Atomic Force Microscopy

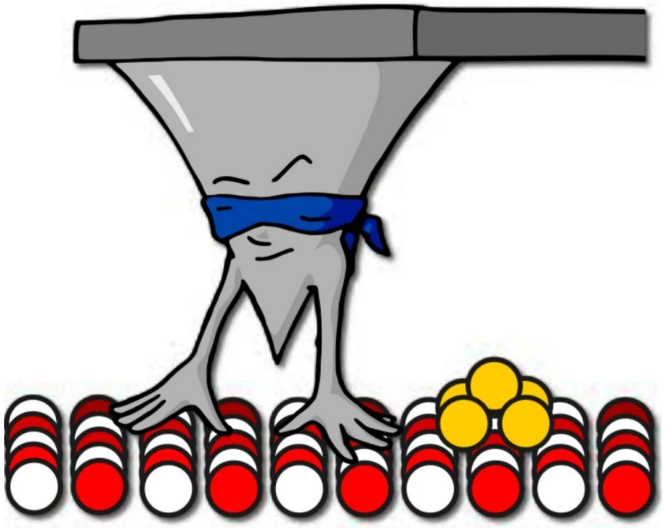
What is AFM

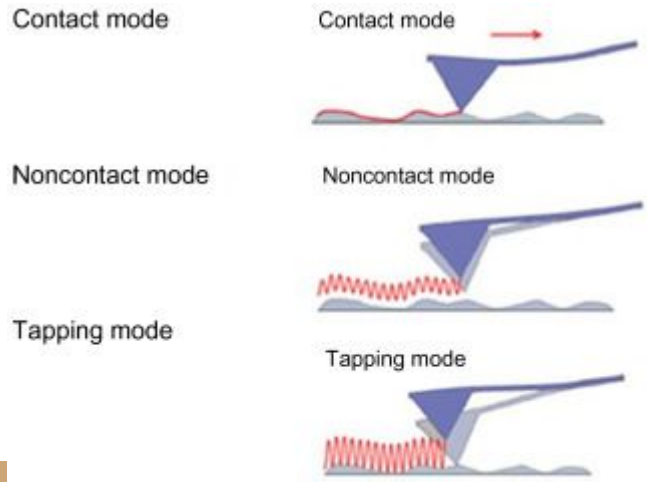
Scanning Probe microscopy

- Forces b/w tip and surface used to measure the characteristics of the surface
- Origin and STM

Resolution:

- Lateral resolution up to few nm
- Vertical resolution up to few Å
- Force resolution up to pN





Advantages and Abilities

Easy sample preparation:

- Can be performed in air, liquid medium or in vacuum
- Can work on conductive or non-conductive, hard or soft material, single protein or living cells

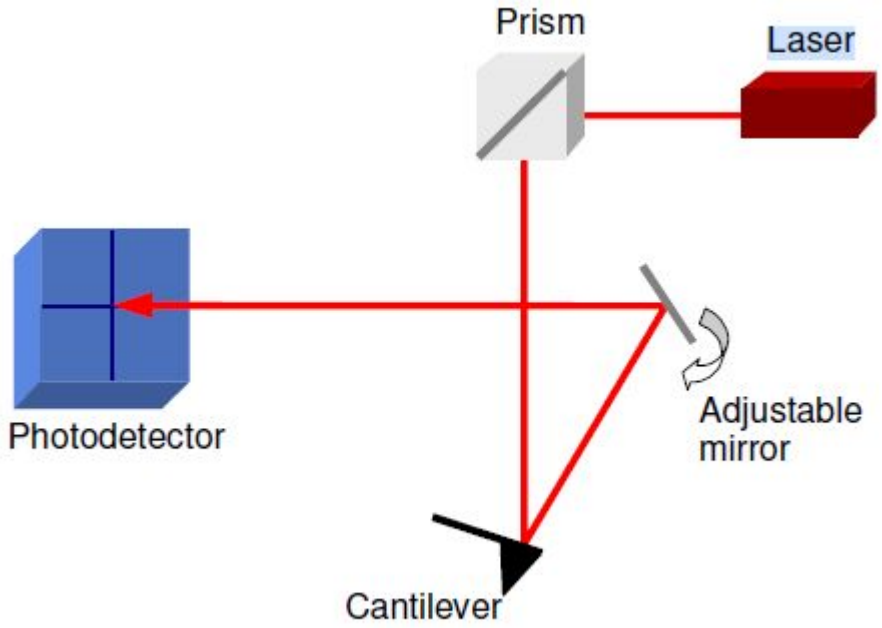
Not just a microscope

- Can provide 3D topography of surface
- Can measure elasticity, viscoelasticity of material
- Can be used as force manipulation tool
- Can measure surface properties

How it works

Measuring force and distances

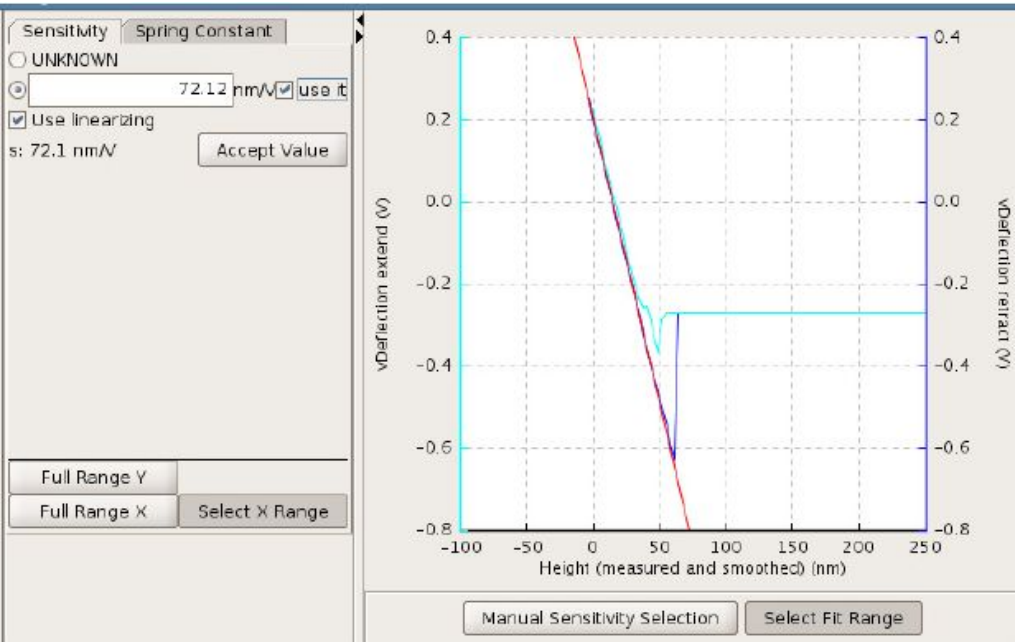
Calibration



Calibration:

Sensitivity(V→F)

By deflection vs approach force curves



Force Constant K

by measuring thermal fluctuations in deflection of cantilever

$$H = p^2/2m + (1/2)m\omega^2 q^2$$

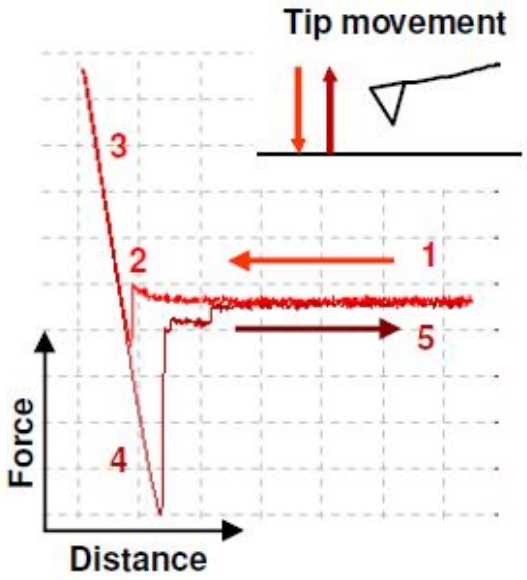
$$< \frac{1}{2} m\omega^2 q^2 >= \frac{1}{2} K_b T$$

$$\omega^2 = k/m,$$

$$k = kBT / < q^2 >$$

- In the spring constant calibration, we assume point mass model which is valid for freely vibrating cantilever.

Force Curves



The z-piezo extends, moving the cantilever towards the surface and retracting it again.

1. Approach of the tip from far distance
2. Tip snaps to the surface (jump to contact)
3. Increase of the repulsive force when the tip in very close contact with the sample. The movement stops when the vertical deflection reaches the **Relative Setpoint** value.
4. Retraction of the cantilever while the tip is still in contact (adhesion)
5. Tip is pulled free from the surface. The movement stops after a total retraction of **Z length**.

Modes of AFM

Contact mode

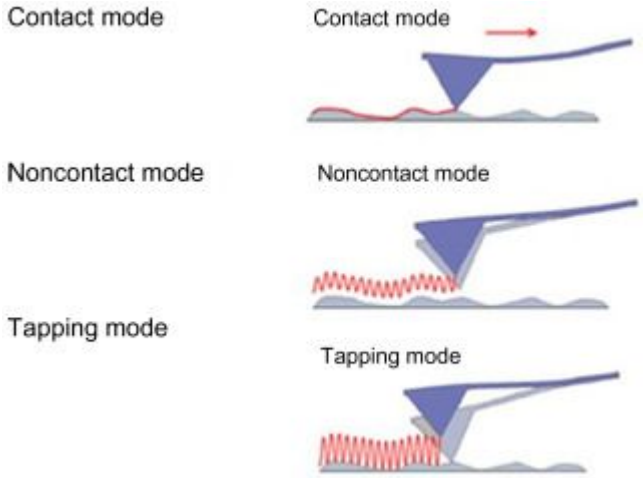
Tapping mode

Non Contact mode

AM AFM

FM AFM

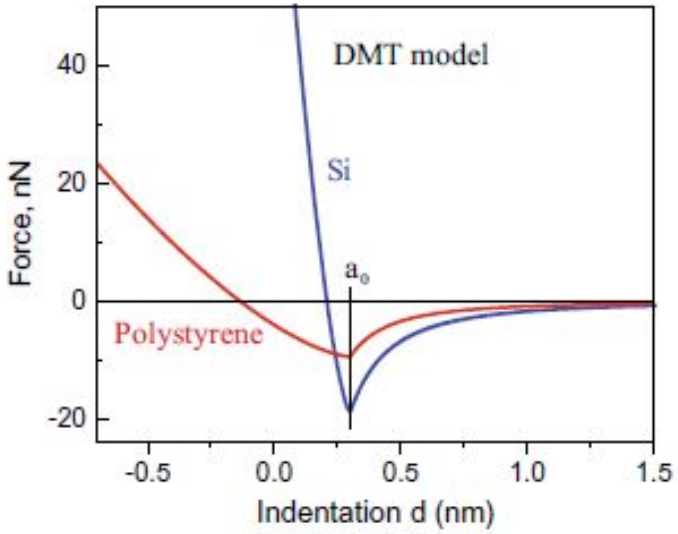
Manipulation

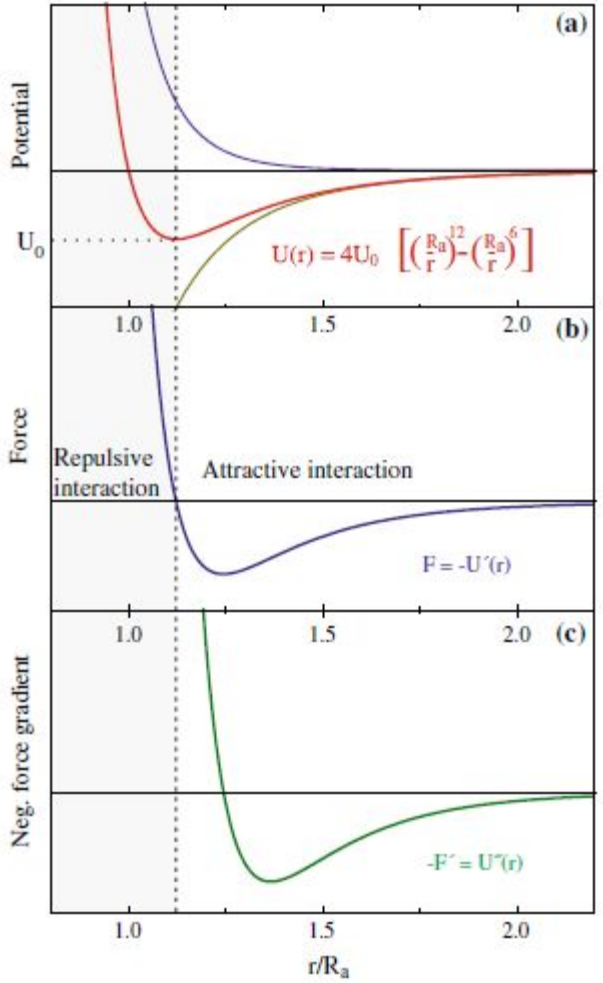


Tip Sample interaction

In Air:

- Van der waal Force
- Short range repulsive force
- Adhesion force
- Capillary forces





Van Der Waal Force

$$U_{\mathrm{vdW}}(r) = -\frac{C}{r^6}$$

interaction energy between an infinitesimal volume element of the tip dVtip and an infinitesimal volume element dVsample

$$dU_{\mathrm{vdW}} = -\frac{C\rho_{\mathrm{tip}}\rho_{\mathrm{sample}}}{\left|\mathbf{r}_{\mathrm{tip}} - \mathbf{r}_{\mathrm{sample}}\right|^6}\mathrm{d}V_{\mathrm{tip}}\mathrm{d}V_{\mathrm{sample}}$$

$$F_{\mathrm{vdW}} = -\frac{\partial U_{\mathrm{vdW}}}{\partial d} = -\frac{H R_{\mathrm{tip}}}{6\mathrm{d}^2}$$

Hamaker constant $H = \pi^2 C \rho_{\mathrm{tip}} \rho_{\mathrm{sample}}$

Capillary Forces

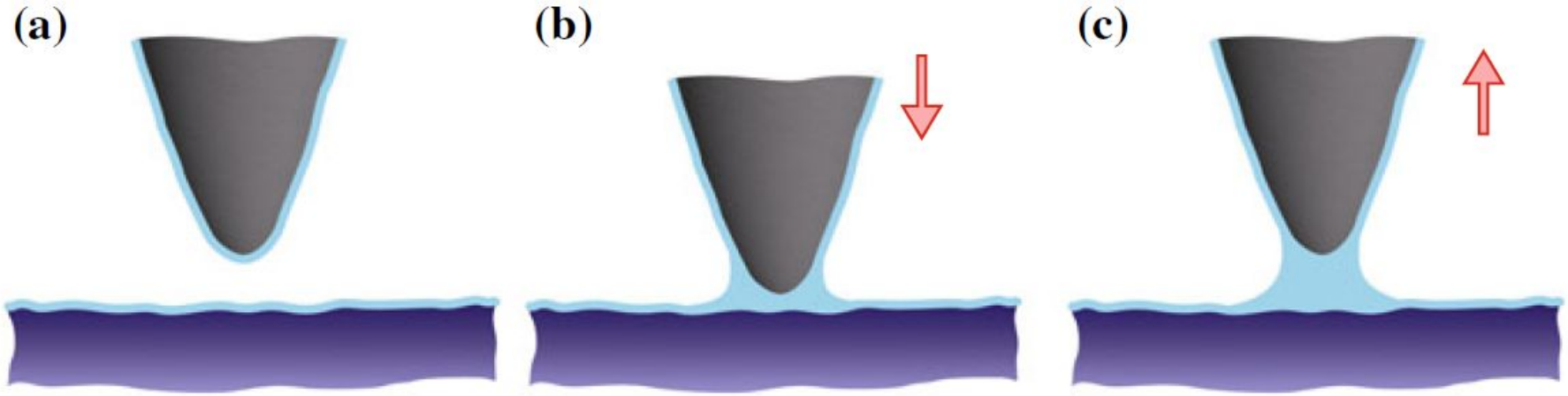
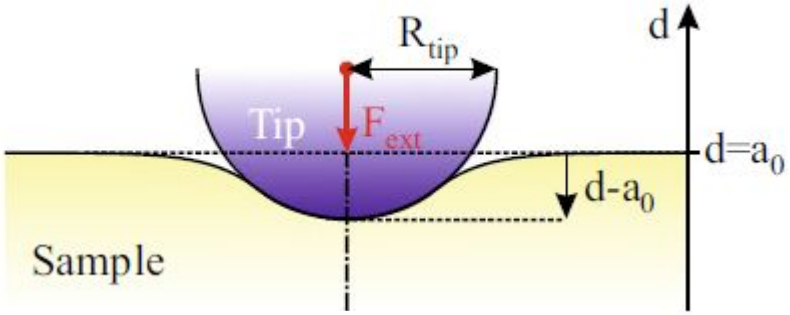


Fig. 10.6 **a** At ambient conditions tip and sample are covered by a thin water layer. **b** If tip and sample touch, the tip-sample gap fills with water, either due to the water films on both or due to capillary condensation **c** The water meniscus between tip and sample remains also if tip and sample disengage, leading to the hysteretic nature of the capillary force

Hertz Model and DMT (Derjaguin, Muller, and Toporov)



$$F_{\text{Hertz}}(d) = -F_{\text{ext}}(d) = \frac{4}{3} E^* \sqrt{R_{\text{tip}}} (a_0 - d)^{3/2} \text{ for } d < a_0$$

$$\frac{1}{E^*} = \frac{1 - \nu_{\text{tip}}^2}{E_{\text{tip}}} + \frac{1 - \nu_{\text{sample}}^2}{E_{\text{sample}}}$$

$$F_{\text{DMT}}(d) = \begin{cases} F_{\text{vdW}} = -\dfrac{H R_{\text{tip}}}{6d^2}. & \text{for } d \geq a_0 \\ \frac{4}{3} E^* \sqrt{R_{\text{tip}}} (a_0 - d)^{3/2} - \dfrac{H R_{\text{tip}}}{6a_0^2} & \text{for } d < a_0 \end{cases}$$

Measuring Elasticity and Viscoelasticity

SLS model

$$E(t) = E_\infty + (E_0 - E_\infty)e^{-\frac{t}{\tau}}$$

Ting's model

$$F(t,\delta(t)) = \begin{cases} \frac{4\sqrt{R}}{3(1-\nu^2)}\int_0^t E(t-\xi)\frac{\partial \delta^{\frac{3}{2}}}{\partial \xi}d\xi, & 0 \le t \le t_m \\ \frac{4\sqrt{R}}{3(1-\nu^2)}\int_0^{t_1(t)} E(t-\xi)\frac{\partial \delta^{\frac{3}{2}}}{\partial \xi}d\xi, & t_m < t \le t_{ind} \end{cases}$$

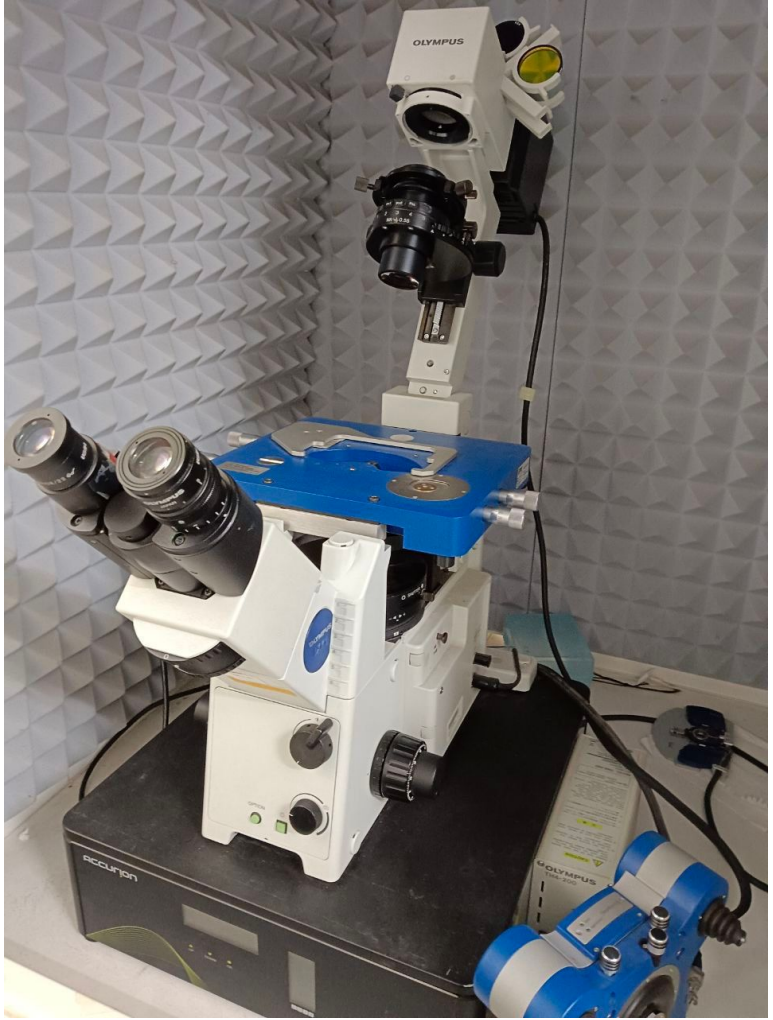
$$\int_{t_1(t)}^{t} E(t-\xi)\frac{\partial \delta}{\partial \xi}d\xi = 0,$$

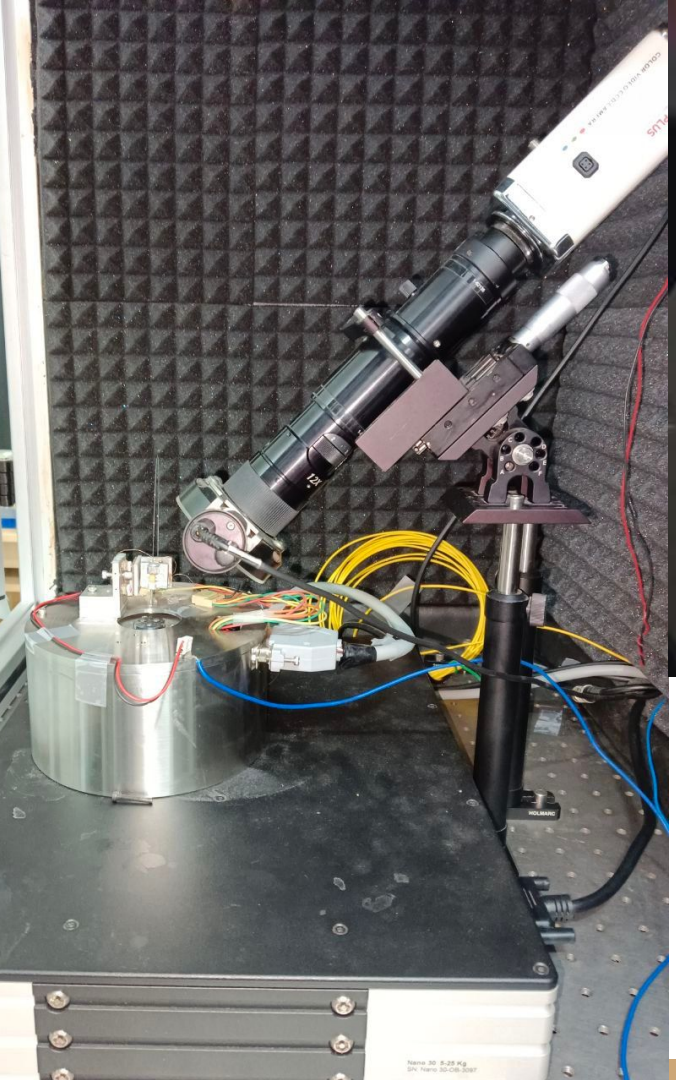
t_1 is the auxiliary function determined by the equation (2); ξ is the dummy time variable required for the integration; $E(t)$ is the Young's relaxation modulus

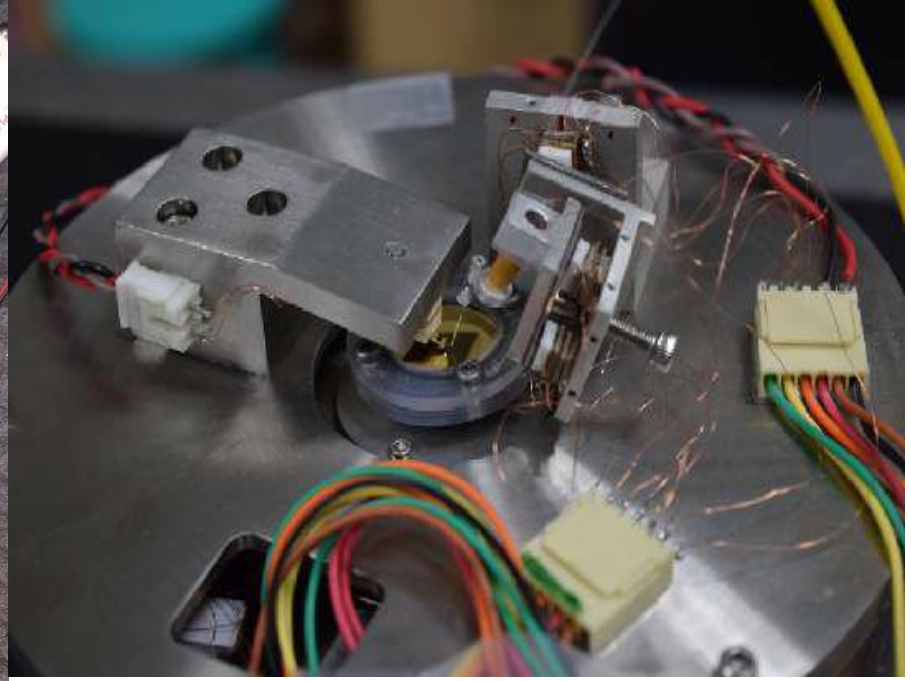
Applications

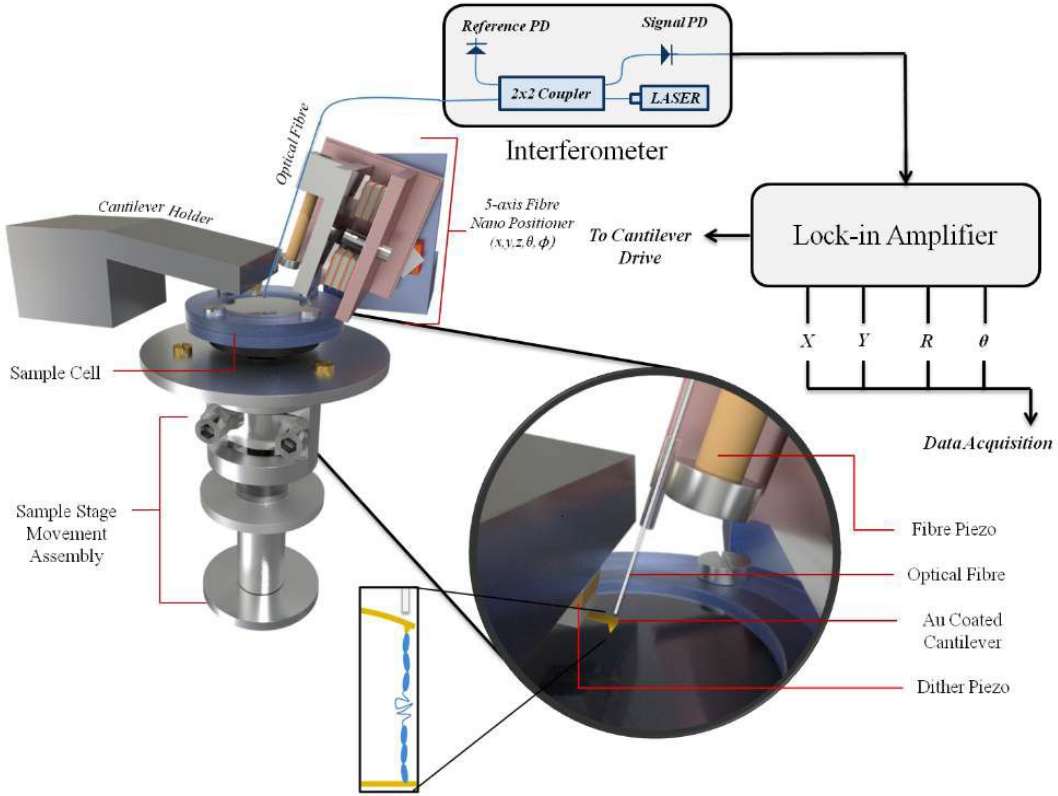
- Topography
- Imaging
- Measuring Elasticity, viscoelasticity, Internal friction
- Force manipulation
- Surface properties

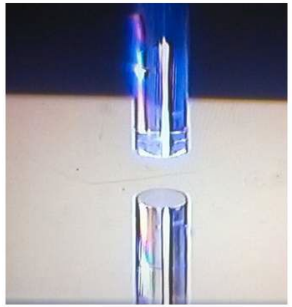
A



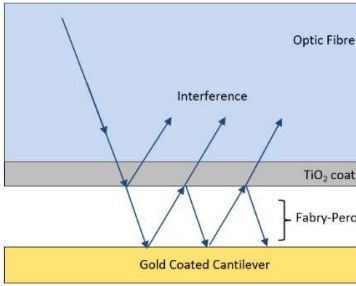




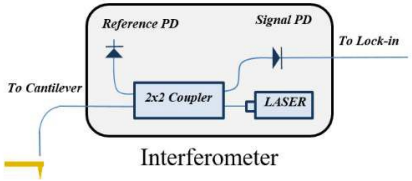

Reference PD
Signal PD
2x2 Coupler
LASER
Interferometer
Optical Fibre
Cantilever Holder
5-axis Fibre
Nano Positioner
(x,y,z,θ,ϕ)
To Cantilever
Drive
Lock-in Amplifier
X
Y
R
θ
Data Acquisition
Sample Cell
Sample Stage
Movement
Assembly
Fibre Piezo
Optical Fibre
Au Coated
Cantilever
Dither Piezo



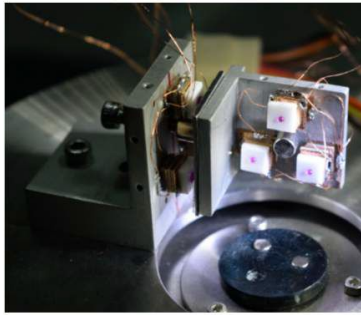
(a)


Optic Fibre
Interference
Fabry-Perc
Gold Coated Cantilever

(b)

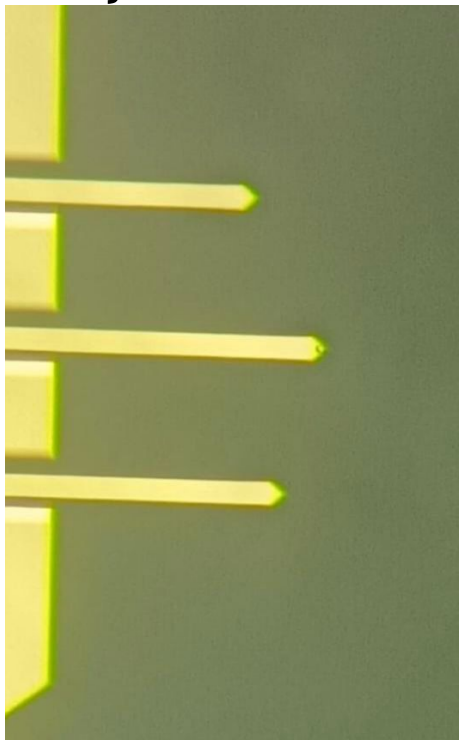

Reference PD
Signal PD
To Lock-in
To Cantilever
2x2 Coupler
LASER
Interferometer

(c)



(d)

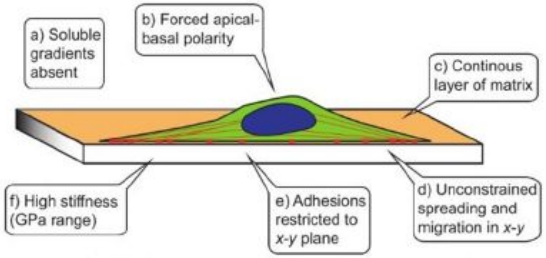
My work



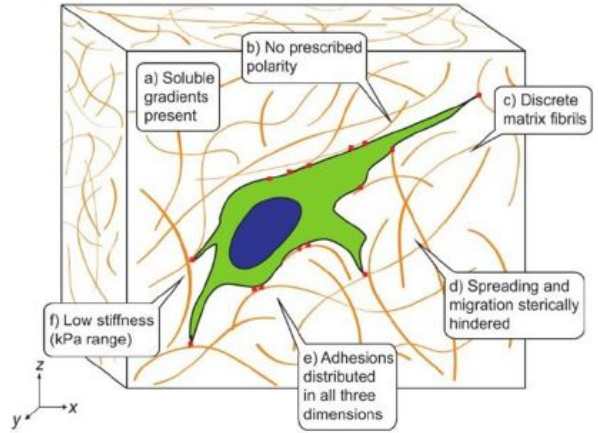
Mechano-sensing in 2D vs 3D microenvironment

Experiments in lab are generally being done in a 2D microenvironment where cells are plated on a stiff cell culture dish.
Here the mechanical cues the cells get is different from the in vivo 3D microenvironment where the cells are surrounded with extracellular matrix and other cells from all of the sides.
Hence comparison is important

Collagen-coated glass (2D)

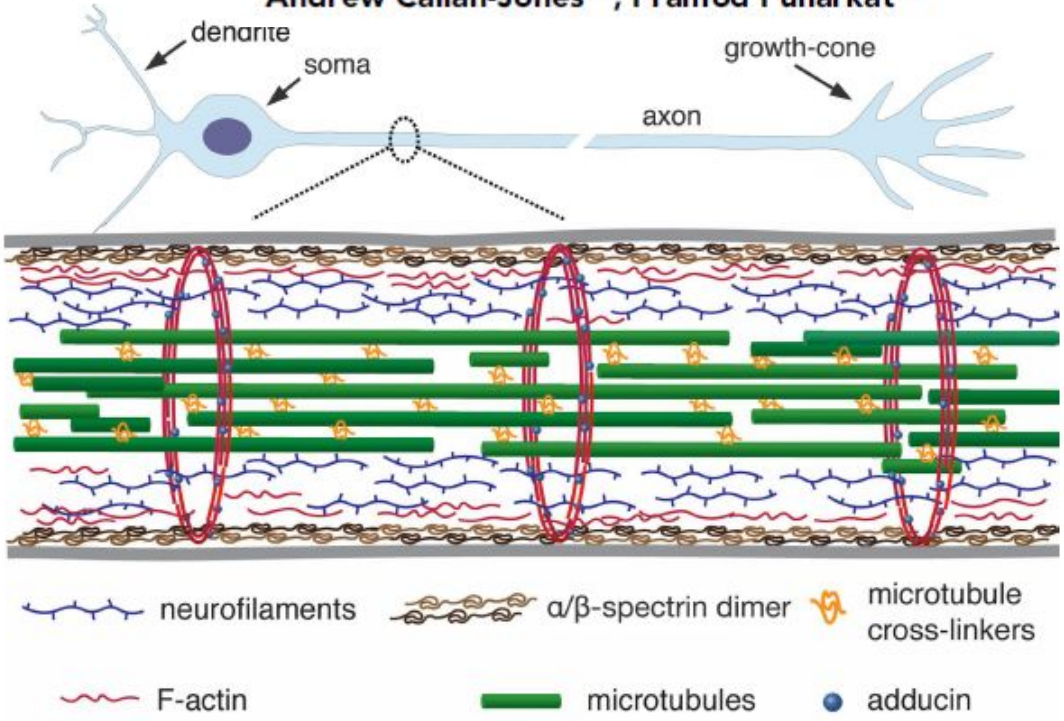


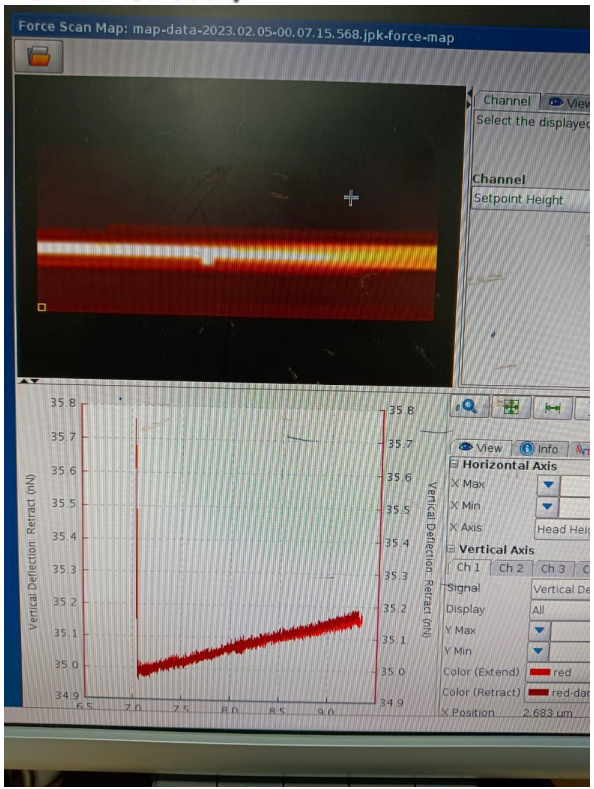
Collagen gel (3D)



The axonal actin-spectrin lattice acts as a tension buffering shock absorber

Sushil Dubey[1], Nishita Bhembre[1], Shivani Bodas[2], Sukh Veer[1], Aurnab Ghose[2], Andrew Callan-Jones[3]*, Pramod Pullarkat[1]*





Sushil Dubey, Nishita Bhembre, Shivani Bodas, Sukh Veer, Aurnab Ghose, Andrew Callan-Jones, Pramod Pullarkat (2020) The axonal actin-spectrin lattice acts as a tension buffering shock absorber eLife 9:e51772

https://doi.org/10.7554/eLife.51772

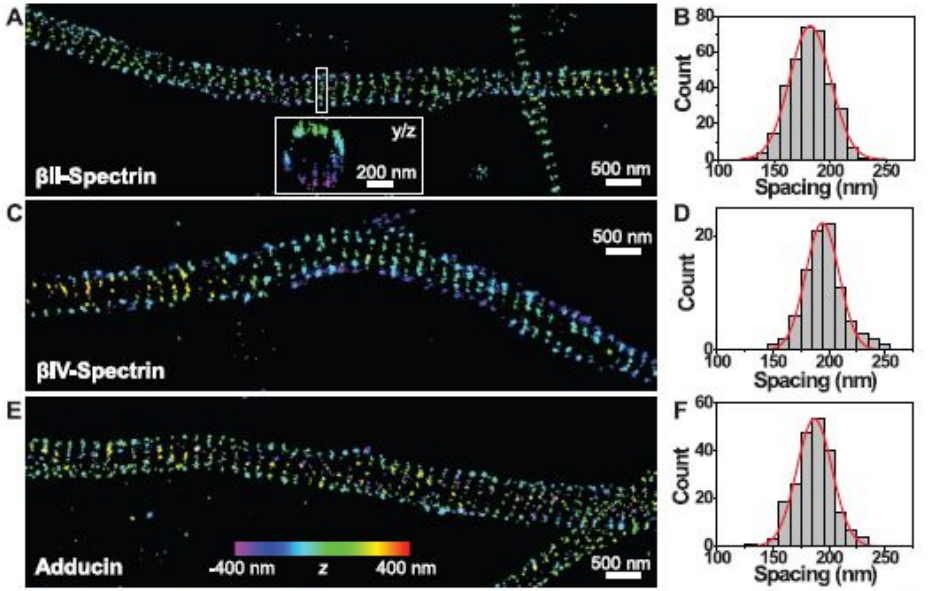


Fig. 3. Spectrin and adducin exhibit quasi-1D, periodic patterns in axons, quantitatively similar to that observed for actin. (**A**) Three-dimensional STORM image of βII-spectrin in axons. βII-spectrin is immunostained against its C-terminal region, which is situated at the center of the rodlike αII-βII spectrin tetramer. (Inset) The *yz* cross section of the boxed region showing the ringlike structure. The smaller white box denotes the position of the inset image. (**B**) Histogram of the spacings between adjacent spectrin rings ($N = 340$ spacings). The red line is a Gaussian fit with a mean of 182 nm and a SD of 18 nm. (**C** and **D**) Same as (A) and (B) but for βIV-spectrin, which is specifically located in the initial segments of axons. βIV-spectrin is immunostained against its N-terminal region, which corresponds to the ends of the spectrin tetramer. The red line superimposed on the histogram is a Gaussian fit with a mean of 194 nm and a SD of 15 nm ($N = 88$ spacings). (**E** and **F**) Same as (A) and (B) but for adducin, an actin-capping protein. The red line superimposed on the histogram is a Gaussian fit with a mean of 187 nm and a SD of 16 nm ($N = 216$ spacings).

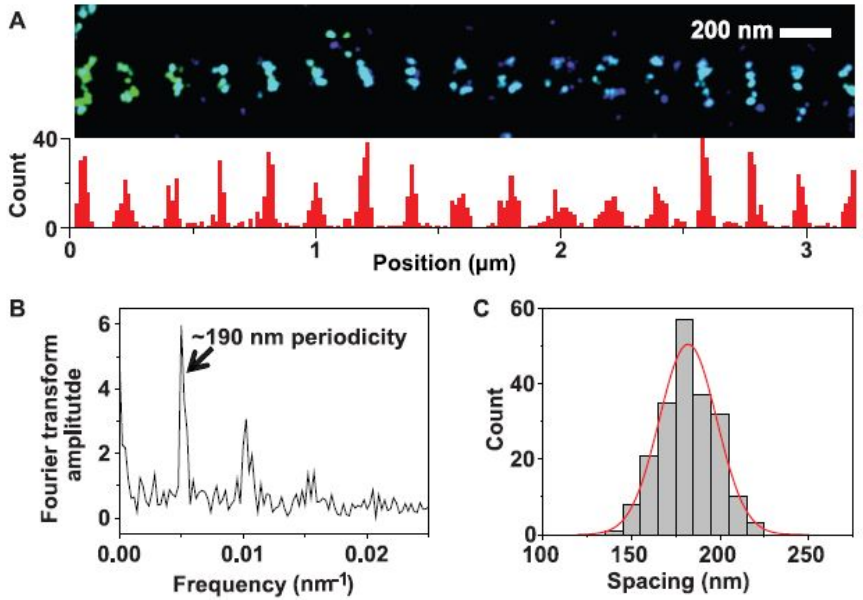


Fig. 2. Actin filaments in axons form a quasi-1D, periodic structure with a uniform spacing of ~180 to 190 nm. (**A**) Three-dimensional STORM image of a segment of axon (top) and the distribution of localized molecules after the 3D image was projected to one dimension along the axon long axis (bottom). (**B**) Fourier transform of the 1D localization distribution shown in (A). The Fourier transform shows a fundamental frequency of $(190\ \text{nm})^{-1}$ and an overtone. (**C**) Histogram of the spacings between adjacent actin ringlike structures ($N = 204$ spacings). The red line is a Gaussian fit with a mean of 182 nm and a SD of 16 nm.

Xu K, Zhong G, Zhuang X. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. Science. 2013 Jan 25;339(6118):452-6. doi: 10.1126/science.1232251. Epub 2012 Dec 13. PMID: 23239625; PMCID: PMC3815867.


MAY THE
FORCE
BE WITH
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