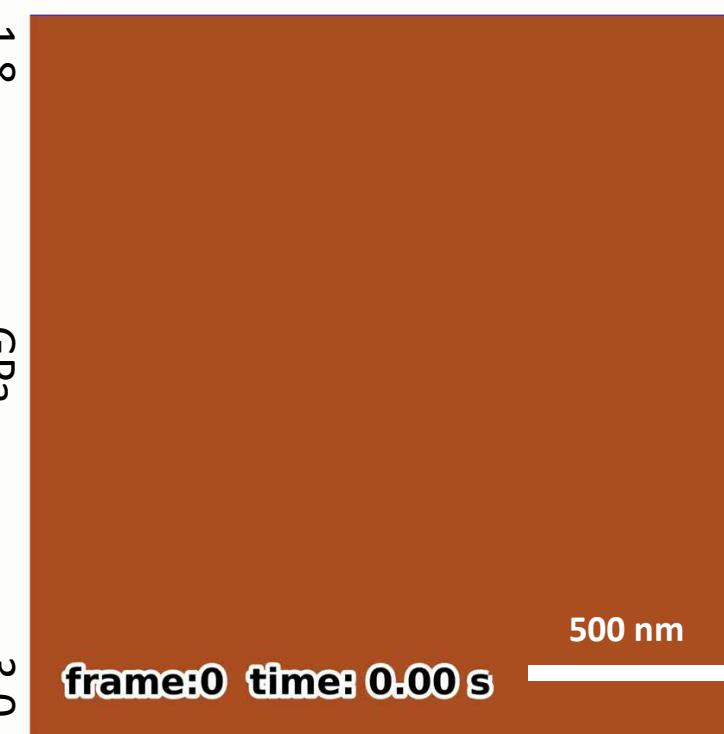
Fast Young's modulus map

10 Hz, 512x512 pix² 51 s/frame

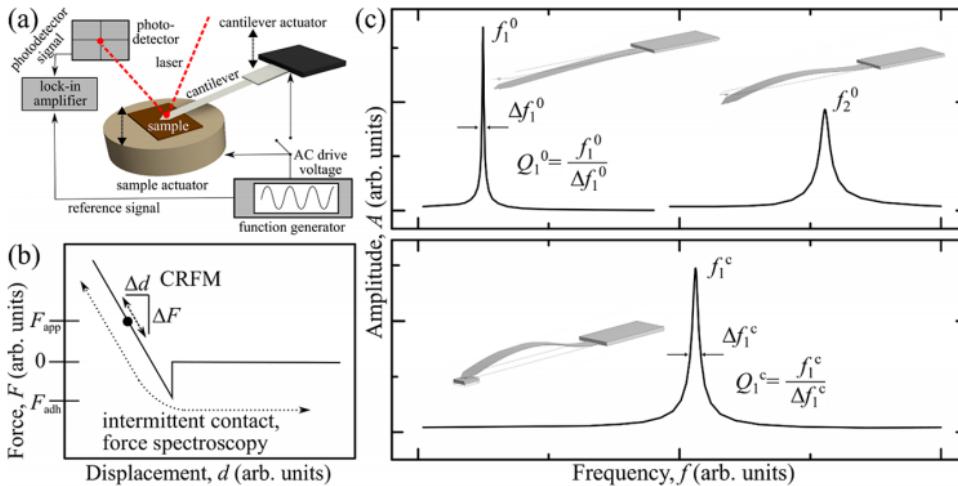


High speed Young's modulus map

200 Hz, 512x512 pix² 2.8 s/frame

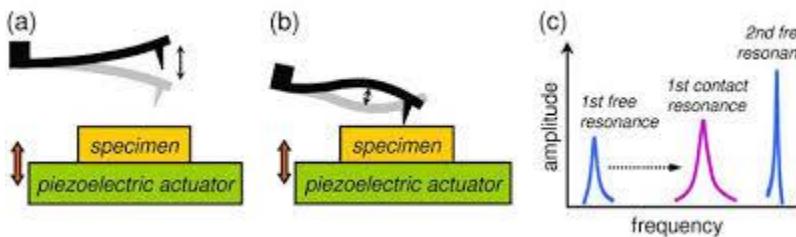
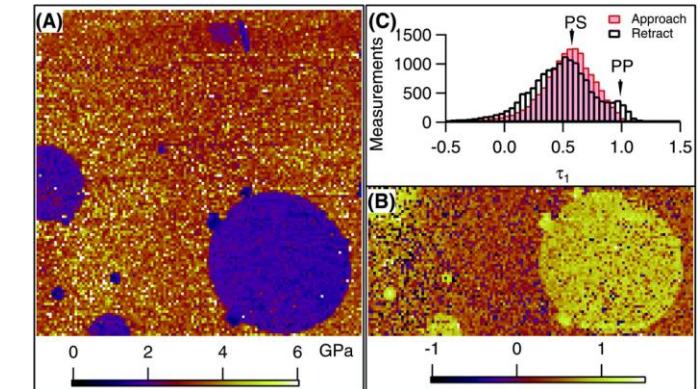
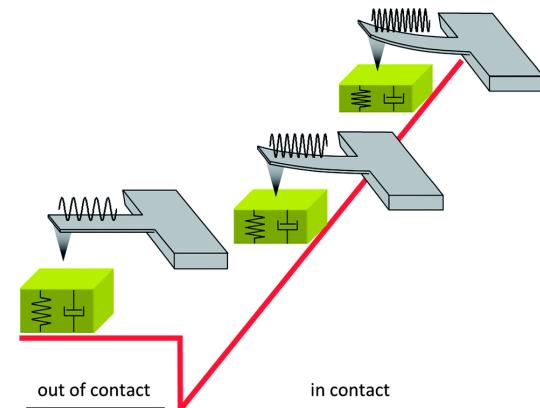


Contact resonance AFM



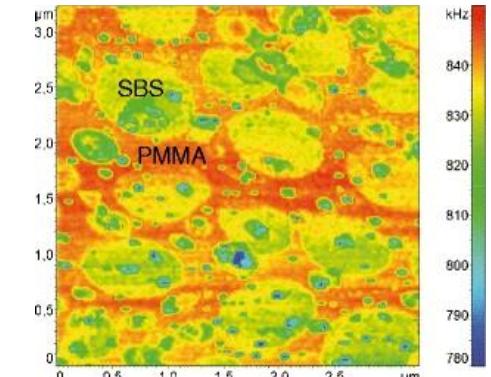
J.P. Killgore and F.W. DelRio, Macromolecules 51, 6977 (2018).

Mechanical parameters deduced by following frequency shifts



G. Stan et al. Nanoscale 6, 962 (2014)

$$E_{eff}^*(x, y) = E'_{eff} + iE''_{eff} = \frac{k_{ts}}{2r_c} + i \frac{\pi f_c c_{ts}}{r_c}$$



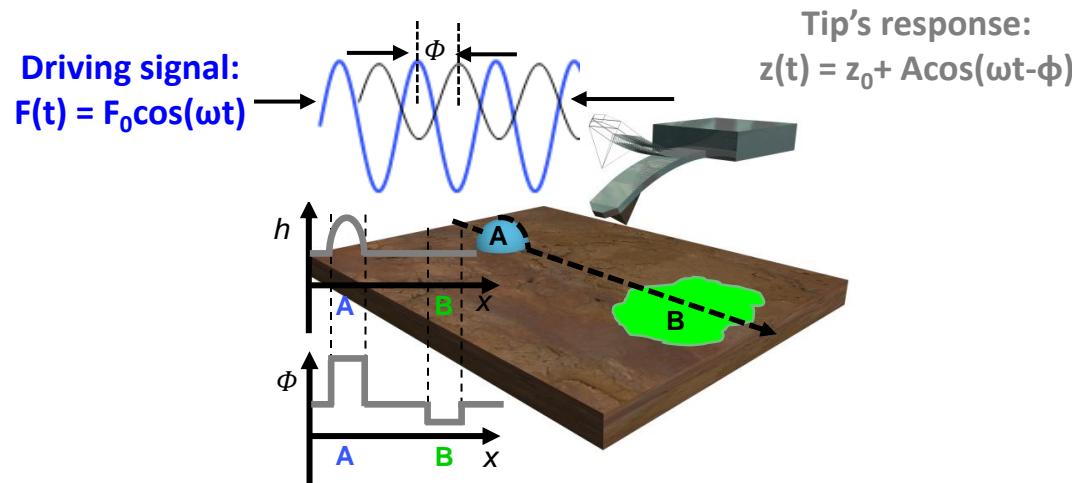
D. Passeri et al. (2013)

References:

- Pioneer:** U. Rabe, I.Turner, W. Arnold, *Appl. Phys. A-Mater.* 66, S277 (1998).
- Theory:** P.A. Yuya, D.C. Hurley and J.A. Turner, *J. Appl. Phys.* 2008, 104, 074916; G.J. Verbiest and M.J. Rost, *Nat. Commun.* 6, 6444 (2015).
- Reviews :** G. Stan & S.W. King (2020); J.P. Killgore and F.W. DelRio, *Macromolecules* 51, 6977 (2018).

AFM phase imaging

Principle: The dynamic response of the cantilever is modified by the tip-surface interactions



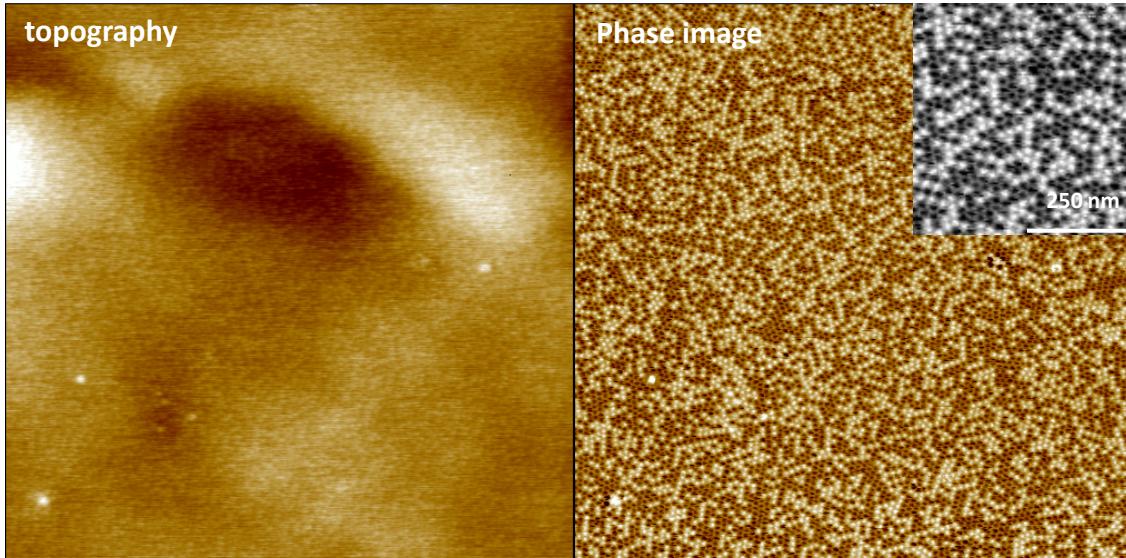
Semi-quantitative method

References:

- Pioneer:** D. Chernoff (1995); J. Tamayo, R. Garcia (1996);
S.N. Magonov (1997); J.P. Cleveland (1998)
Theory: R. Garcia *et al.* Phys. Rev. Lett. (2006)
Reviews : R. Garcia, Amplitude modulation AFM, Wiley (2010);
R. Garcia, Chem. Soc. Rev. (2020)

Polymers: Morphology and Structure

PEO-PB diblock copolymer

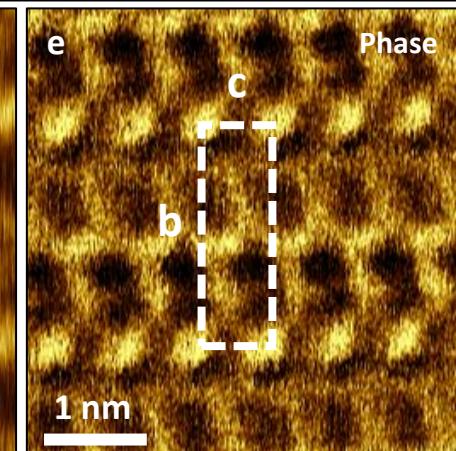
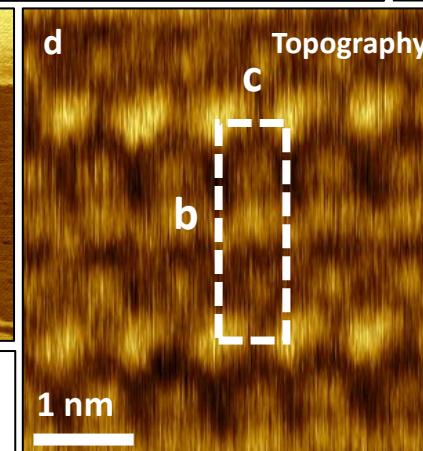
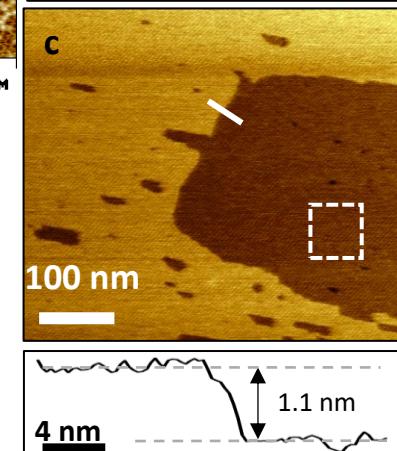
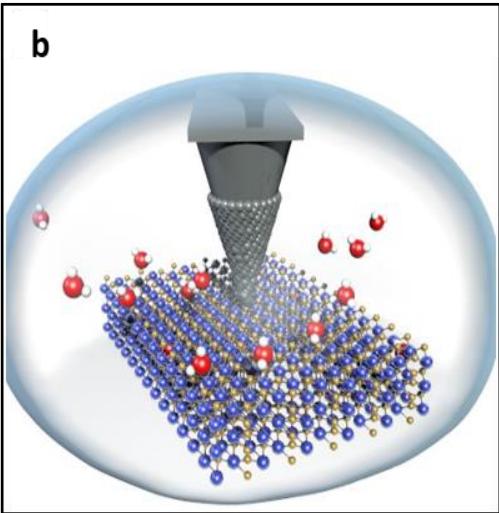
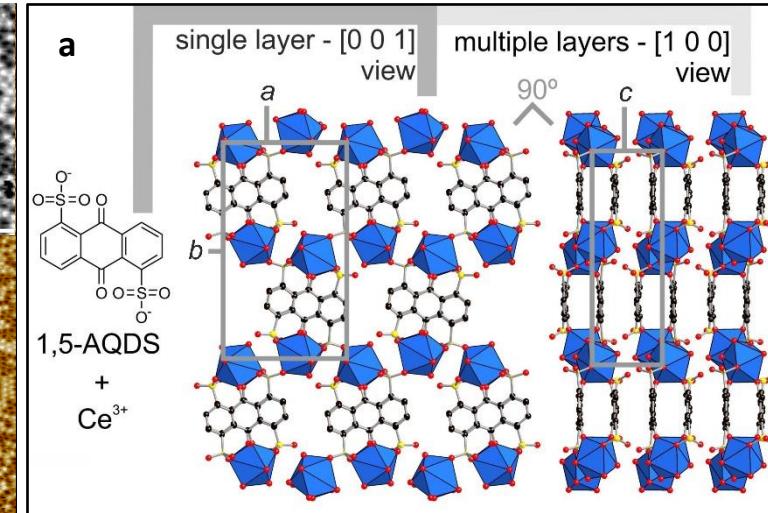


G. Reiter et al., Phys. Rev. Lett. 87, 2261 (2001)



Data type
Z range
2.00 μm

Phase
15.00 de



S. Chioldini et al. Sci. Rep. 7, 11088 (2017)

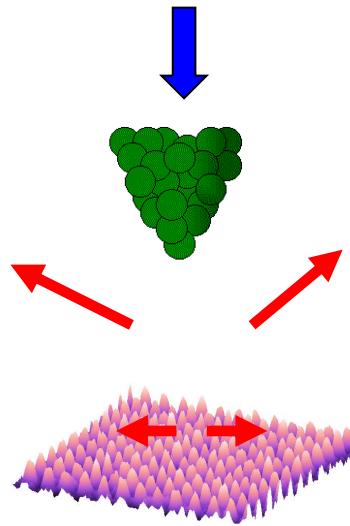
SBS triblock copolymer

A. Knoll, R. Magerle, G. Kraush,
J. Chem. Phys. 120, 1105 (2004)

Polymer morphology and structure as a function of temperature. Hydrogenated diblock copolymer (PEO-PB). Crystallisation of PEO blocks occurs individually for each sphere (light are crystalline, dark amorphous). **Reiter et al.**, Phys. Rev. Lett. 87, 2261 (2001)

Theory of AFM phase imaging

$$E_{dis} = \oint (F_{ts}) \frac{dz}{dt} dt$$



$$m \frac{d^2 z}{dt^2} = -kz - \frac{m\omega_0}{Q} \frac{dz}{dt} + F_{ts} + F_0 \cos \omega t \quad z = z_0 + A \cos(\omega t - \phi)$$

Energy balance per period

$$\bar{E}_{ext} = \bar{E}_{med} + \bar{E}_{dis}$$

$$E_{dis}(x, y) = E_{med} \left(\frac{A_0 \omega_0 \sin \phi(x, y)}{A_1 \omega_1} - 1 \right)$$

$$\sin \phi(x, y) = \frac{A_1 \omega}{A_0 \omega_0} \left(1 + \frac{E_{dis}(x, y)}{E_{med}} \right)$$

J. Tamayo and R. Garcia, Appl. Phys. Lett. 73, 2926 (1998)

Phase changes are associated to changes in the dissipated energy



S. Benaglia, C.A. Amo, R. Garcia, Nanoscale 11, 15289 (2019)

Loss tangent imaging

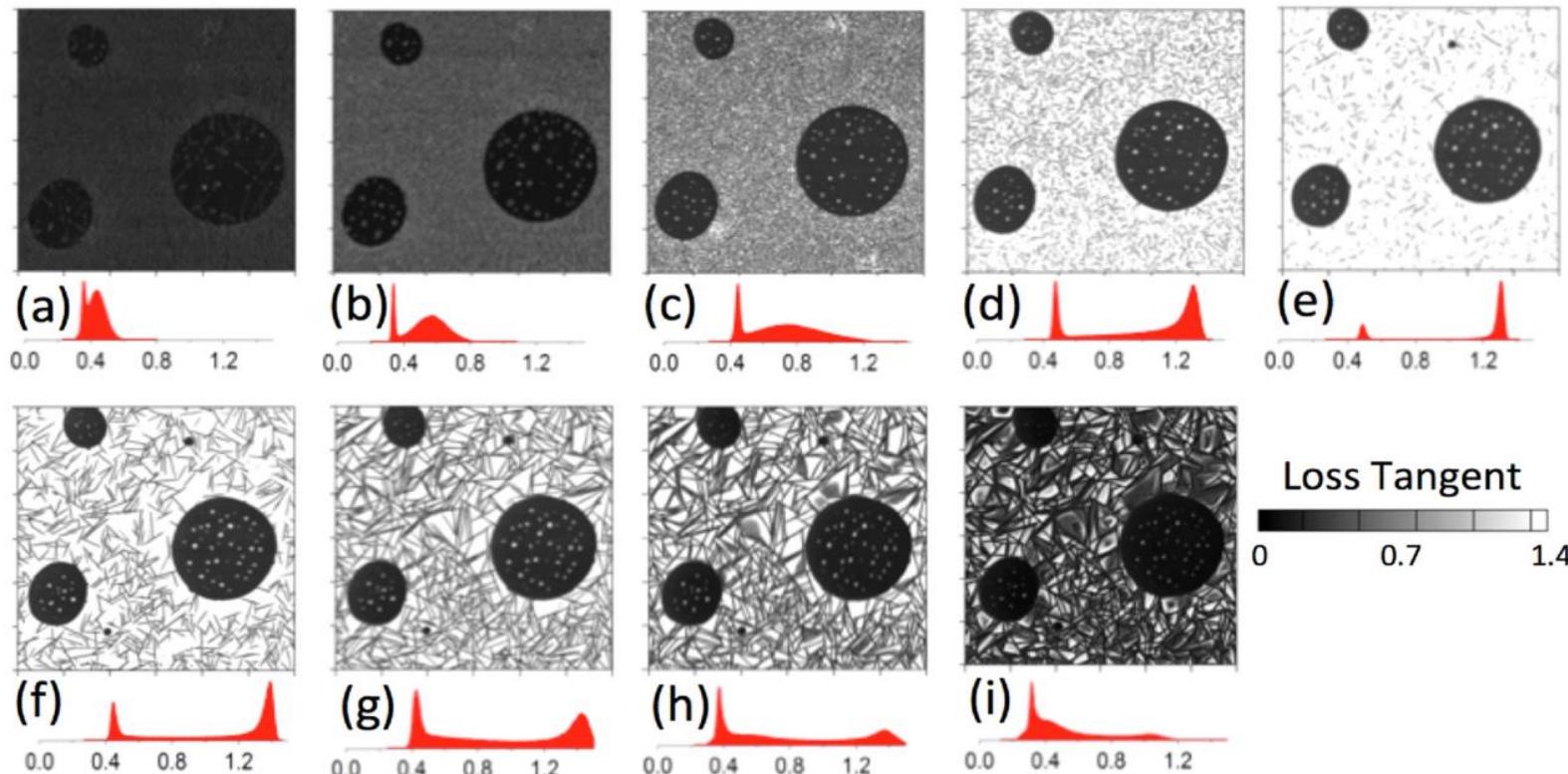
Dynamic mechanical analysis

$$\tan\delta = \frac{G''(\omega)}{G'(\omega)}$$

Amplitude-modulated AFM

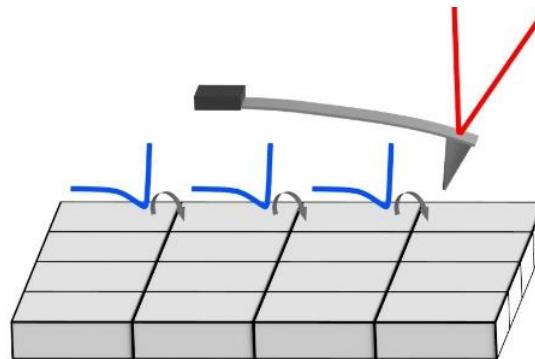
$$\tan\delta = \frac{\text{loss energy}}{\text{store energy}} = \frac{\oint F_{ts} \dot{z} dt}{\frac{1}{T} \oint F_{ts} z dt} = \frac{\sin\phi - \frac{A}{A_0}}{\cos\phi}$$

Energy balance/virial principle
A. San Paulo, R. Garcia Phys. Rev. B 64, 193411 (2001)

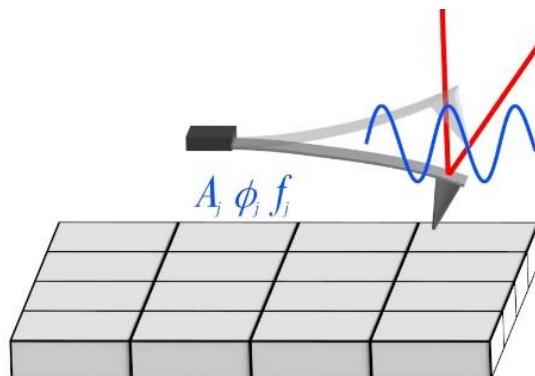


Thank you for your attention !

Force-distance curve vs parametric



On each pixel a FDC is measured



Parametric: observables are directly related to mechanical properties

Method	Material properties	Resolution	High-speed	Compatible with low forces (≤ 100 pN)
FV	Complex modulus; elastic modulus; loss tangent	1 nm	To be demonstrated	Yes
Bimodal AFM	Complex modulus; elastic modulus; loss tangent	0.5 nm	Yes	Yes
Torsional harmonics	Elastic modulus	5 nm	No	Yes
CR-AFM	Complex modulus; elastic modulus; loss tangent	≥ 20 nm	No	No
AFM phase imaging	Semi-quantitative; Loss tangent	0.5 nm	Yes	Yes