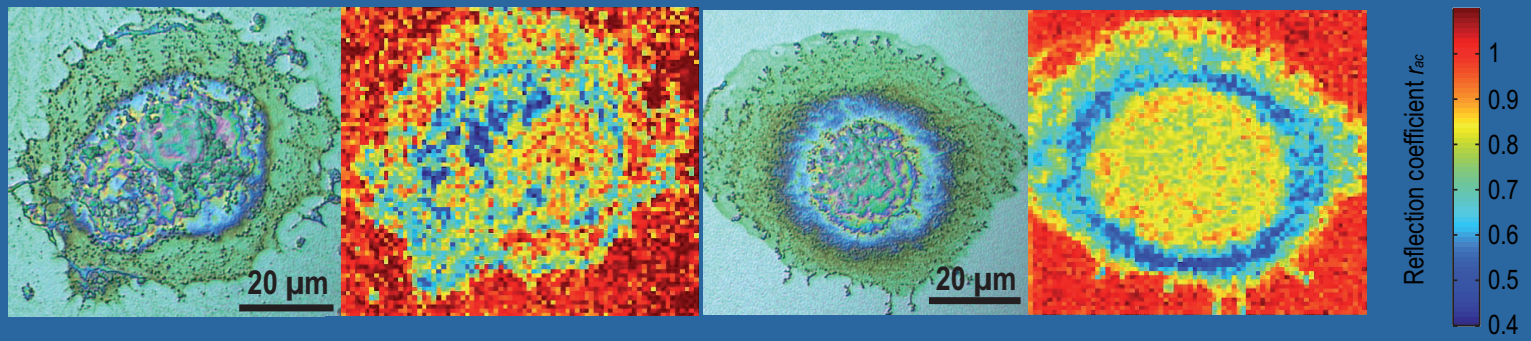
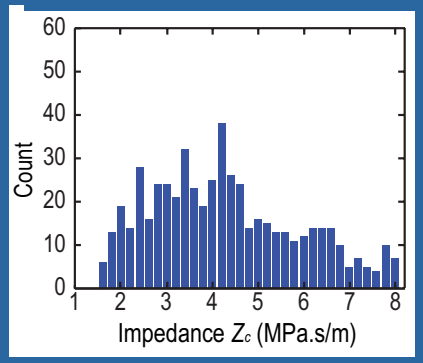
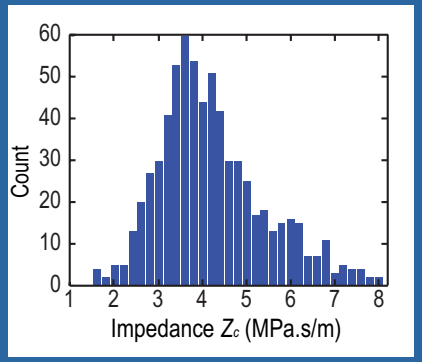
Single cell optical ultrasonography







B. Audoin *et al.*

I_2M, Physical Acoustics Department, UMR CNRS 5295, Univ. de Bordeaux, France

Cell mechanics in fundamental processes

Differentiation alters stem cell nuclear architecture, mechanics, and mechano-sensitivity

Su-Jin Heo[1,2], Tristan P Driscoll[1,2], Stephen D Thorpe[3], Nandan L Nerurkar[4], Brendon M Baker[5,6], Michael T Yang[5], Christopher S Chen[5,6], David A Lee[3], Robert L Mauck[1,2*]

Abstract Mesenchymal stem cell (MSC) differentiation is mediated by soluble and physical cues. In this study, we investigated differentiation-induced transformations in MSC cellular and nuclear biophysical properties and queried their role in mechanosensation. Our data show that nuclei in differentiated bovine and human MSCs stiffen and become resistant to deformation. This attenuated nuclear deformation was governed by restructuring of Lamin A/C and increased heterochromatin content. This change in nuclear stiffness sensitized MSCs to mechanical-loading-induced calcium signaling and differentiated marker expression. This sensitization was reversed when the 'stiff' differentiated nucleus was softened and was enhanced when the 'soft' undifferentiated nucleus was stiffened through pharmacologic treatment. Interestingly, dynamic loading of undifferentiated MSCs, in the absence of soluble differentiation factors, stiffened and condensed the nucleus, and increased mechanosensitivity more rapidly than soluble factors. These data suggest that the nucleus acts as a mechanostat to modulate cellular mechanosensation during differentiation.

DOI: 10.7554/eLife.18207.001 RESEARCH ARTICLE Nov 30, 2016

Role of cell nucleus to modulate mechano-sensation during differentiation.

Cell mechanics is key player in many fundamental processes: mechano-transduction, morphogenesis, motility, mitosis, apoptosis, **differentiation**, progression of degenerative diseases.

Cell mechanics in fundamental processes

LETTERS

Nanomechanical analysis of cells from cancer patients

SARAH E. CROSS[1,2†], YU-SHENG JIN[3†], JIANYU RAO[3*†] AND JAMES K. GIMZEWSKI[1]

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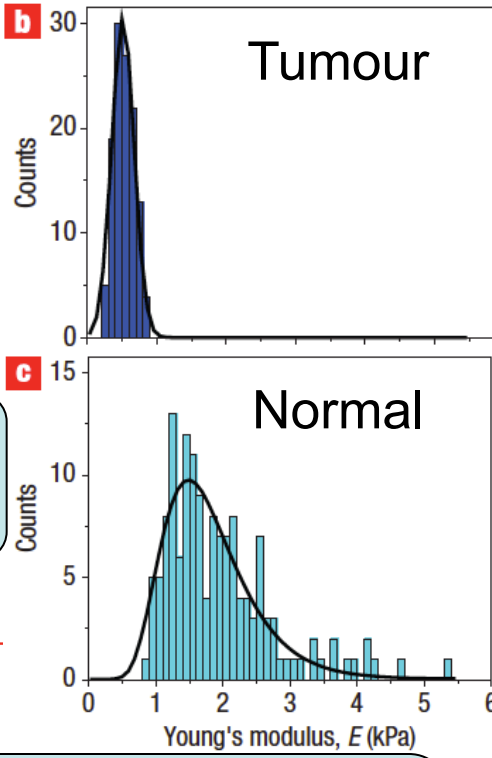
nature nanotechnology

Published online: 2 December 2007; doi:10.1038/nnano.2007.388



Cancer cells softer

Cell mechanics is key player in many fundamental processes: mechano-transduction, morphogenesis, motility, mitosis, apoptosis, differentiation, progression of **degenerative diseases**.

Cell mechanics in fundamental processes

Acta Materialia 55 (2007) 3989–4014

Biomechanics and biophysics of cancer cells ☆

Subra Suresh *

Department of Materials Science and Engineering, Division of Biological Engineering, and Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139-4307, USA

Received 2 April 2007; accepted 16 April 2007

Abstract

The past decade has seen substantial growth in research into how changes in the biomechanical and biophysical properties of cells and subcellular structures influence, and are influenced by, the onset and progression of human diseases. This paper presents an overview of the rapidly expanding, nascent field of research that deals with the biomechanics and biophysics of cancer cells. The review begins with some key observations on the biology of cancer cells and on the role of actin microfilaments, intermediate filaments and microtubule biopolymer cytoskeletal components in influencing cell mechanics, locomotion, differentiation and neoplastic transformation. In order to set the scene for mechanistic discussions of the connections among alterations to subcellular structures, attendant changes in cell deformability, cytoadherence, migration, invasion and tumor metastasis, a survey is presented of the various quantitative mechanical and physical assays to extract the elastic and viscoelastic deformability of cancer cells. Results available in the literature on cell mechanics for different types of cancer are then reviewed. Representative case studies are presented next to illustrate how chemically induced cytoskeletal changes, biomechanical responses and signals from the intracellular regions act in concert with the chemomechanical environment of the extracellular matrix and the molecular tumorigenic signaling pathways to effect malignant transformations. Results are presented to illustrate how changes to cytoskeletal architecture induced by cancer drugs and chemotherapy regimens can significantly influence cell mechanics and disease state. It is reasoned through experimental evidence that greater understanding of the mechanics of cancer cell deformability and its interactions with the extracellular physical, chemical and biological environments offers enormous potential for significant new developments in disease diagnostics, prophylactics, therapeutics and drug efficacy assays.

PERSPECTIVES

NATURE REVIEWS | CANCER VOLUME 11 | JULY 2011 | 513

OPINION

The physics of cancer: the role of physical interactions and mechanical forces in metastasis

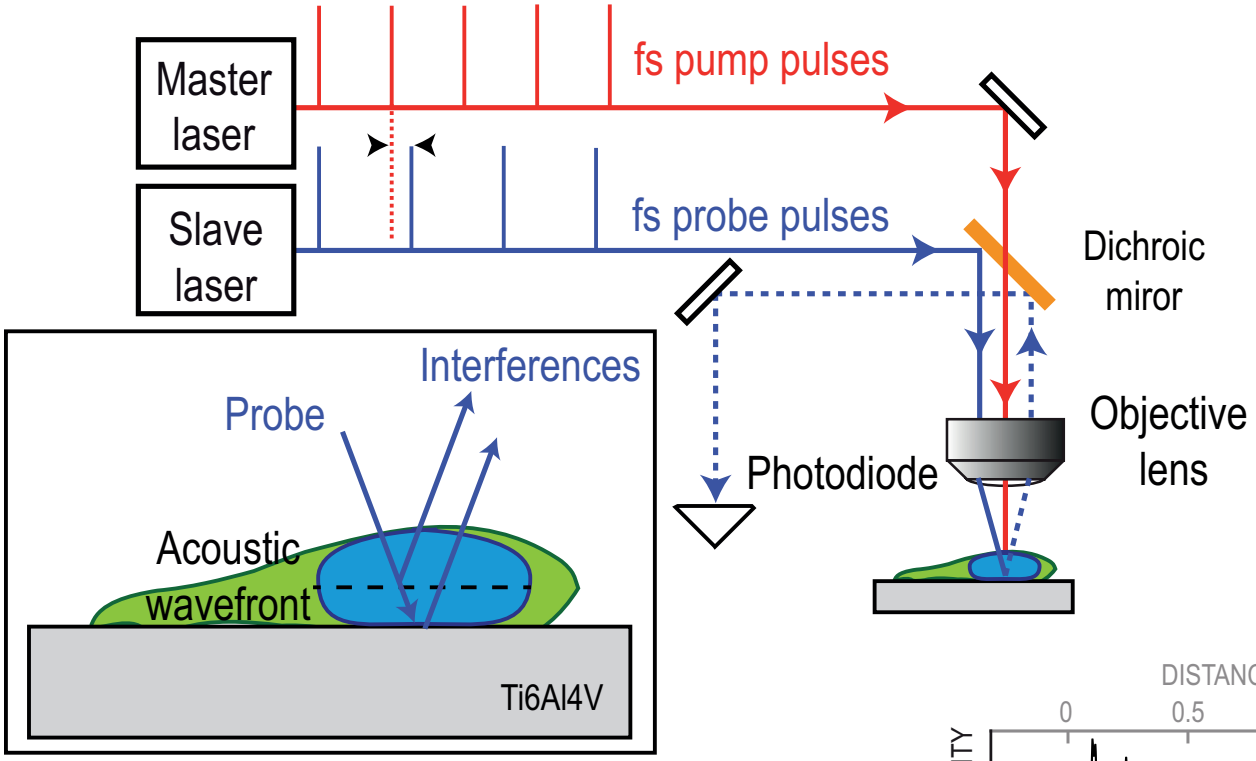
Denis Wirtz, Konstantinos Konstantopoulos and Peter C. Searson

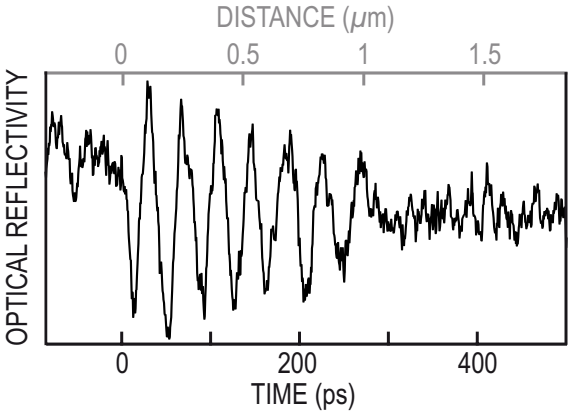
Abstract | Metastasis is a complex, multistep process responsible for >90% of cancer-related deaths. In addition to genetic and external environmental factors, the physical interactions of cancer cells with their microenvironment, as well as their modulation by mechanical forces, are key determinants of the metastatic process. We reconstruct the metastatic process and describe the importance of key physical and mechanical processes at each step of the cascade. The emerging insight into these physical interactions may help to solve some long-standing questions in disease progression and may lead to new approaches to developing cancer diagnostics and therapies.

"Understanding of the mechanics of cancer cells… offers enormous potential for significant developments in desease diagnostics therapeutics and drug efficiency assays"

Cell mechanics is key player in many fundamental processes: mechano-transduction, morphogenesis, motility, mitosis, apoptosis, differentiation, progression of **degenerative diseases**.

Probing single cell mechanics





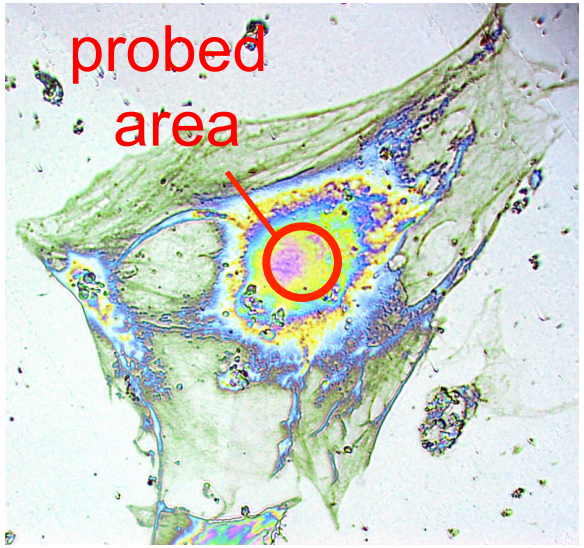
Time resolved coherent Brillouin scattering:

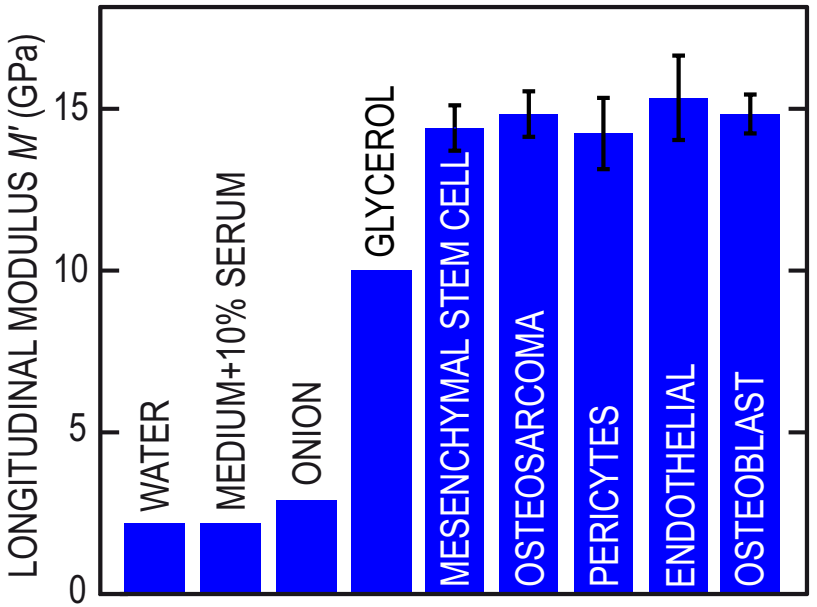
Needs cell thickness > $\frac{\lambda_{opt}}{2n} \approx 200 \text{ nm}$

Sound velocity: in vegetal cell organels, **in human cell nuclei.**

Appl. Phys. Lett., 93, 123901, 2008

In the nucleus of single human cells: discussion





Nucleus: a network of chromatin fibers randomly oriented in a cytosol like fluid (viscous).

M' ≈ 15 GPa, **not affected by intranuclear fluid**.

Properties of an elastic chromatin network common among several cell type.

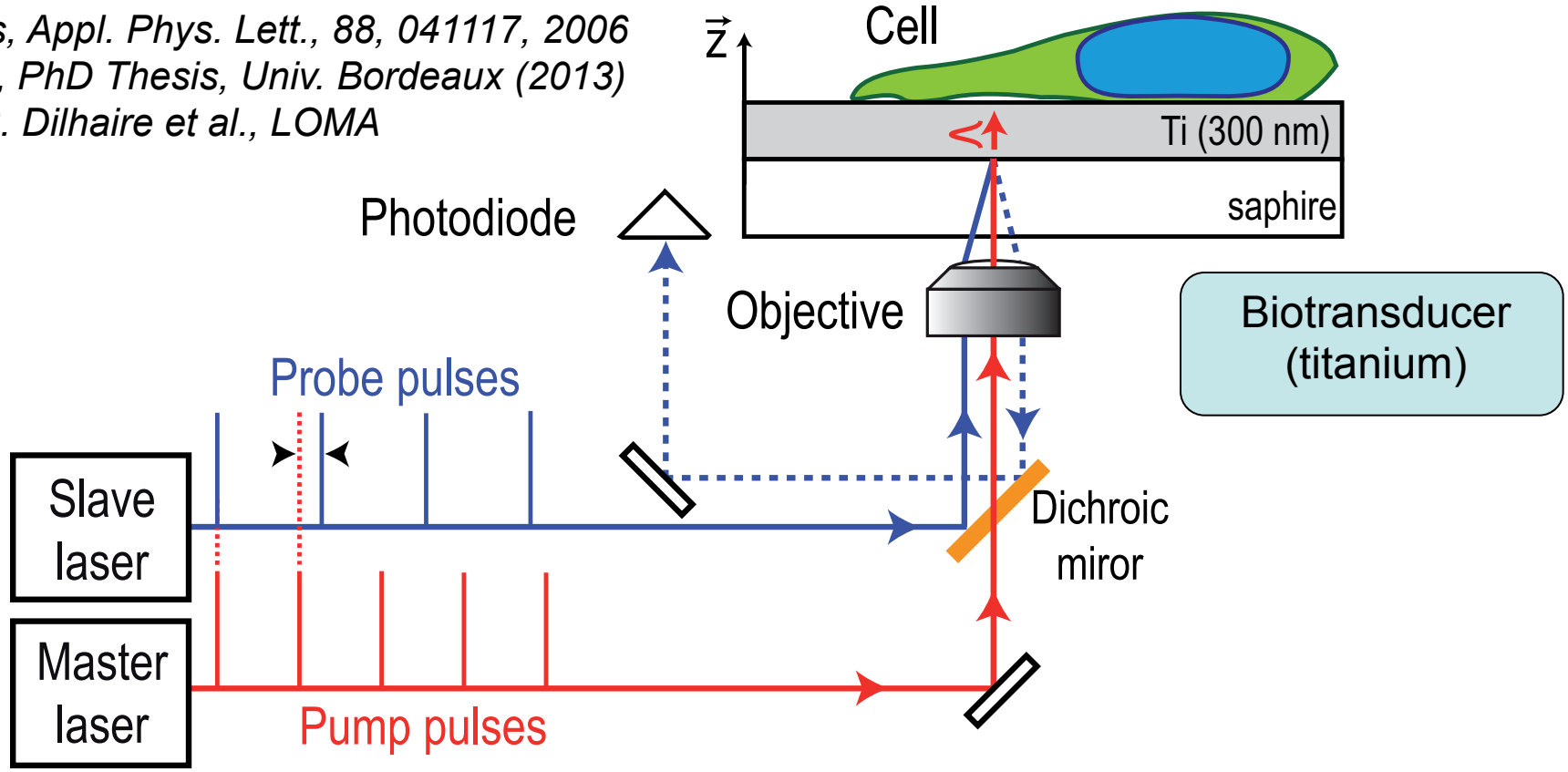
A volume fraction of 0.7 yields a chromatin fibre modulus of 22 GPa close to globular proteins. Assuming a glass behaviour of intra-nuclear fluid, with negligible elastic modulus compared to that of fibres.

contribution of the rigid scaffold

Soft Matter, 10, 8737, 2014

iPOM (inverted Pulsed Opto-acoustic Microscope)

Bartels, Appl. Phys. Lett., 88, 041117, 2006
Abbas, PhD Thesis, Univ. Bordeaux (2013)
Coll. S. Dilhaire et al., LOMA

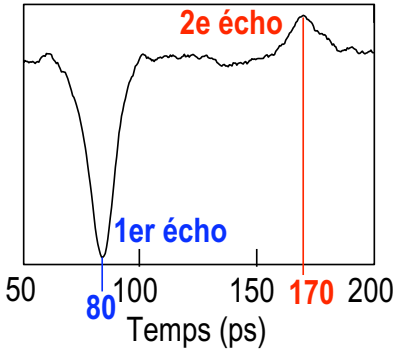


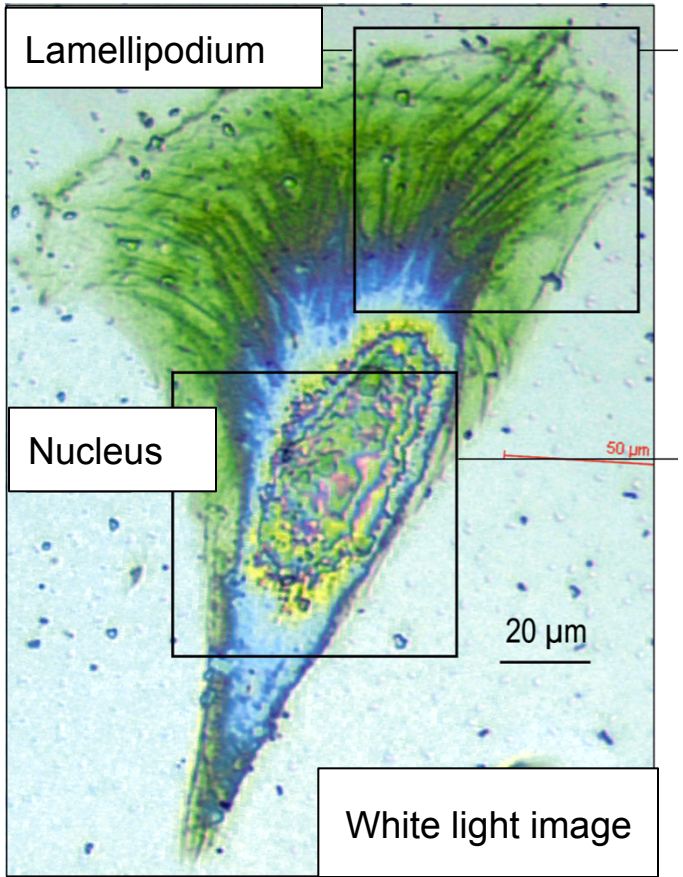
Asynchroneous set-up
16 pixels per minute

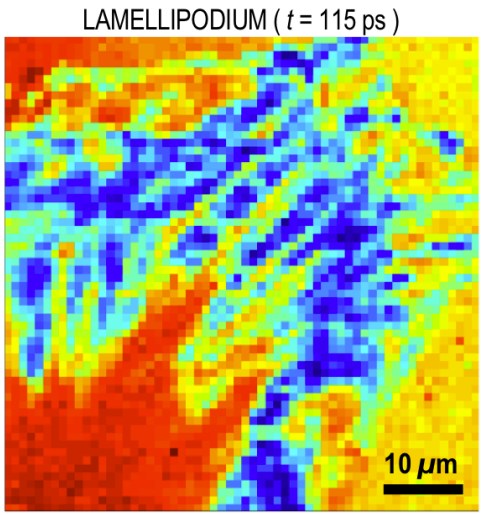
An opto-acoustic microscope suited for single cell imaging
Micron résolution **in plane** *(optics)*
Sub-micron résolution **in depth** *(acoustics)*

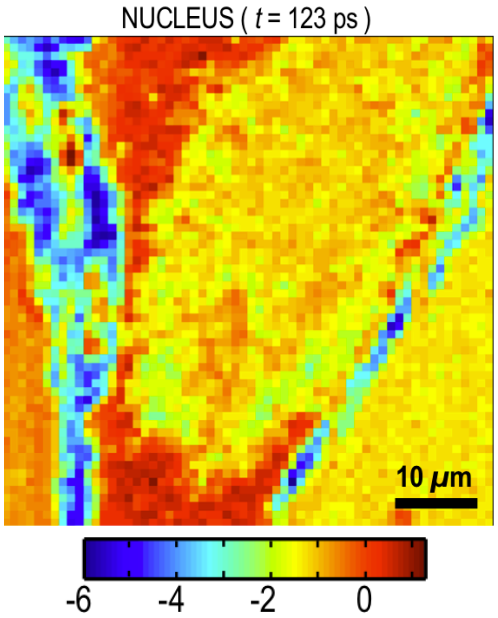
Single cell ultrasonography (hMSC)

Acoustic strain at one point

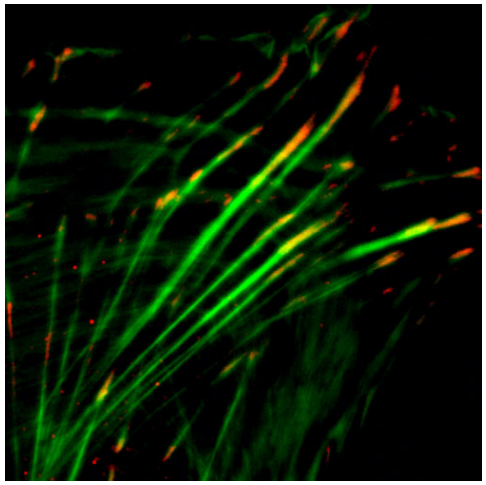


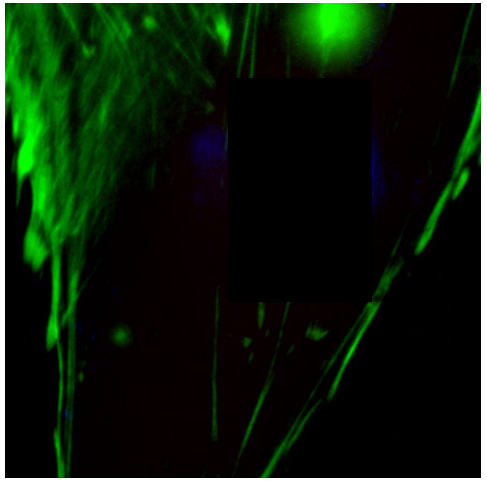






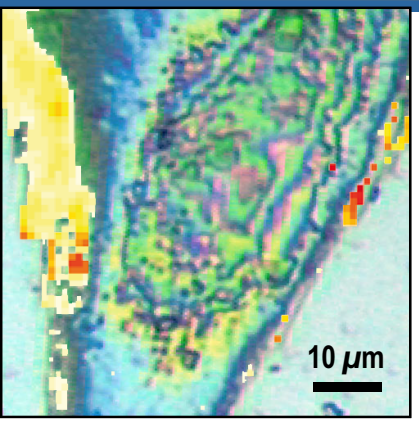
Fluorescence

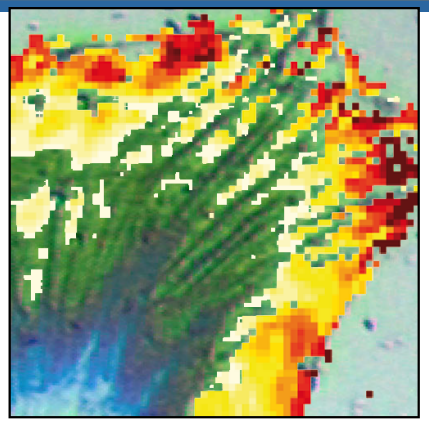


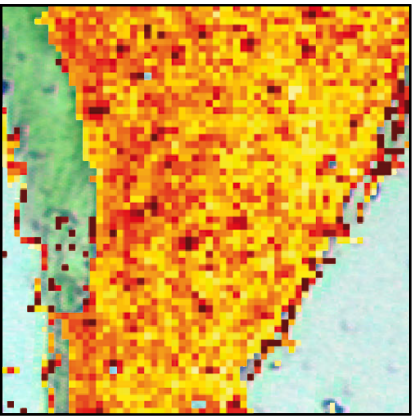


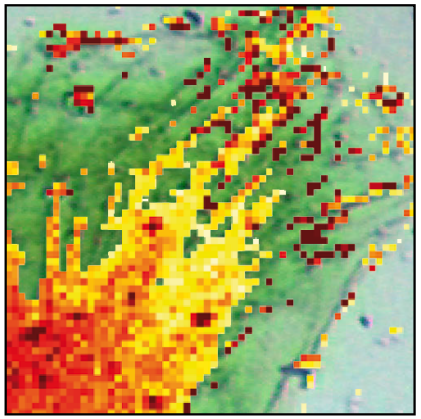
Scient. Reports, ***5****, 8650, 2015.*

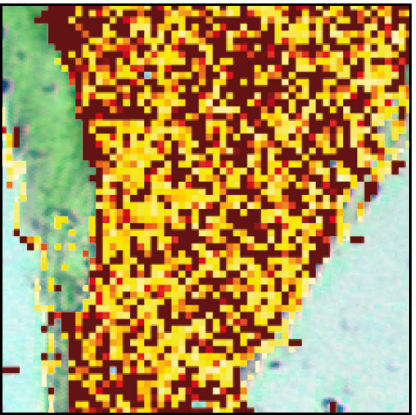
Imaging thickness, impedance, contact stiffness

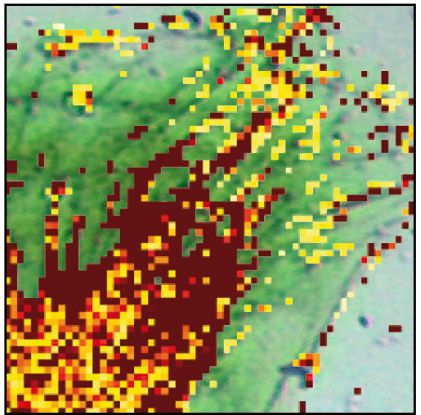


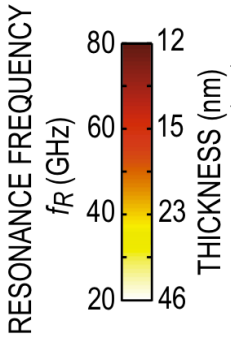


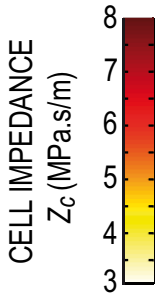


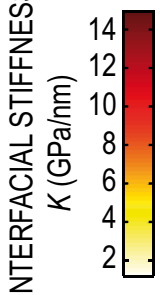












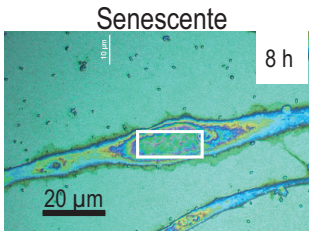
Identification of cytosqueleton main structures: **actin fibers, arcs, focal points of adhesion**

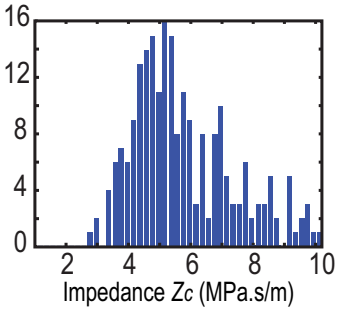
Impedance increases gradually to 5 MPa.s/m in the nucleus due to **molecular crowding**

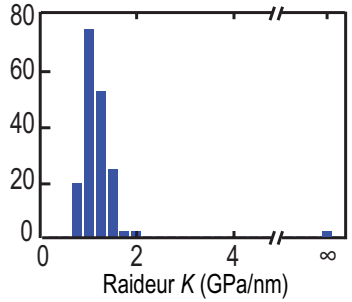
In area where K reaches max. value, the adhesion of the cell with the metal is the strongest to provide **anchor for** the tread-milling **motion during migration**

Scientific Reports, ***5***, *8650, 2015.*

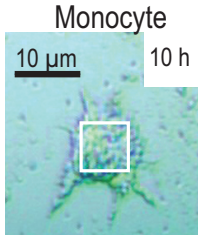
Several cell types: senescent, monocytes, hMSC

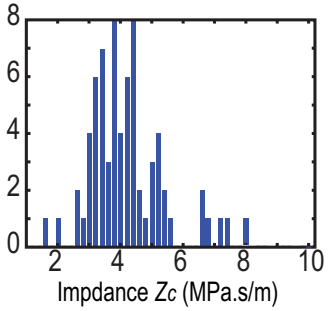


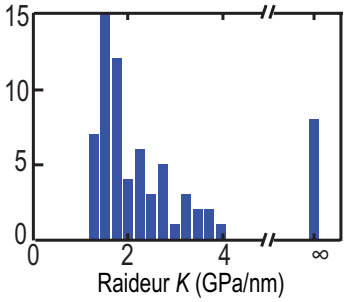




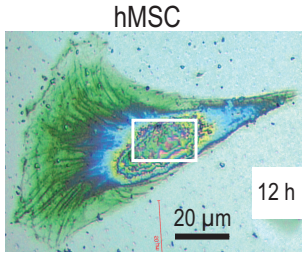
Fibroblast senescent cells:
stop to divide after about 50 cycles.
fibroblasts: cells of connective tissues;

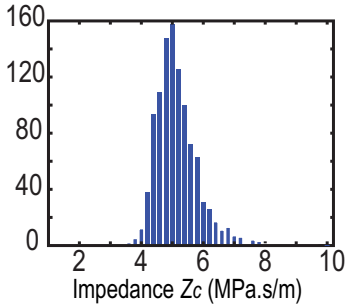


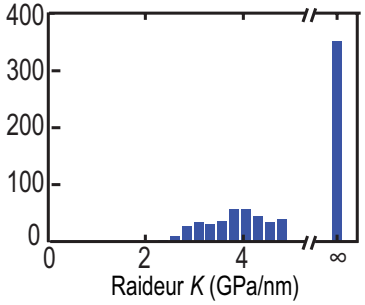




Monocytes:
a type of white blood cells;







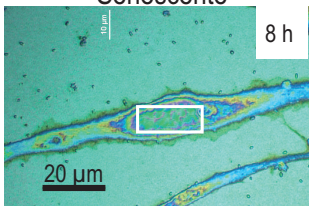
hMSC: Human mesenchymal stem cells:
multipotent cells that can differentiate into osteoblast (bone), chondrocytes (cartilage), adipocytes (fat)…

Acoustic impedance

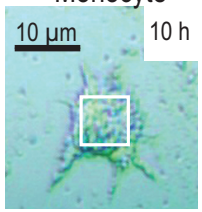
Contact stiffness

Several cell types: senescent, monocytes, hMSC

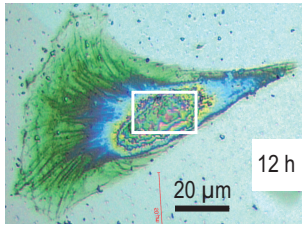
Senescente



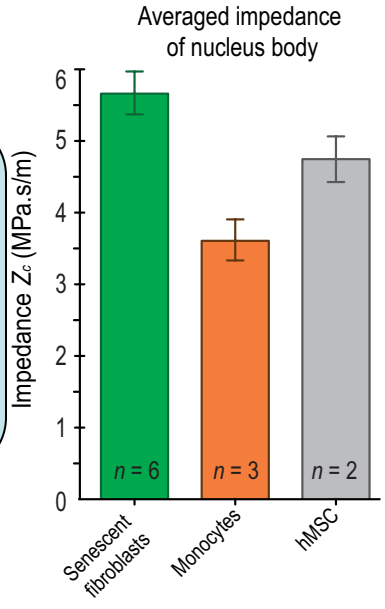
Monocyte

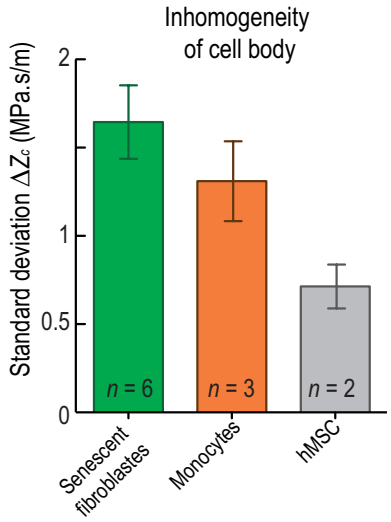


hMSC



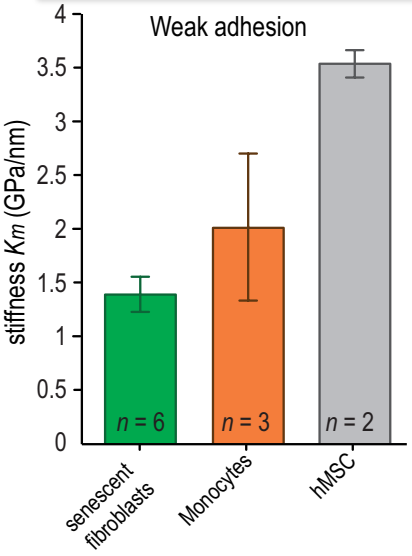
Impedance mean value: density, ***stiffness of chromatine network***

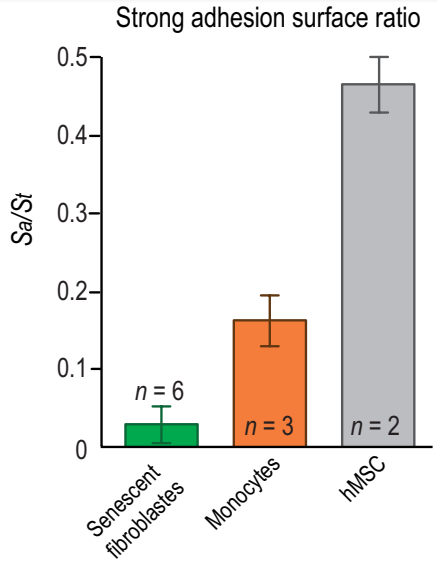




standard deviation: ***inhomogeneity of the nucleus*** body

Mean weak adhesion: ***strength of molecular links***

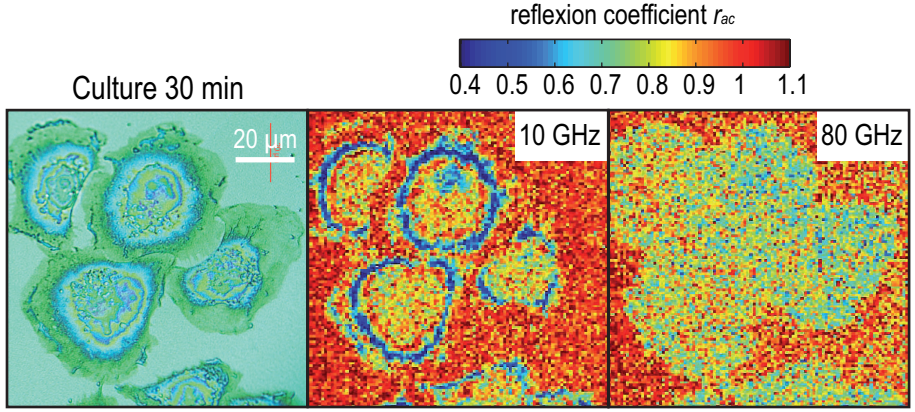


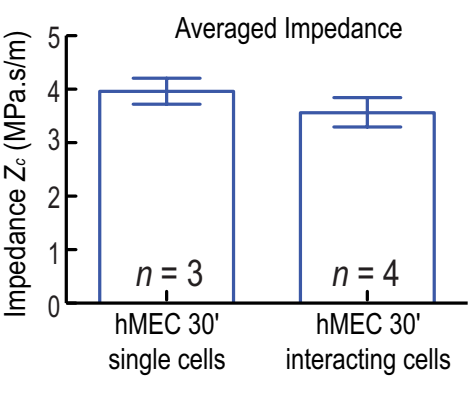


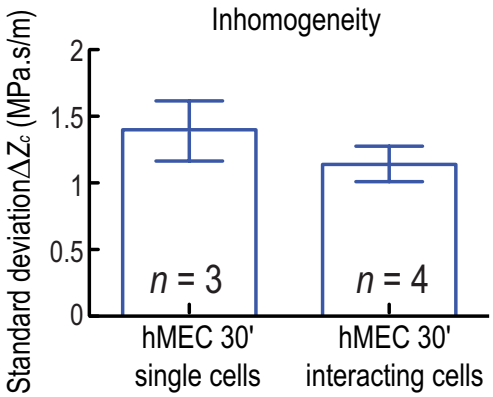
Surface ratio of strong adhesion: ***number and size of adhesion focii***

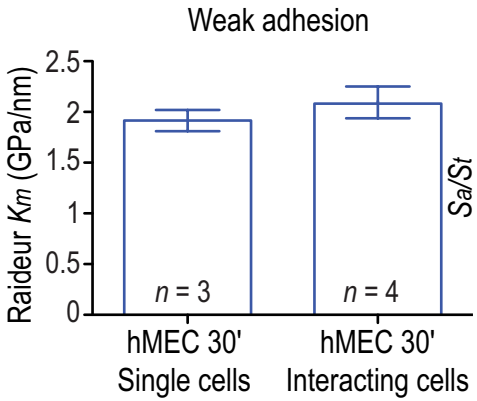
Correlation of adhesion with impedance inhomogeneity

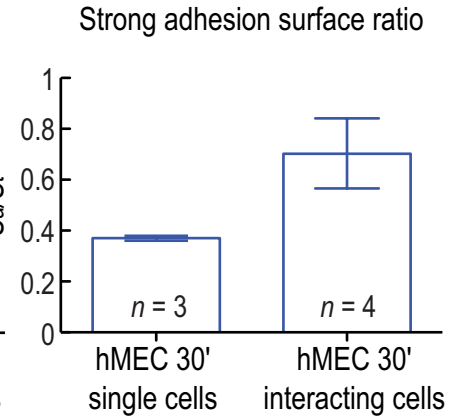
Cell-cell interaction (hMEC endothelial cells)











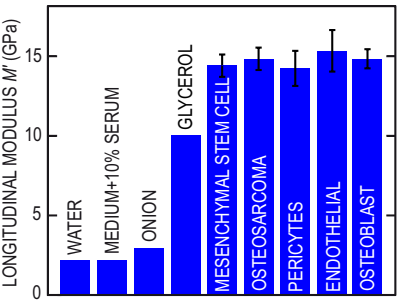
Impedance ***mean value*** and ***standard deviation***.
Decrease of nucleus homogeneity

Mean ***weak adhesion;*** and surface ratio of ***strong adhesion***.
Increase of strong adhesion

Increase of the surface ratio of strong adhesion sites

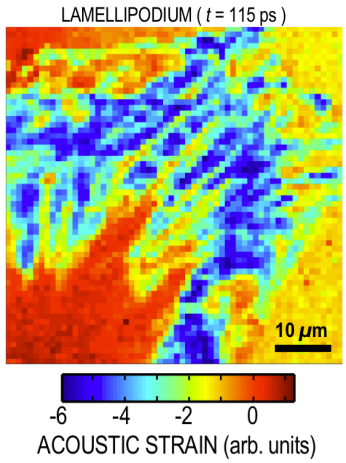
Interaction with other cells favors building of strong adhesion sites

Remote, label-free optical ultrasonography



Quantitative evaluation (functional information: **metabolic processes**),

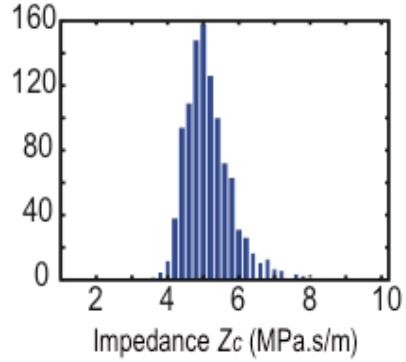
We measured the stiffness of single cell nuclei in the previously unexplored GHz frequency range.



Nucleus behaves as a solid network at this regime. The stiffness of this scaffold is common across several cell types.

The inverted Picosecond Opto-acoustic Microscope (iPOM) allows:

- **mapping** of the fibrilar structure of the cell, adhesion sites
- Assessing -thickness, impedance locally
  -adhesion strength of each adhesion site.



Statistical analysis of large data number for several cell types shows robustness to qualify mechanical properties changes from one cell type to another and to **differentiate active/passive proceses**

Partners and fundings

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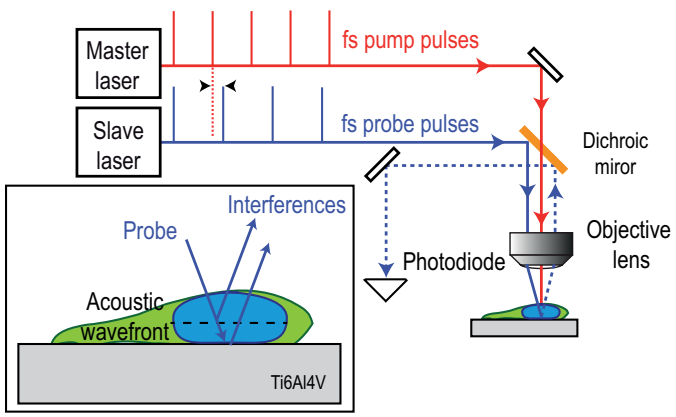
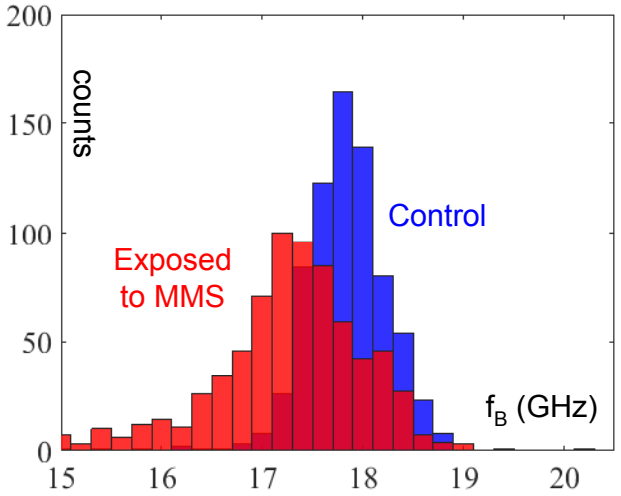
Probing DNA damage mechanics (coll. CENBG)

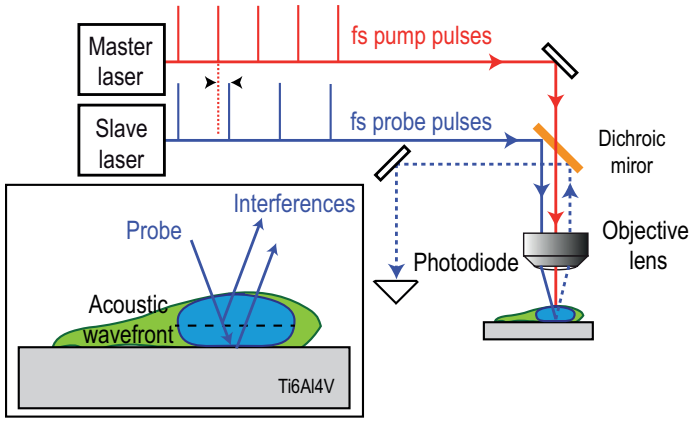


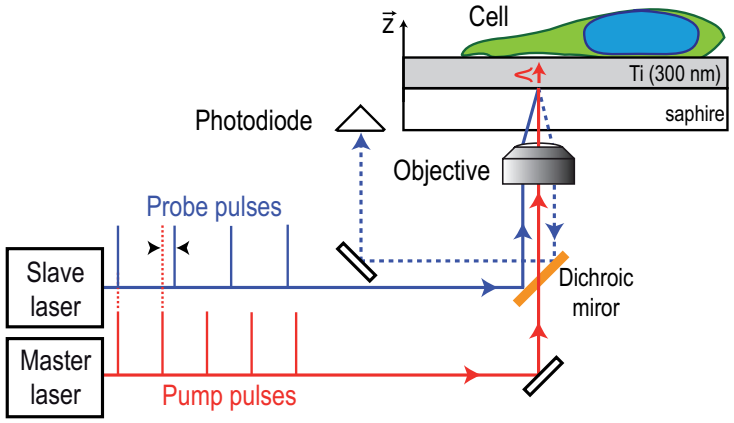


Preliminary results. Exposure to methyl Methanesulfonate (MMS)

Occurrence of the Brillouin frequencies measured ***in nuclei of osteosarcoma cells****. 720 measurements performed in 20 nuclei for control cells (blue) and cells exposed to MMS (red). The mean frequencies are 17.8 and 17.3 GHz and standard deviations are 0.4 and 0.8 GHz for control and exposed cells, respectively.* ***Cells appear softer after MMS exposure*** *(DNA damage inducer).*

Probing cell mechanics





Coherent Brillouin scattering:

Needs cell thickness > $\frac{\lambda_{opt}}{2n} \approx 200\ \text{nm}$

Sound velocity
in vegetal cell organels,
in human cell nuclei.

Pulse ultrasonography:

Measures GHz sound reflection at the metal/cell interface

Maps of cell impedance, adhesion stiffness, cell thickness