In salute e in malattia, finché morte non ci separi:
impronta biomeccanica delle patologie
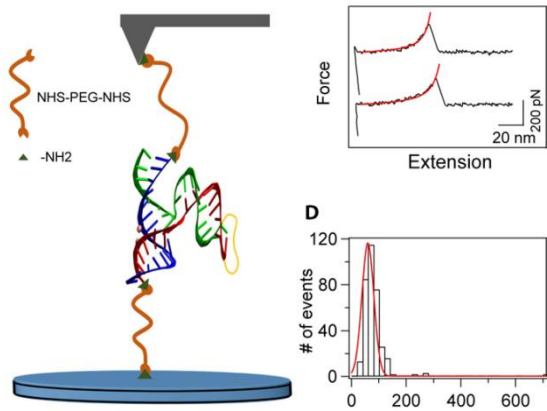
Marco De Spirito

Atomic Force Microscopy nano-mechanics in biology and medicine
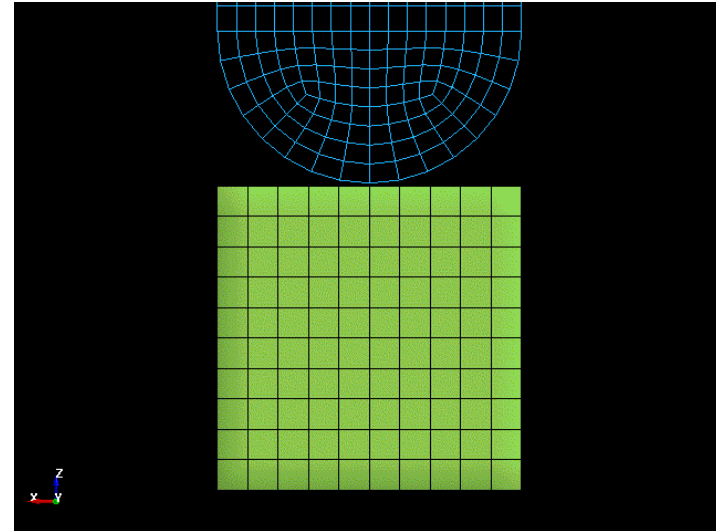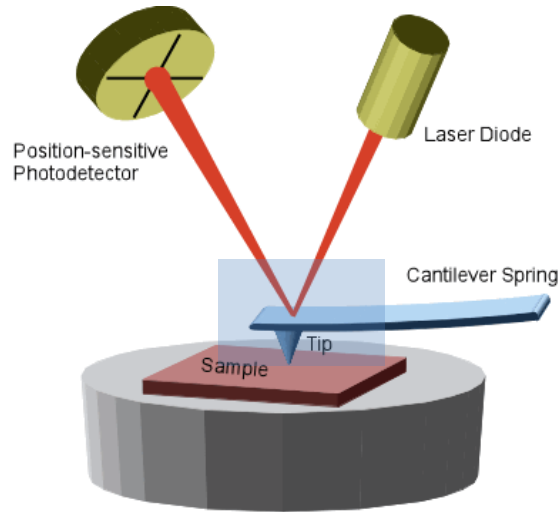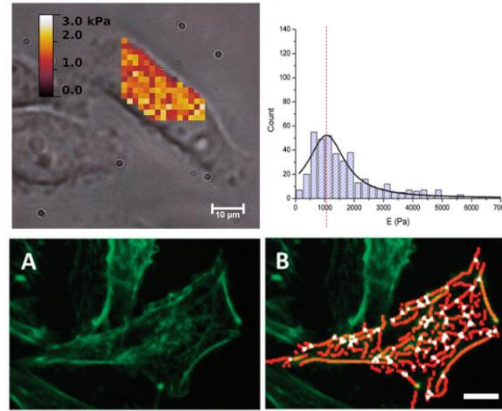

Papi M et al, Applied Physics Letters. 2014;104(10):103703.

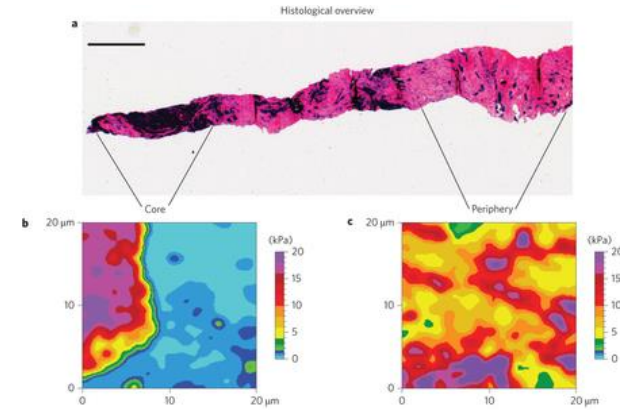# Atomic Force Microscopy nano-mechanics in biology and medicine

Single molecule

Xu, Z. et al. Science Advances (2017)

Cells

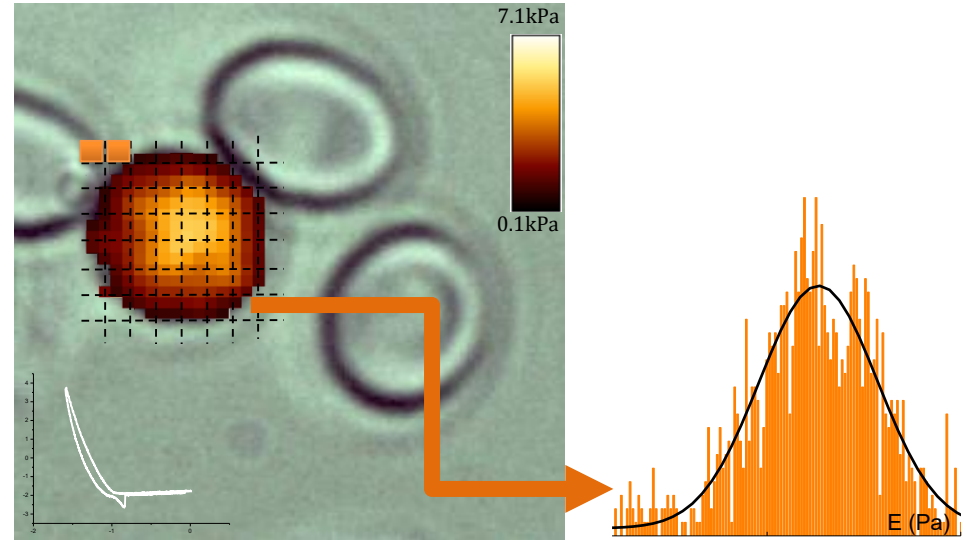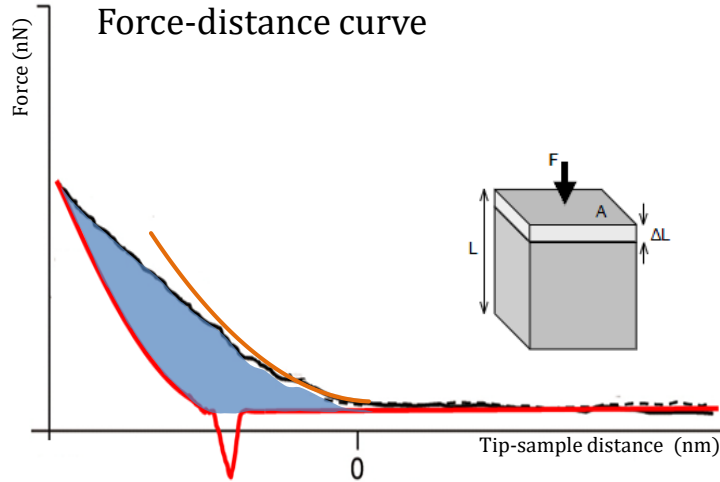Palmieri, V. et al. Applied Physics Letter (2014)
Palmieri, V. et al. Soft matter (2015)

Tissues

Plodinec, M. et al. Nature nanotechnology (2012)

How can we image mechanical properties with AFM?

Force-distance curve

Young's modulus:
Cell/Tissue stiffness
Sneddon's model

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

Hysteresis:
Viscoelastic behavior

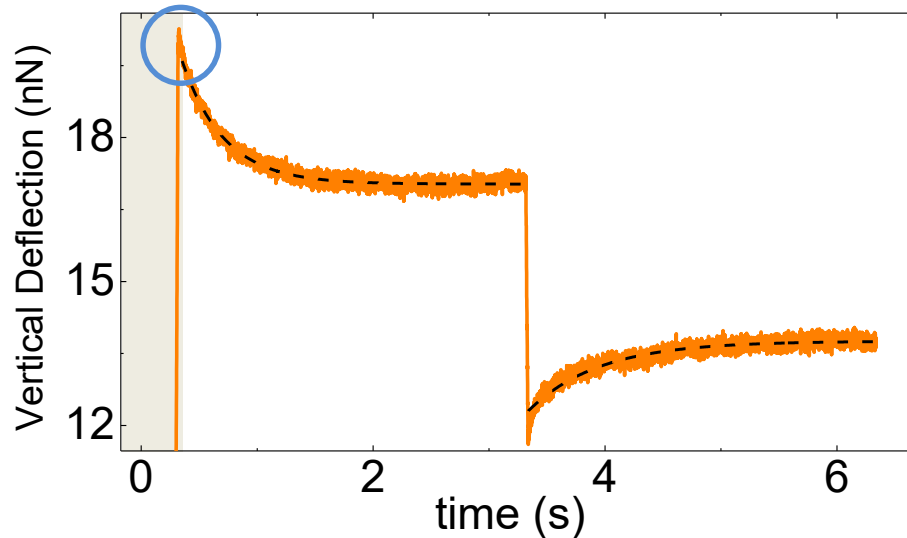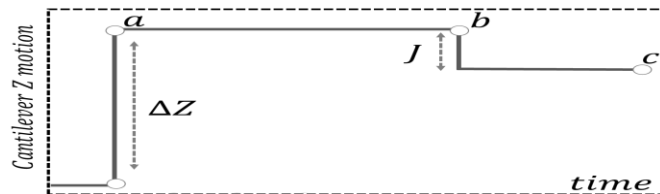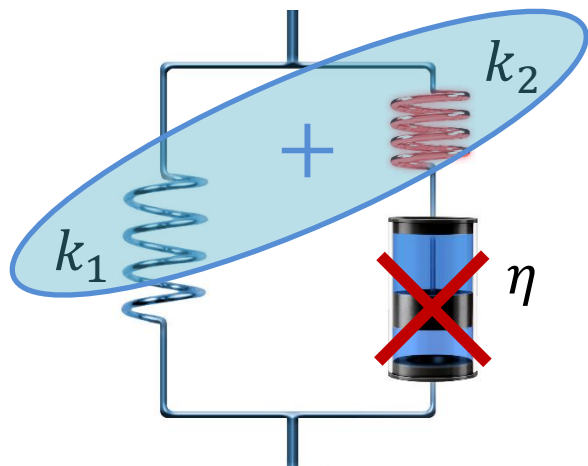$$H = \frac{\int_0^\delta F_E(\delta)d\delta - \int_0^\delta F_R(\delta)d\delta}{\int_0^\delta F_E(\delta)d\delta}$$
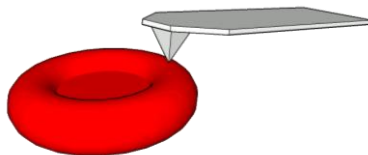
Minelli et al. Appl. Phys. Lett. 111, 143701 (2017)
Very interesting paper (not from us) Müller et al. *BMC Bioinformatics* 20.1 (2019): 1-9.

We assume that the sample has a purely elastic behavior × Viscoelastic behavior

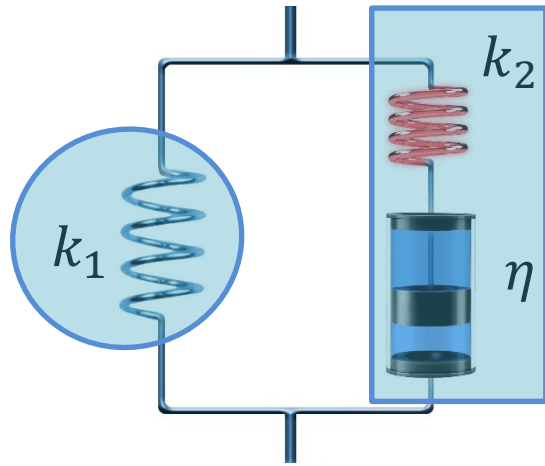# More complex models for mechanical imaging
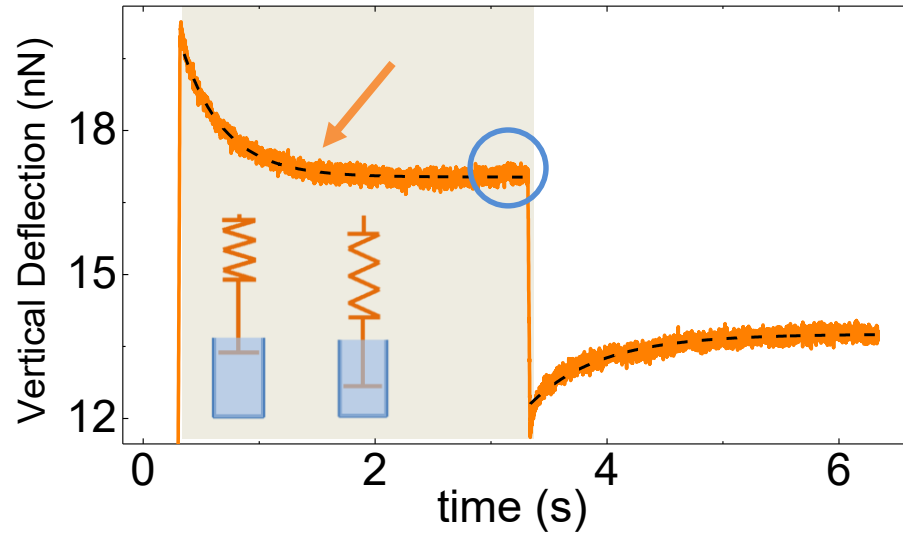
Standard Linear Solid

The cantilever touches the sample
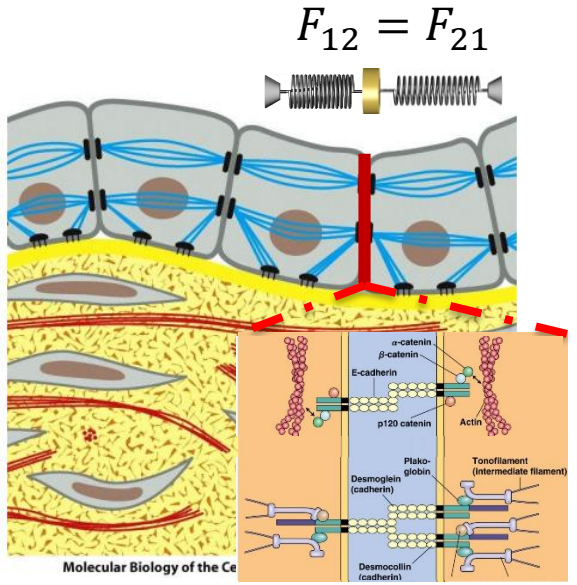
More complex models for mechanical imaging

Standard Linear Solid

The time-dependent relaxation gives information on the viscosity η

More complex models for mechanical imaging

Sample deformation

k_2

k_1

η

Cantilever deflection (elastic behavior)

In order to retrieve k_1, k_2 and η, we need to adjust the AFM set up to the model

(the model is a slight modification of the one demonstrated by C. Riethmuller, & M. Radmacher, Eur BioPhys J. (2017) 46:309 - 324)

Why should we image the mechanical properties in diagnostics ?

$$F_{12} = F_{21}$$

Mechanical balance of forces among cells

Why should we image the mechanical properties in diagnostics ?

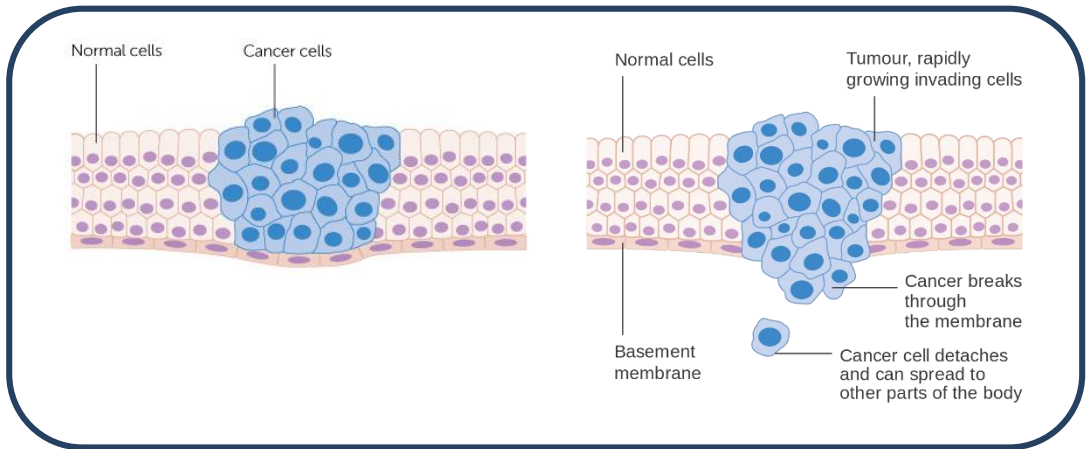$$F_{12} = F_{21}$$

$$F_{12} = F_{21}$$
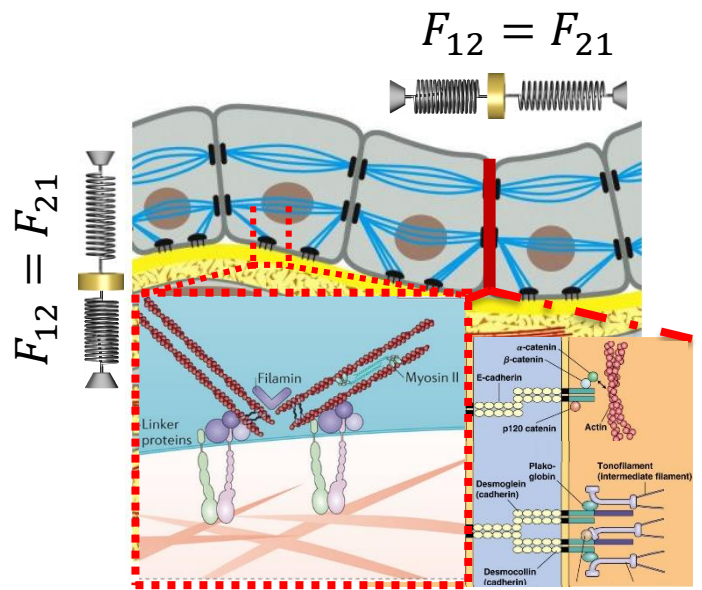
Mechanical balance of forces among cells

Mechanical balance of forces between cells and their ECM

# Why should we image the mechanical properties in diagnostics ?

$$F_{12} = F_{21}$$

$$F_{12} = F_{21}$$

Normal cells

Cancer cells

Normal cells

Tumour, rapidly growing invading cells

Cancer breaks through the membrane

Basement membrane

Cancer cell detaches and can spread to other parts of the body

Molecular Biology of the Cell 5/e (© Garland Science 2008)
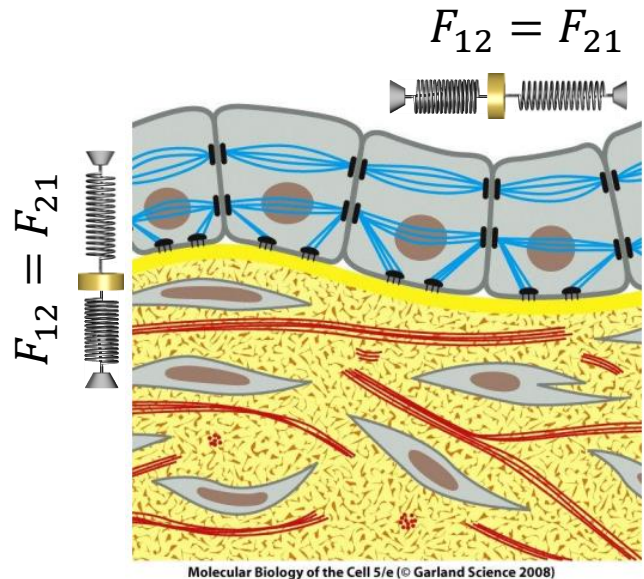
Mechanical homeostasis

In pathological conditions, such as cancer, the mechanical homeostasis is disrupted

Mechanical fingerprint of brain tumors

GLIOBLASTOMA
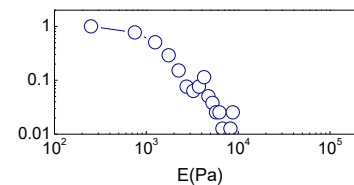
A highly aggressive and infiltrating tumor
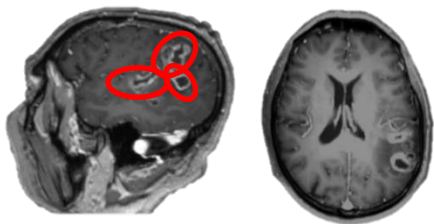
Necrosis
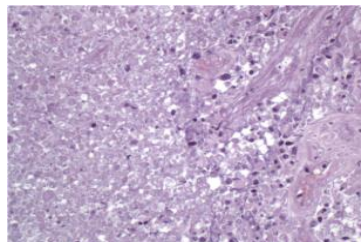
Tumor tissues
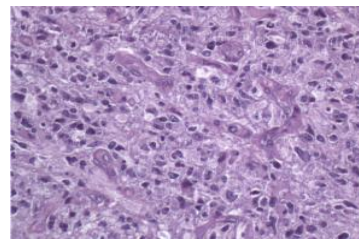
Healthy tissues
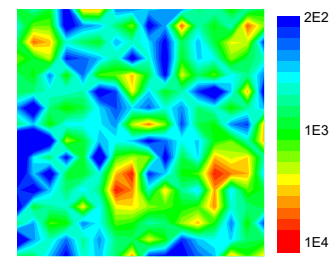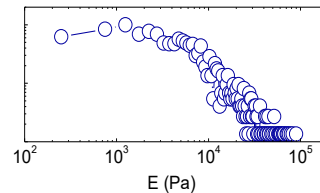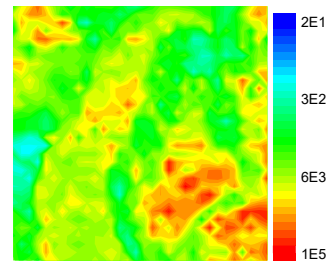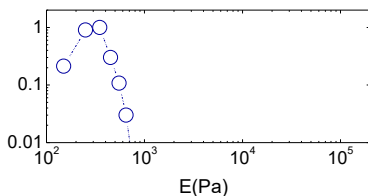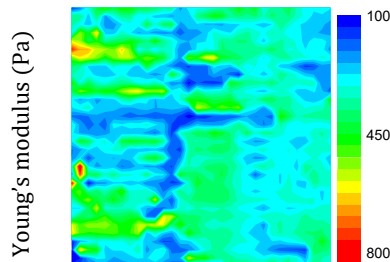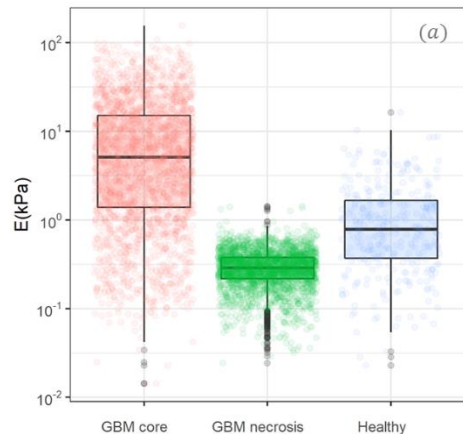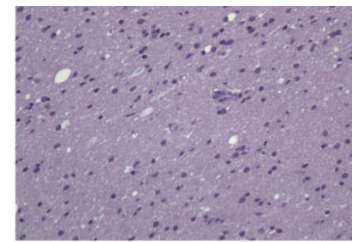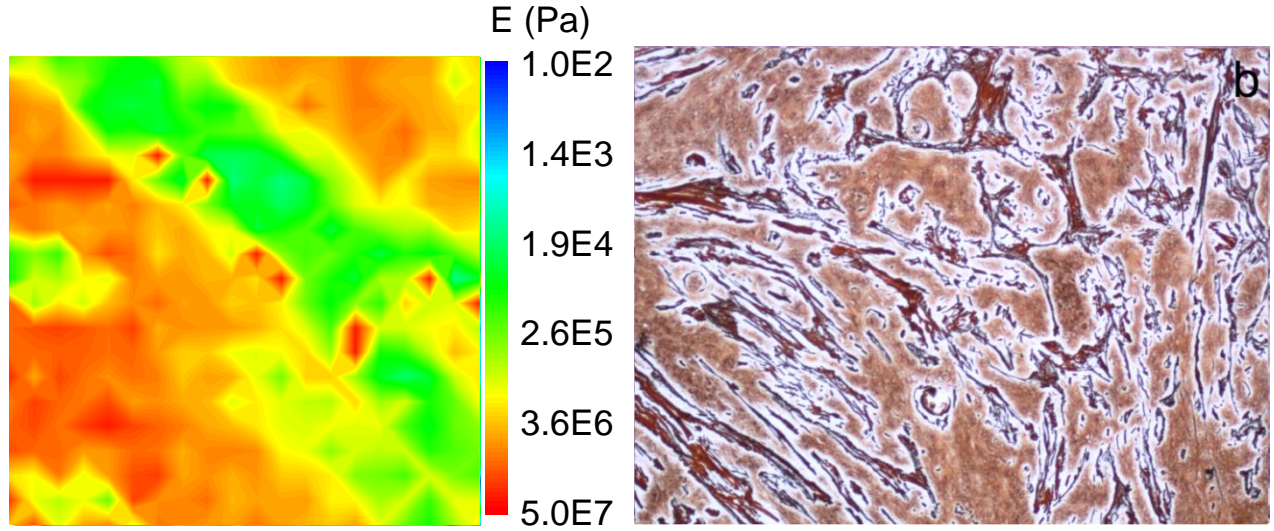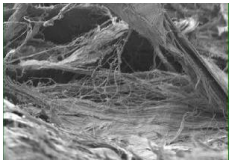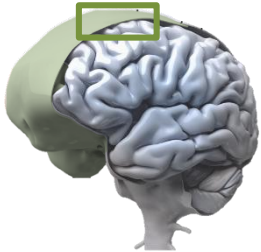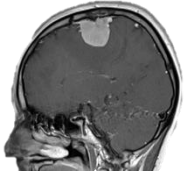
Mechanical fingerprint of brain tumors

MENINGIOMA
A slowly growing tumor originating from the meninges

E (Pa)
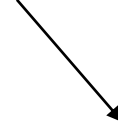
Casca, G. et al. Nanoscale (2016)
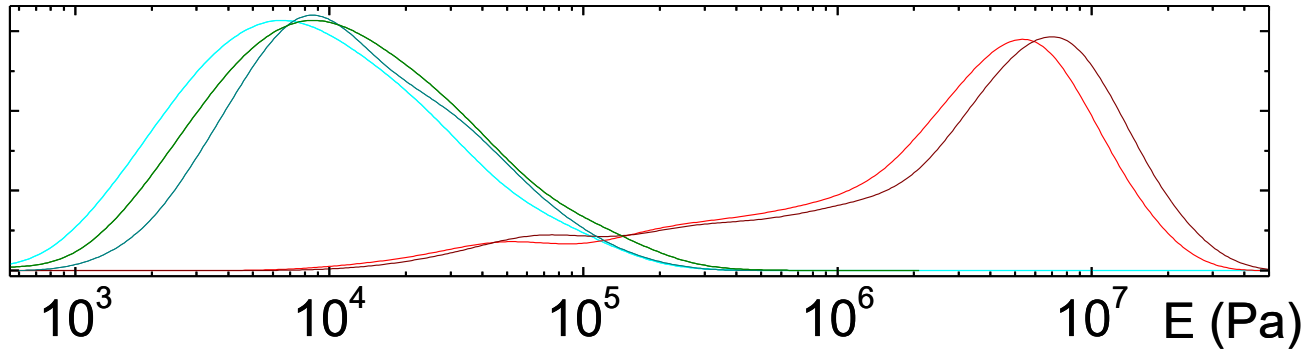
AFM is a time-consuming technique in terms of measurement and analysis time

Neural network and machine learning?

Minelli et al. APL **111**, 143701 (2017)

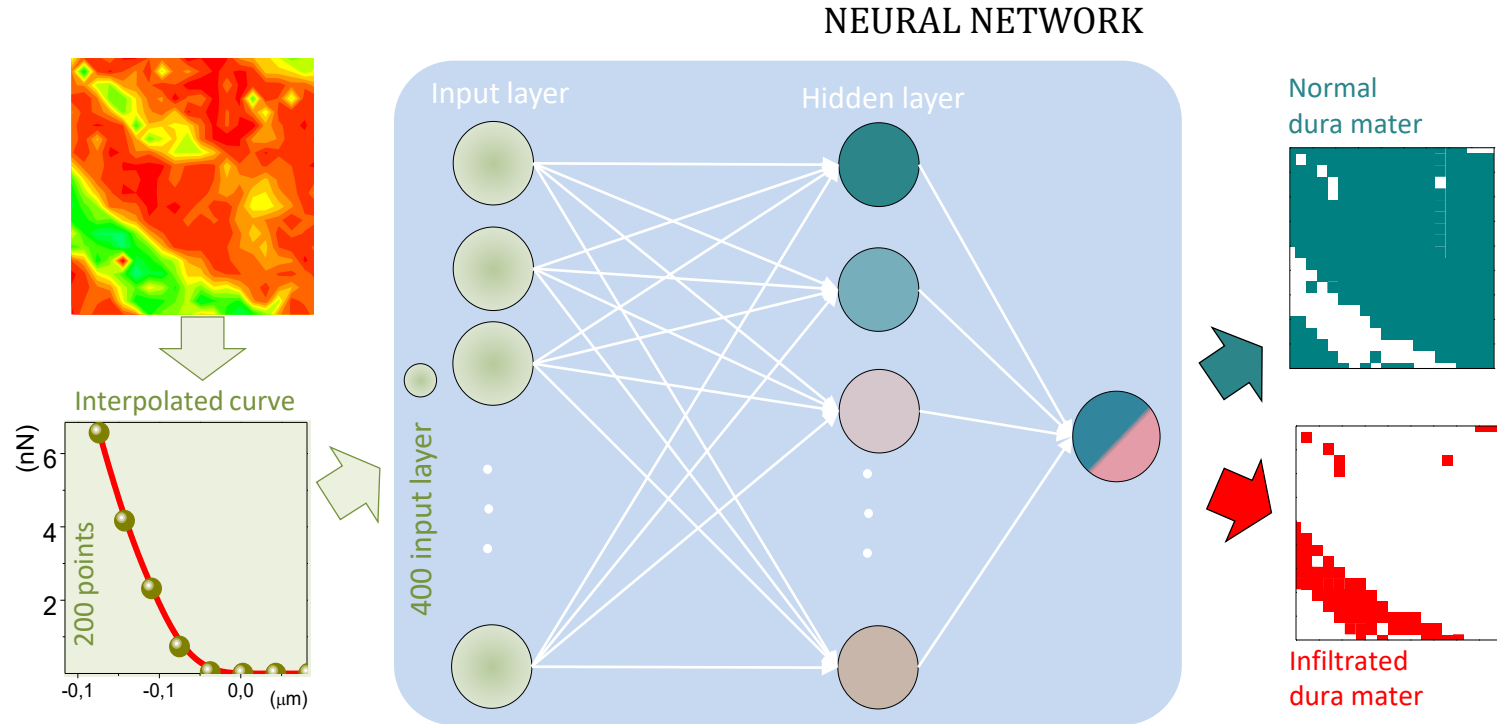Efficient Spatial Sampling for AFM-Based Cancer Diagnostics

Ciasca et al. Condens. Matter **2019**, *4*(2), 58
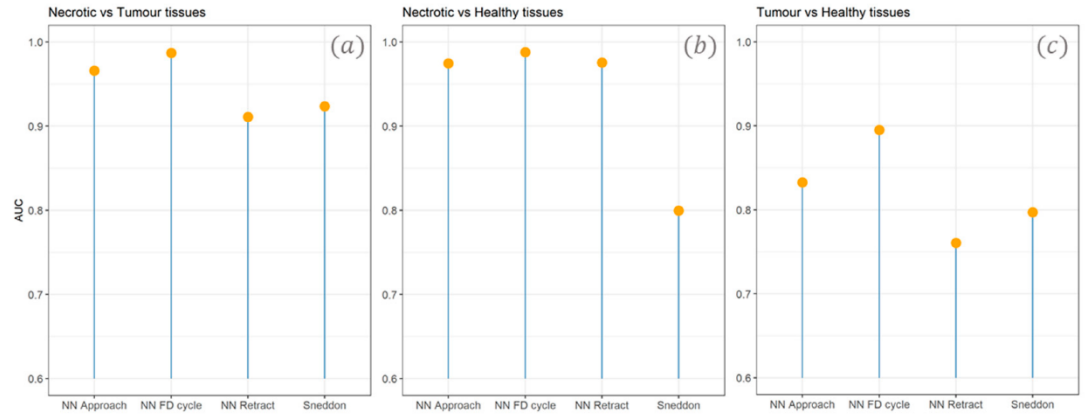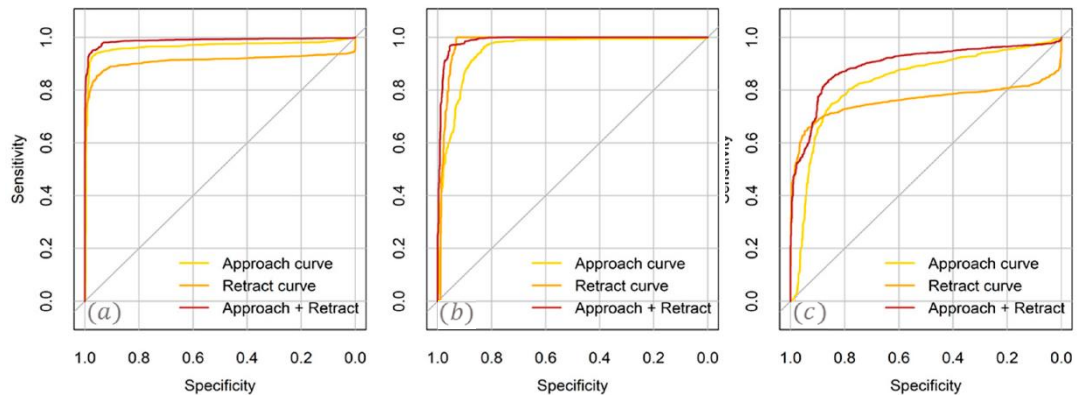
Exciting breaktrough in the field :

Müller et al. "nanite: using machine learning to assess the quality of atomic force microscopy-enabled nano-indentation data." *BMC Bioinformatics* 20.1 (2019): 1-9.

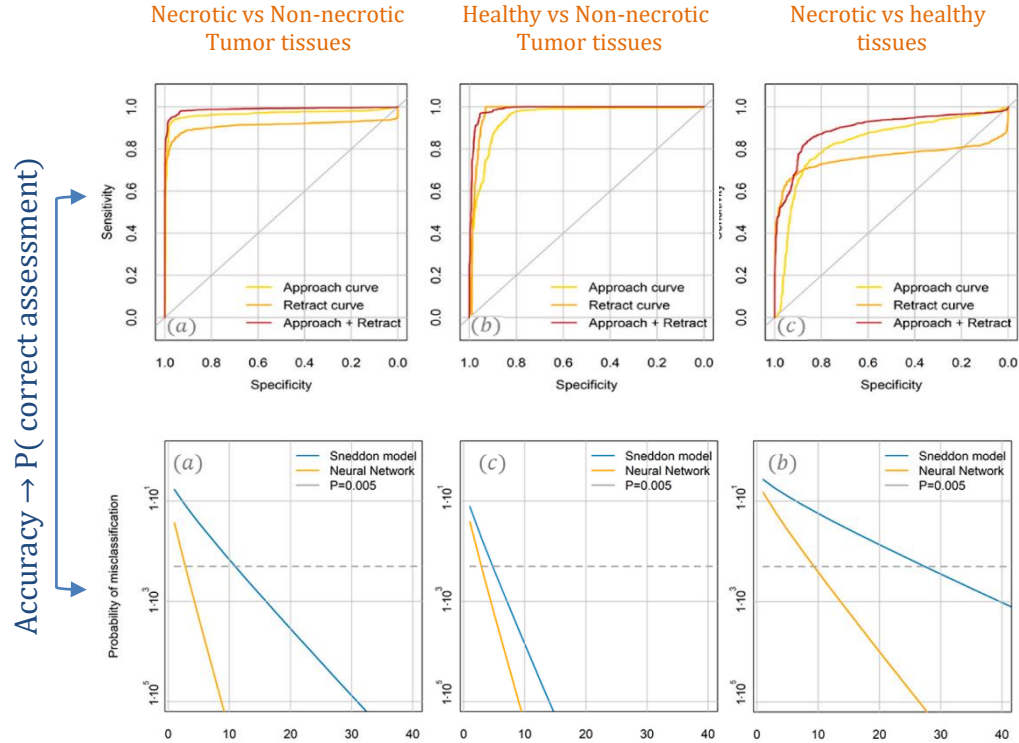Neary-Zajiczek et al.Whole-Sample Mapping of Cancerous and Benign Tissue Properties arXiv preprint arXiv (2019)

AFM is time-consuming in terms of data analysis and acquisition

NEURAL NETWORK

Input layer

Hidden layer

Interpolated curve

(nN)

200 points

6

4

2

-0,1   -0,1   0,0

(µm)

400 input layer

Normal
dura mater

Infiltrated
dura mater

Ciasca et al. Condens. Matter 2019, 4(2), 58 ; Minelli et al.APL 111, 143701 (2017)

# Neural Network's performance in distinguishing glioblastoma tissues

AFM is time-consuming in terms of data analysis and acquisition

Necrotic vs Non-necrotic Tumor tissues

Healthy vs Non-necrotic Tumor tissues

Necrotic vs healthy tissues

Accuracy → P(correct assessment)

Ciasca et al. Condens. Matter 2019, 4(2), 58 ; Minelli et al.APL 111, 143701 (2017)

Efficient sampling: few FD curves are needed to properly assign the class membership to a 40 µm x 40 µm tissue region at the 0.005 significance level
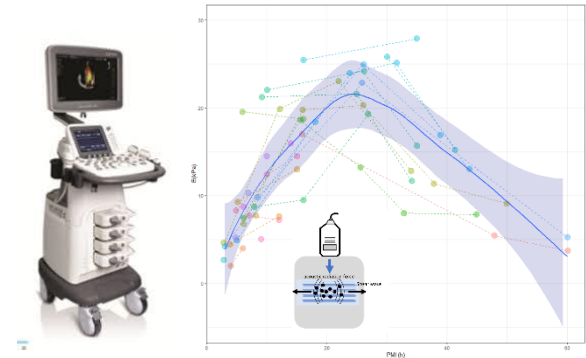
Quantitative evaluation of Rigor Mortis for the determination of the time of the death in forensic sciences

1) Absent

2) Partially developed

3) Fully developed
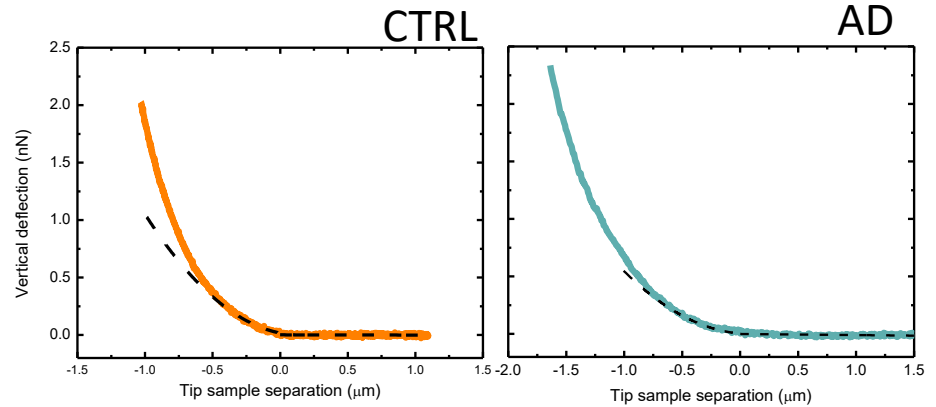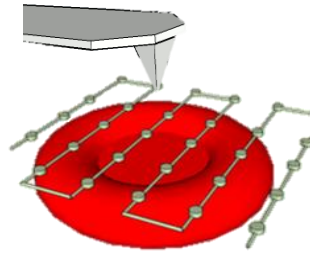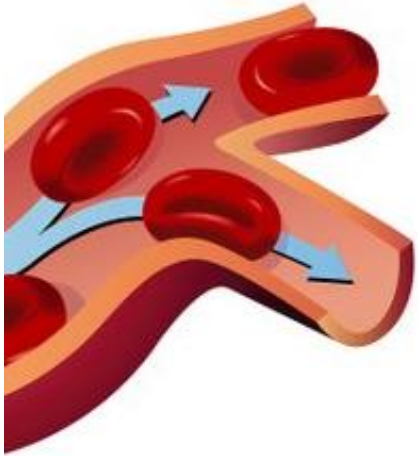
4) Partially relaxed

5) Fully relaxed
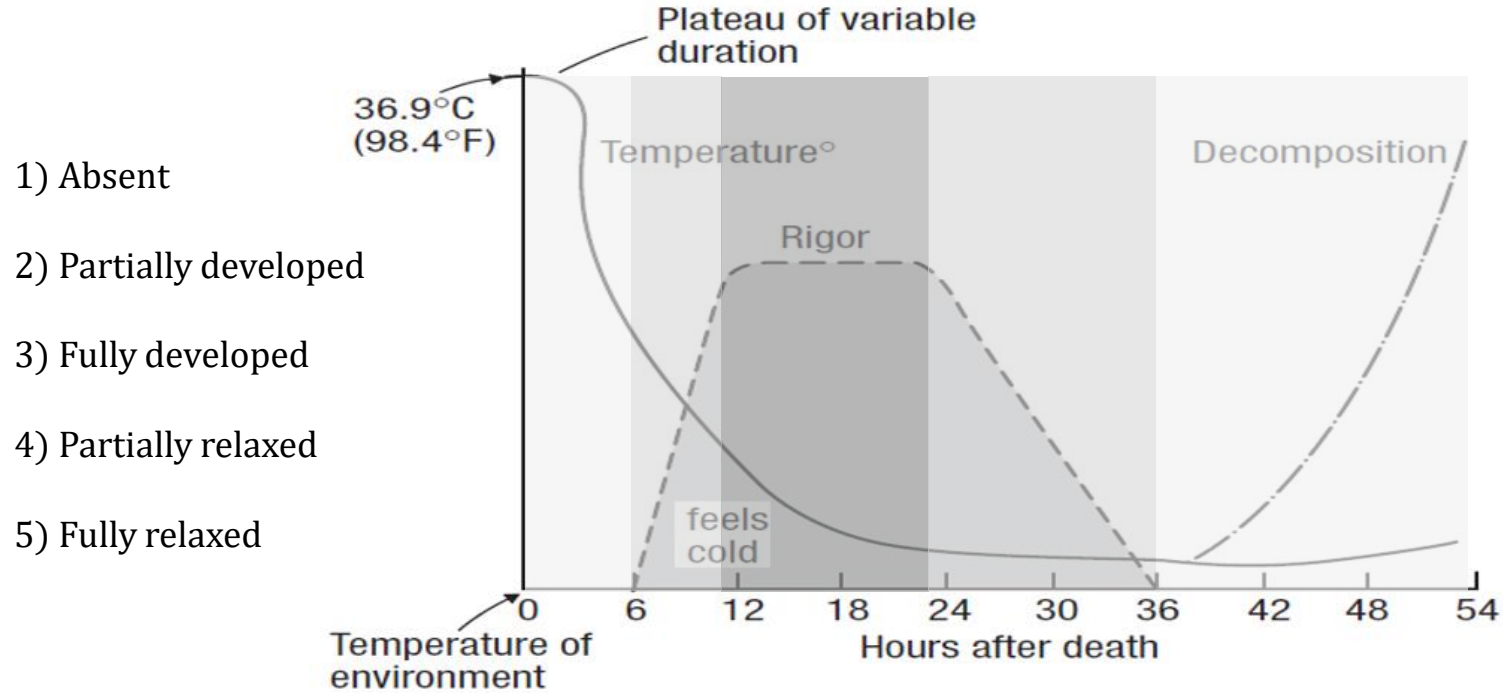
AFM
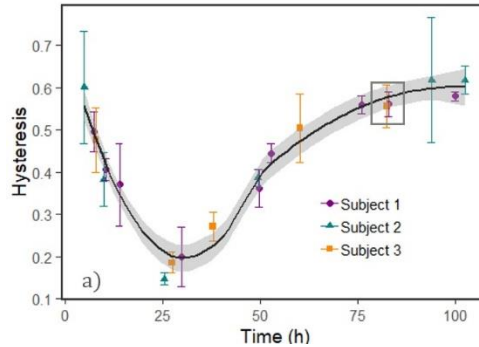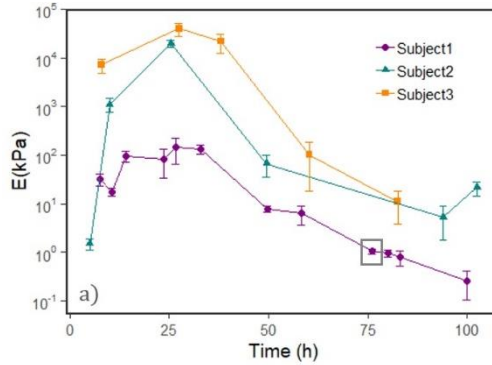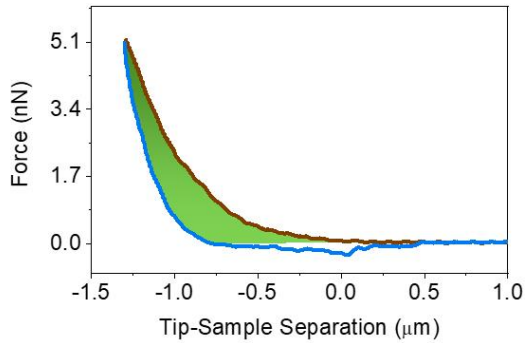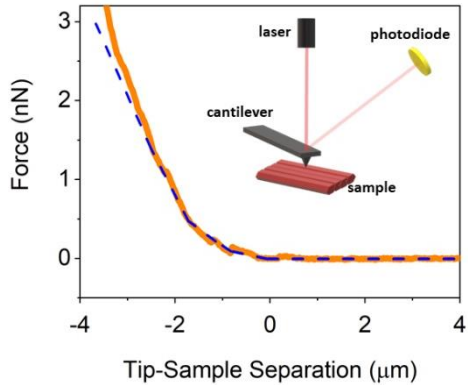
Ultra Sound-SWE

$$H = \frac{\int_0^\delta F_E(\delta)d\delta - \int_0^\delta F_R(\delta)d\delta}{\int_0^\delta F_E(\delta)d\delta}$$

Hysteresis helps getting rid of the inter-individual variability

F. De Giorgio, IJLM (2019) **133**, 1133–1139

Red Blood Cells obtained from Alzheimer's Disease patients have an altered biomechanical response

Altered Deformability

Blood flow and micro-circulation
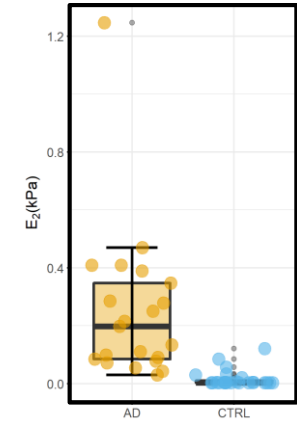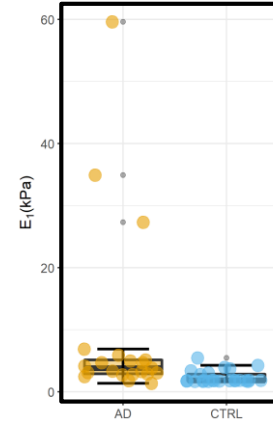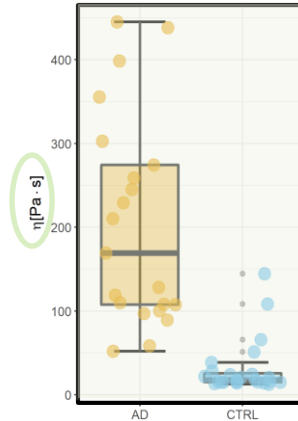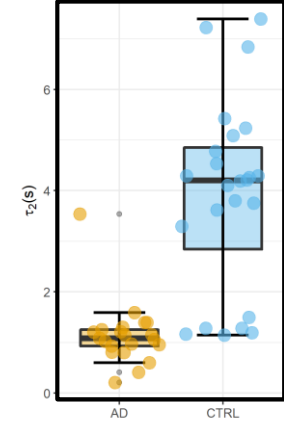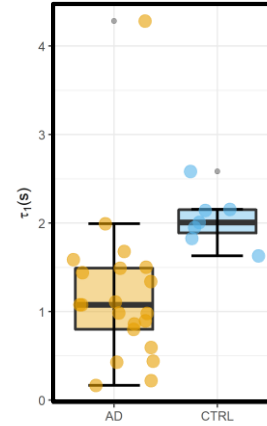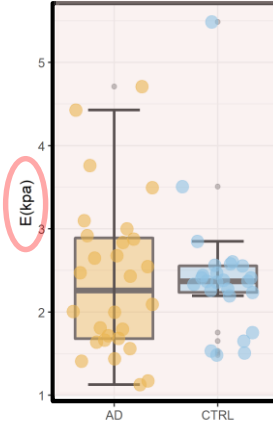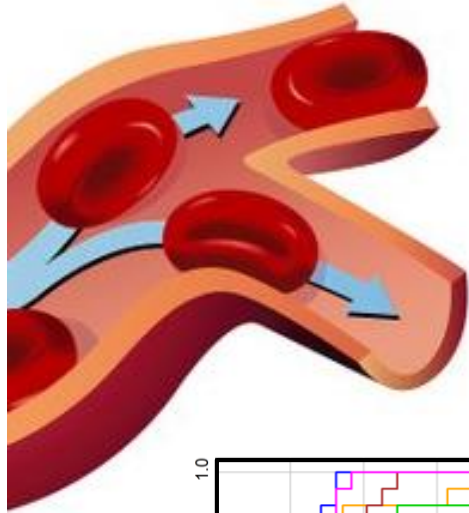
Vascular pathologies
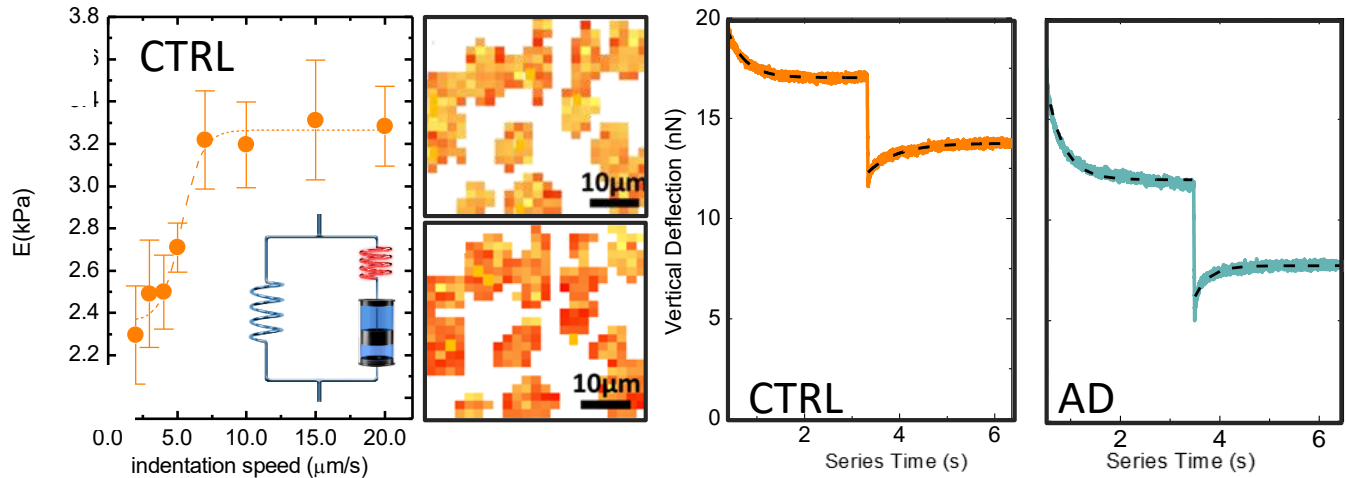
Red Blood Cells obtained from Alzheimer's Disease patients have an altered biomechanical response
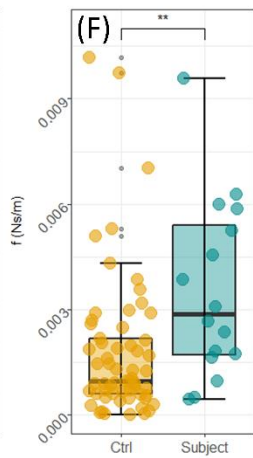
In conclusion: is this technique really diagnostics and/or useful for early detection?

In 2017, when we started to study the mechanical properties of RBCs in AD and diabetes,

A healthy male subject, aged 38,
with normal blood sugar levels
was included among CTRLs
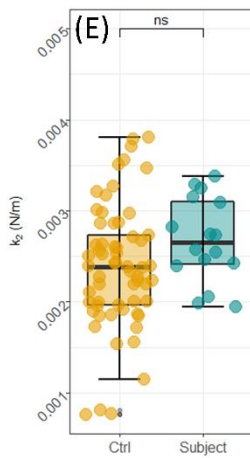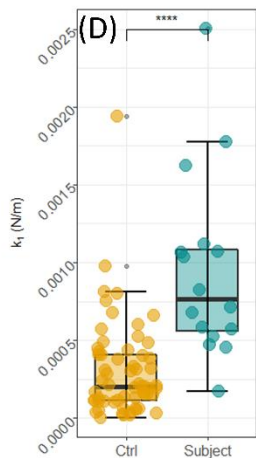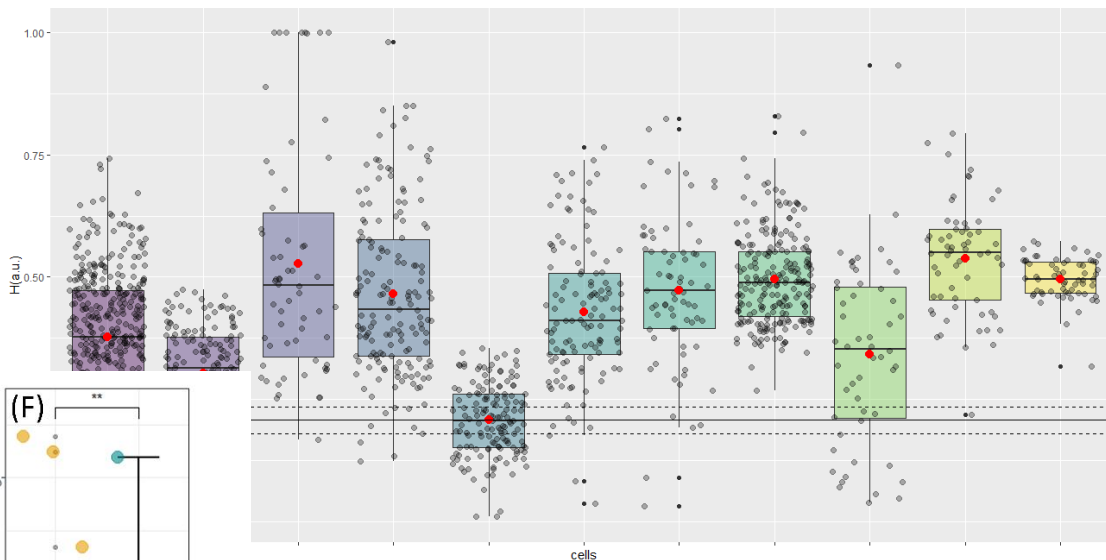

Ciasca et al. Nanoscale 2015 7(40)

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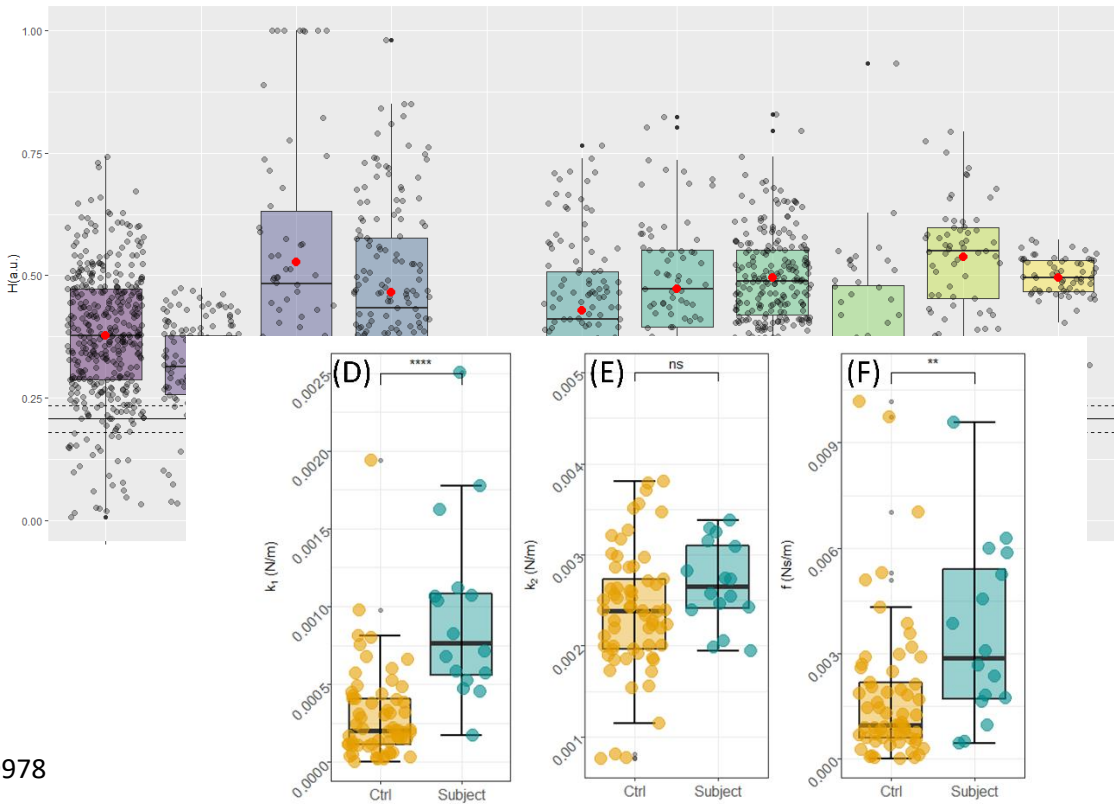Di Giacinto F, et al. Front. Bioeng. Biotechnol. 2020, 8:569978

In conclusion: is this technique really diagnostics and/or useful for early detection?

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A healthy male subject, aged 38,
with normal blood sugar levels
was included among CTRLs

In diagnostics, we usually search for morphological
(e.g. histological or radiological findings) or biochemical
(e.g. blood tests) markers

Morphological and biochemical changes are deeply
connected with mechanical modifications of cells and
tissues at the nanoscale level

Mechanical biomarkers have the potential to be used
in combination with the conventional ones for
diagnostic purpose

Acknowledgements

Università Cattolica del Sacro cuore

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- Dr. Eleonora Minelli
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- Prof. Massimiliano Papi

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Ministero della Salute

UNIVERSITÀ CATTOLICA del Sacro Cuore

**Thank you
for your attention!**