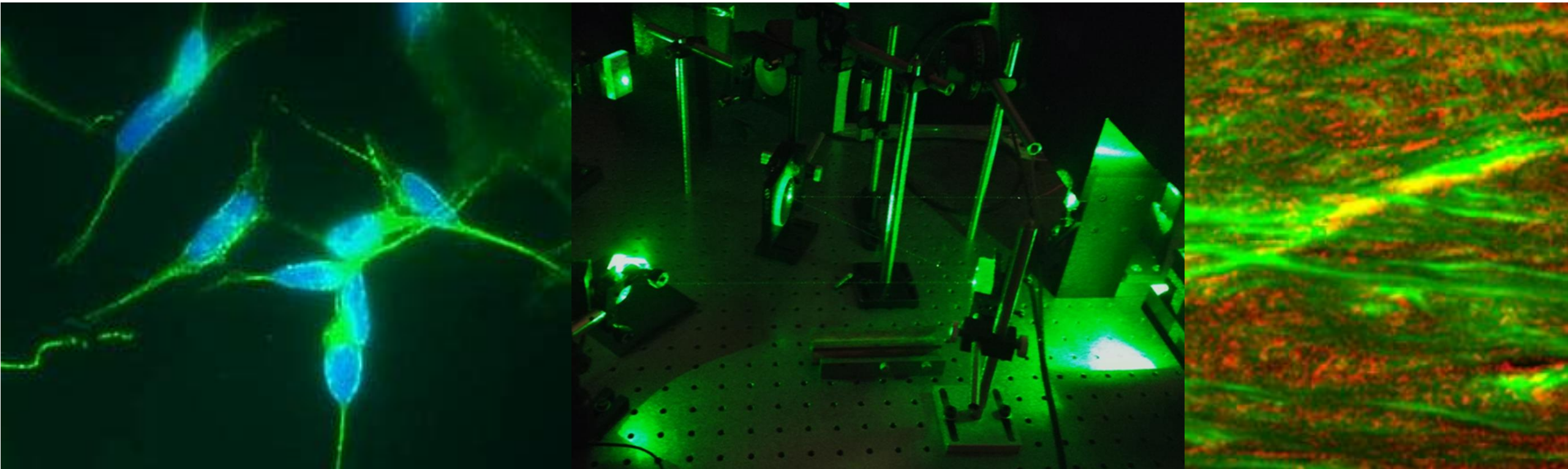


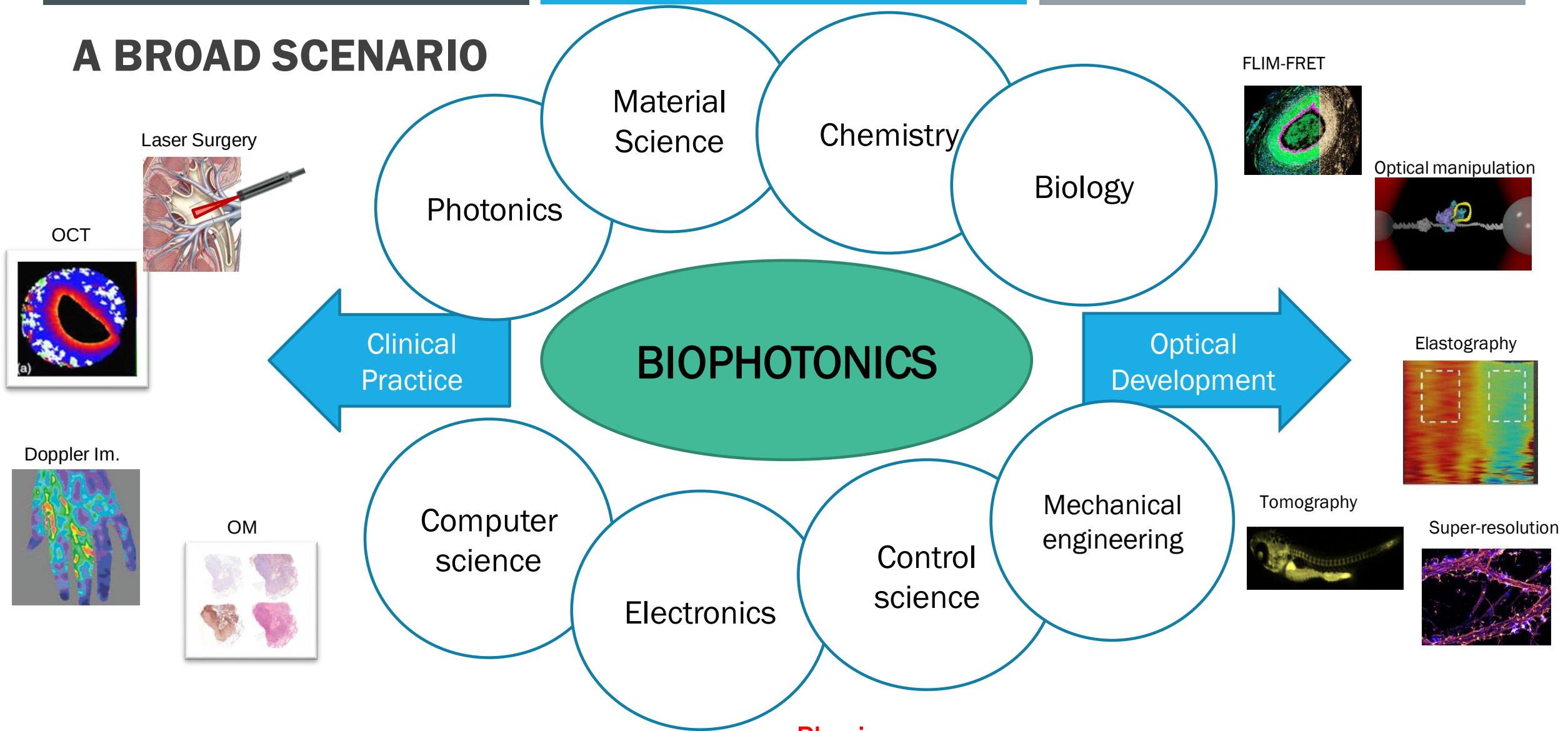
BIOPHOTONICS

MAURIZIO MATTARELLI, DANIELE FIORETTO (UNIPG)

SILVIA CAPONI (CNR)

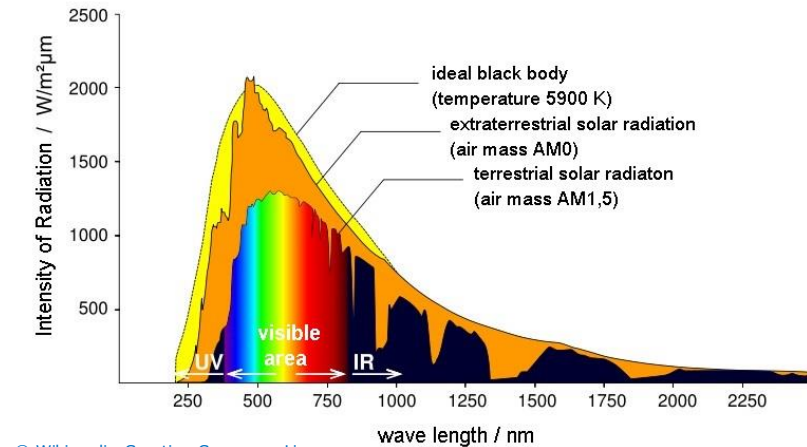


A BROAD SCENARIO



Physics touches many different aspects of this field:
from the optics of the instrument design, to the interaction mechanism of
light and matter, to biophysics, to the design of biomimetic materials

WHY THE LIGHT TO STUDY BIOLOGICAL MATTER?

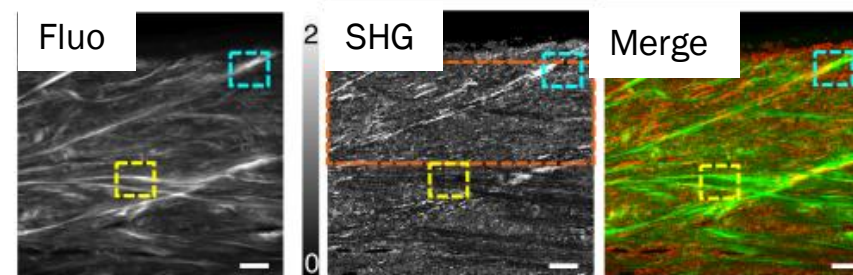


© Wikimedia Creative Commons Licence

ONE FUNDAMENTAL REASON:
It can interact with biological matter,
without destructing it

MANY PRACTICAL ONES:

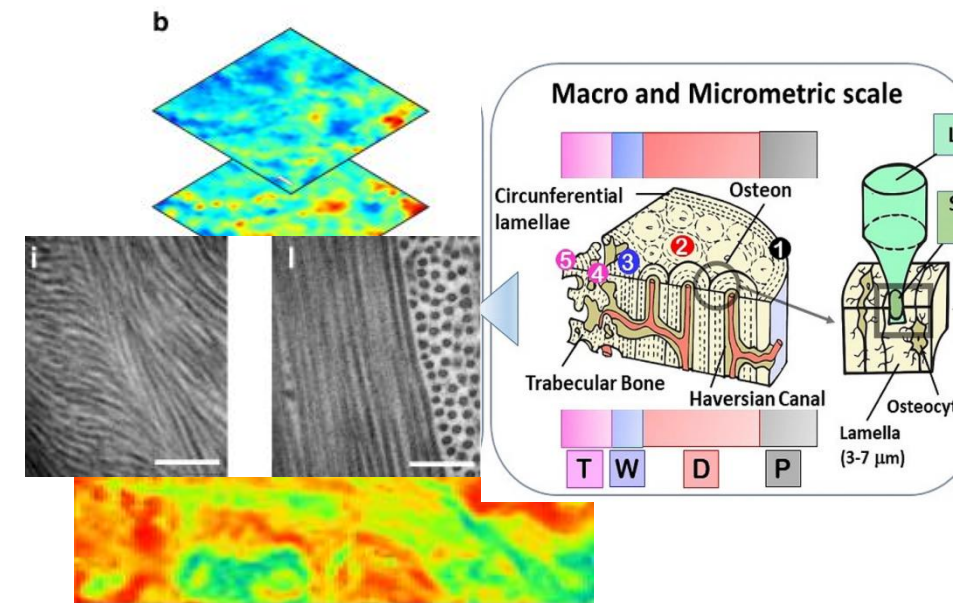
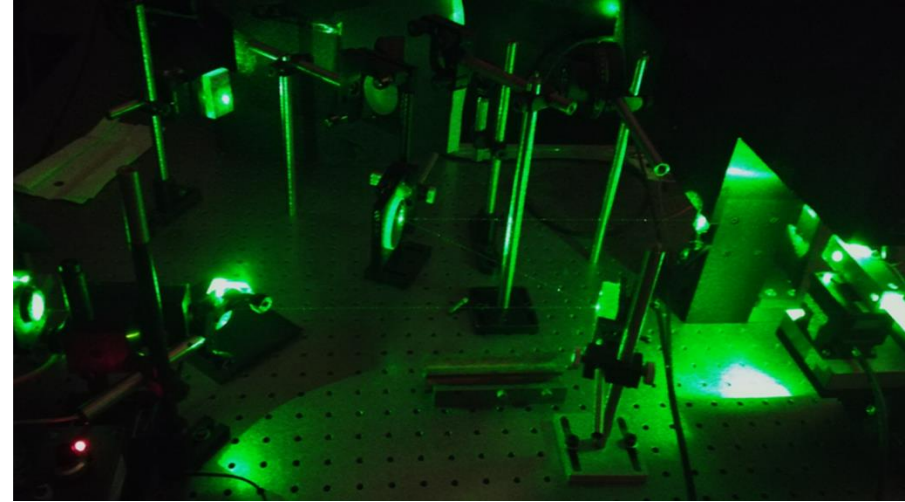
- Wide temporal and spatial scales
- Multifunctional (morphology, chemistry, structure)
- Compatibility (use of more simultaneous techniques)
- Practicity (price, invasiveness)



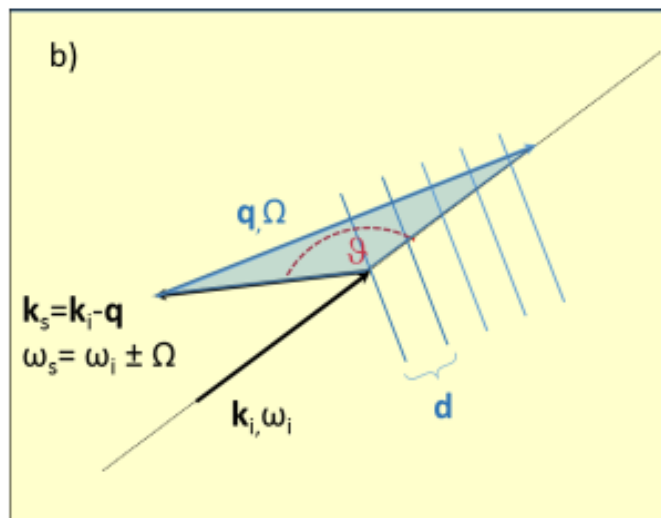
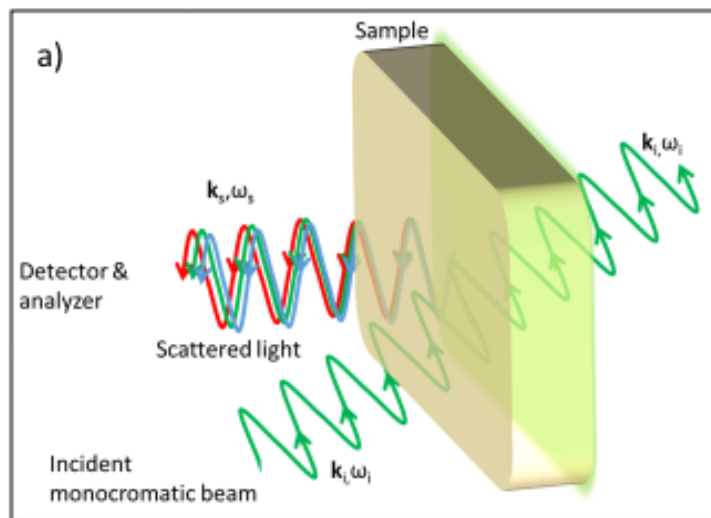
Mercatelli *et al.*, *Commun Biol* 2, 117 (2019).

BIOPHOTONICS IN PERUGIA

- Instruments development:
phenomenology of the light matter
interaction and new experimental strategies
- Study of relevant biological structures
(from tissues to single cells)



INELASTIC LIGHT SCATTERING

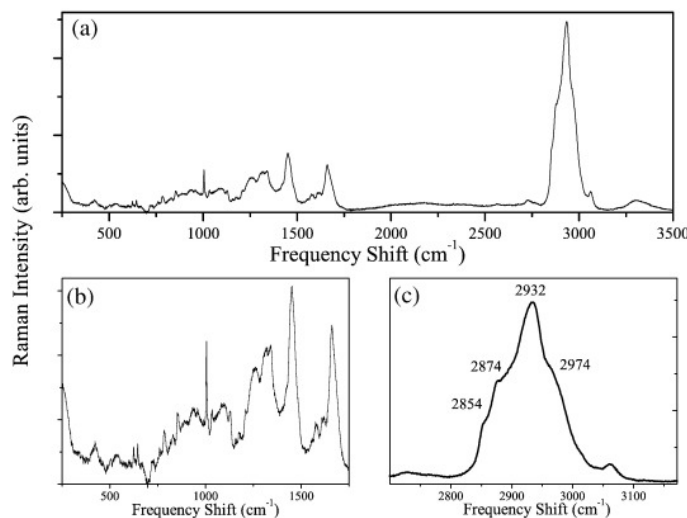


Sound waves and molecular vibrations thermally activated induce a modulation of the refractive index

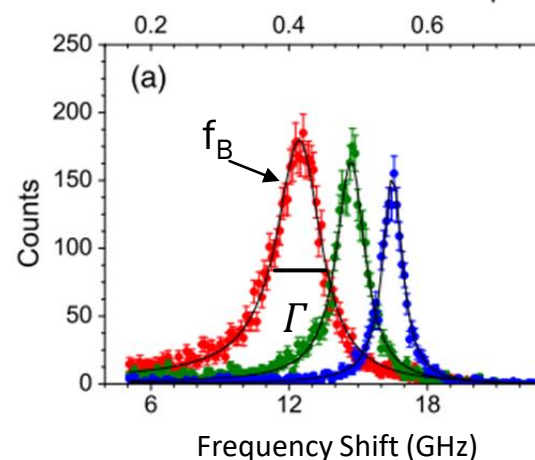
$$I_{\alpha\beta}(\mathbf{q}, \omega) \propto \int dt e^{-i\omega t} \langle \delta\epsilon_{\alpha\beta}^*(\mathbf{q}, 0) \delta\epsilon_{\alpha\beta}(\mathbf{q}, t) \rangle,$$

Frequency of the normal modes

$$\sim \nu = \frac{1}{2\pi} \sqrt{\frac{k}{m}}$$



S. Caponi et al. Biophysical Chemistry 182, 1 58 (2013)



Viscoelastic properties

$$\nu = \sin(\theta/2) f_B \lambda_0 / (2n)$$

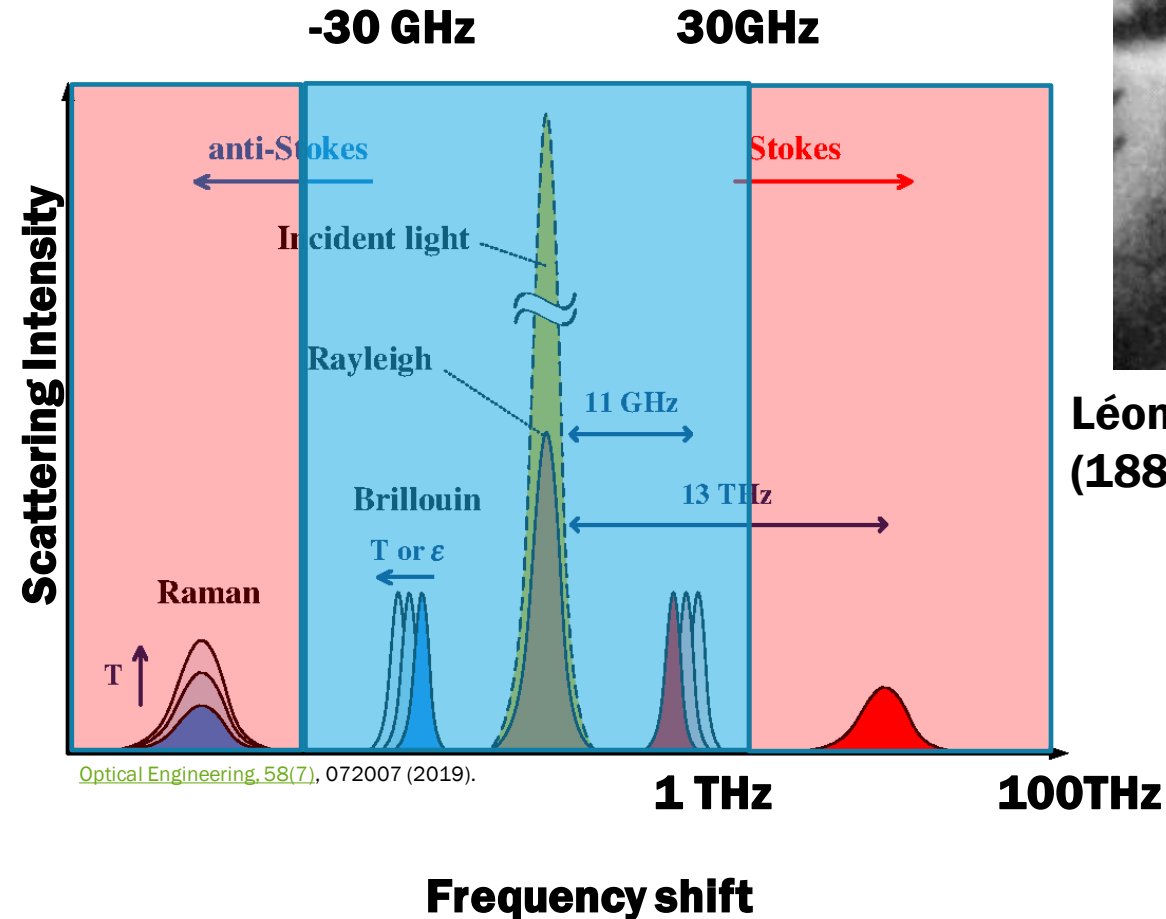
$$M' = \nu^2 \rho$$

$$M'' = f_B \eta_L = \frac{f_B \rho \Gamma}{(4\pi n)^2}$$

BRILLOUIN AND RAMAN SPECTROSCOPY

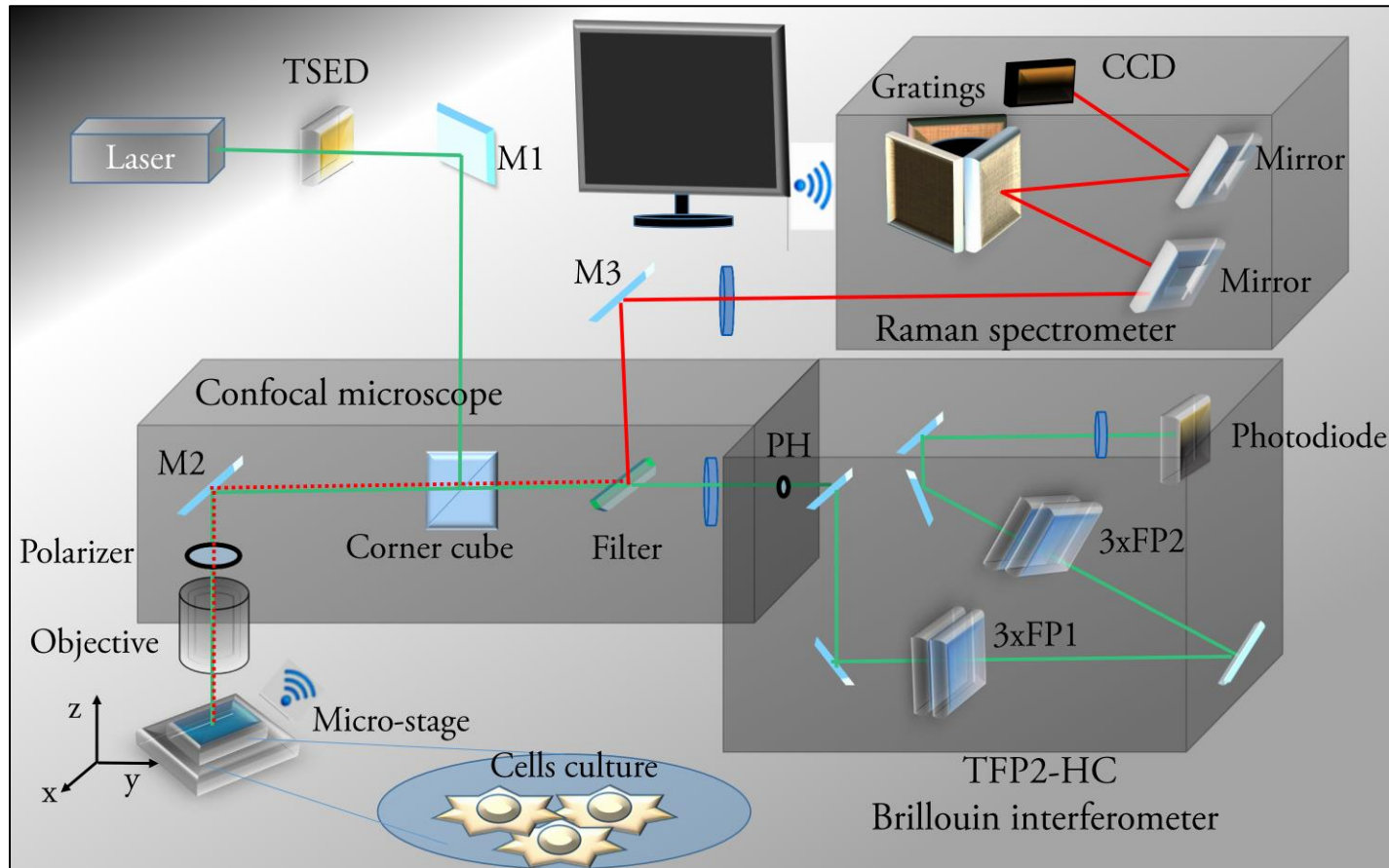


**Sir C. V. Raman
(1888 –1970)**

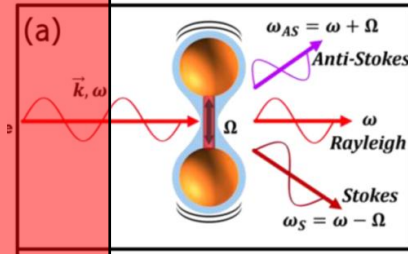


**Léon Nicolas Brillouin
(1889–1969)**

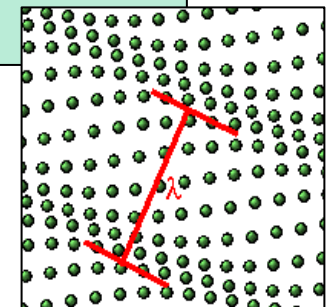
NEW SETUP FOR SIMULTANEOUS μ -BRILLOUIN AND μ -RAMAN SPECTROSCOPY



**vibrational modes
of molecules**
*Chemical
properties -
composition,
structure*



**thermally activated
acoustic waves**
*Mechanical
properties
In the continuum
description*

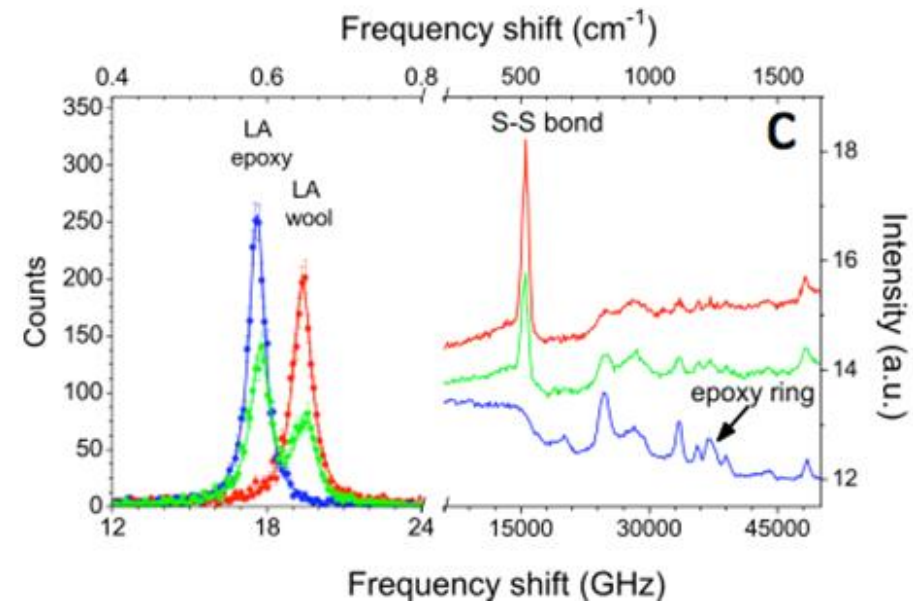
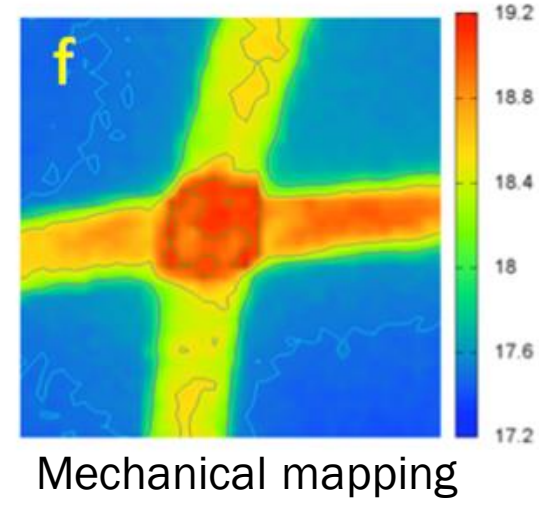
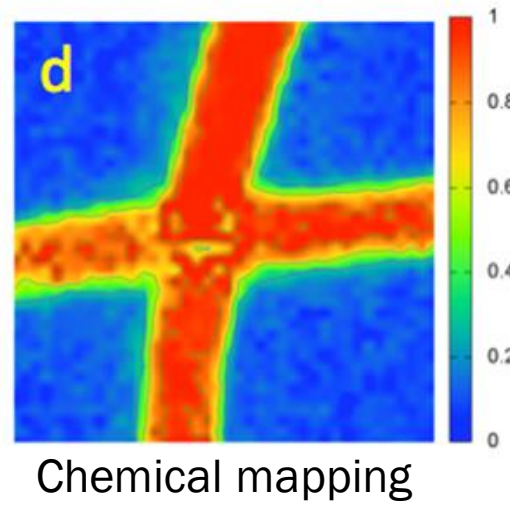
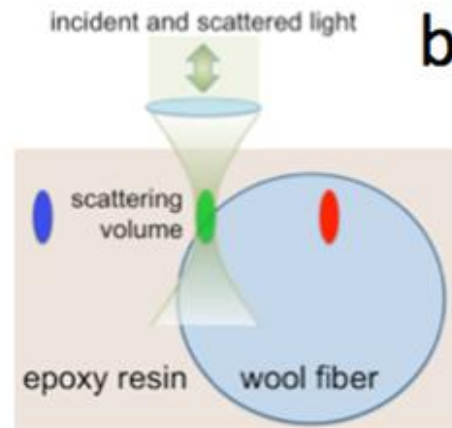
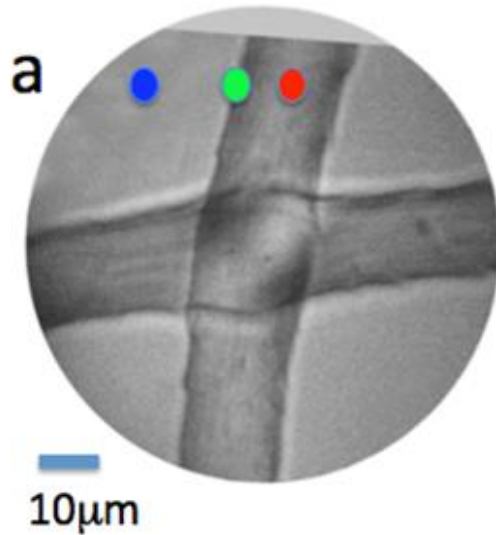


F. Scarponi et al. PRX 7, 031015 (2017);
S. Mattana et al. Nature Light: Science & Applications 7, 17139 (2018);
R. Mercatelli et al. Nature: Comms Biology 2 117 (2019).

BRILLOUIN AND RAMAN MAPPING

Two wool fibers embedded
into epoxy film

2 μ m step, 40 $\times$ 40 points
10 s
P=5mW

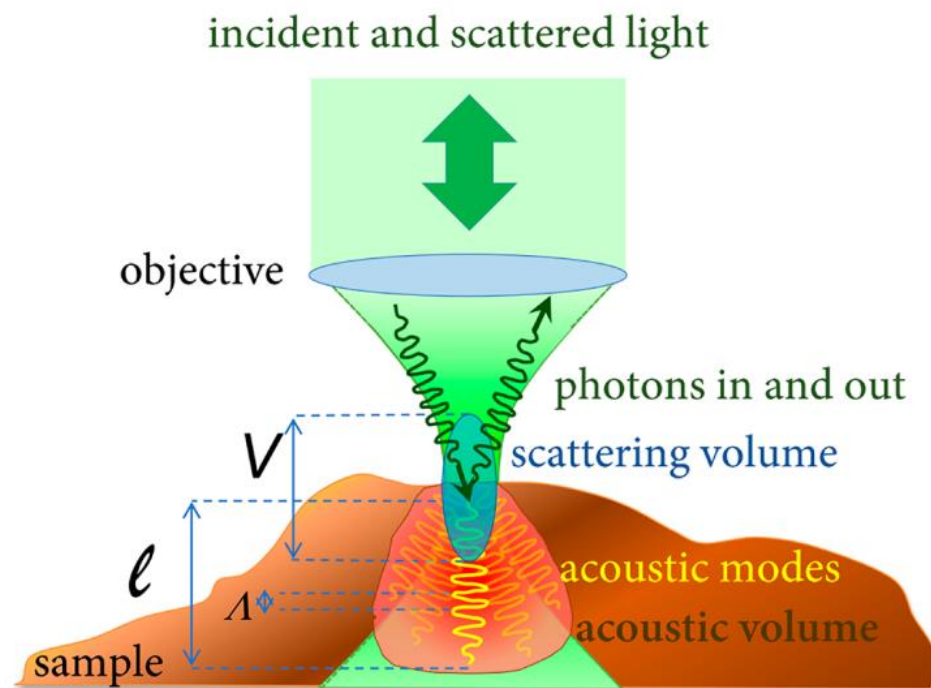


Fioretto et al. Biomedical Optics Express (2019)

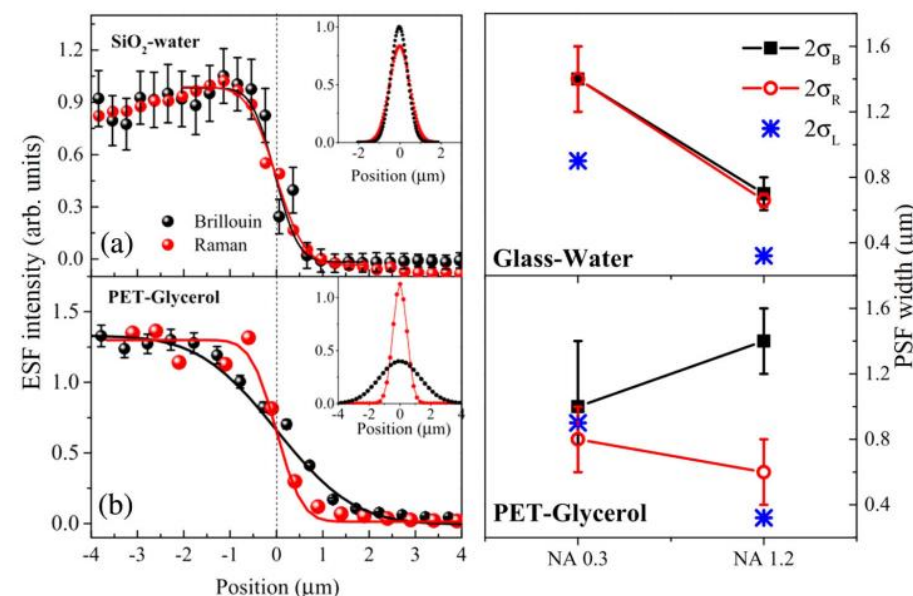
SPATIAL RESOLUTION IN BRILLOUIN IMAGING

The propagating acoustic phonon is the probe of the sample visco-elastic properties

- Λ = phonon wavelength (200 nm using $\lambda=532\text{nm}$)
- ℓ = mean free path of the acoustic phonons $\ell = (\omega_B/2\Gamma) \Lambda$
- V = the dimension driven by the optical system

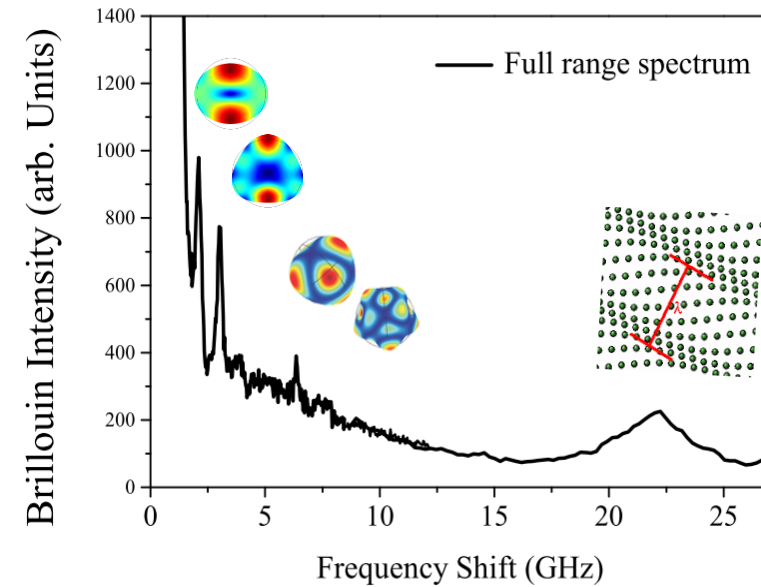
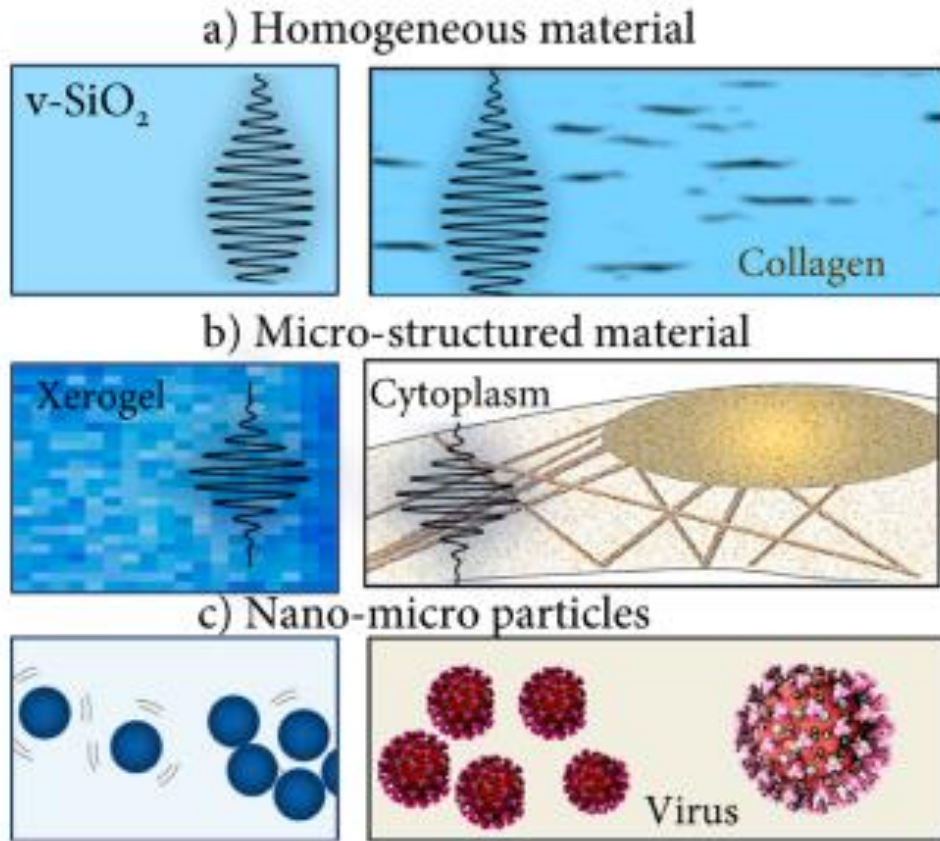


The acoustic volume (red) takes into account the broadening induced by the phonon propagation



M. Mattarelli, M. Vassalli, S.Caponi, ACS Photonics (2020)
S.Caponi, D. Fioretto, M. Mattarelli, Optics Letters (2020)

WHAT IF THE SIZE DECREASES?



$$\omega_B^2 = M/\rho \cdot q^2$$

$$\omega_k^2 = C_k M/\rho \cdot 1/d^2$$

$$C_k = C_k(M, G)$$

The normal modes are sensitive to the environment



Particles as elastic probes (on a much reduced length-scale!)

SCIENTIFIC REPORTS

OPEN

Efficient Structure Resonance Energy Transfer from Microwaves to Confined Acoustic Vibrations in Viruses

Received: 22 June 2015
Accepted: 05 November 2015
Published: 09 December 2015

Szu-Chi Yang¹, Huan-Chun Lin², Tzu-Ming Liu¹, Jen-Tang Lu¹, Wan-Ting Hung¹, Yu-Ru Huang¹, Yi-Chun Tsai¹, Chuan-Liang Kao², Shih-Yuan Chen¹ & Chi-Kuang Sun^{1,5}

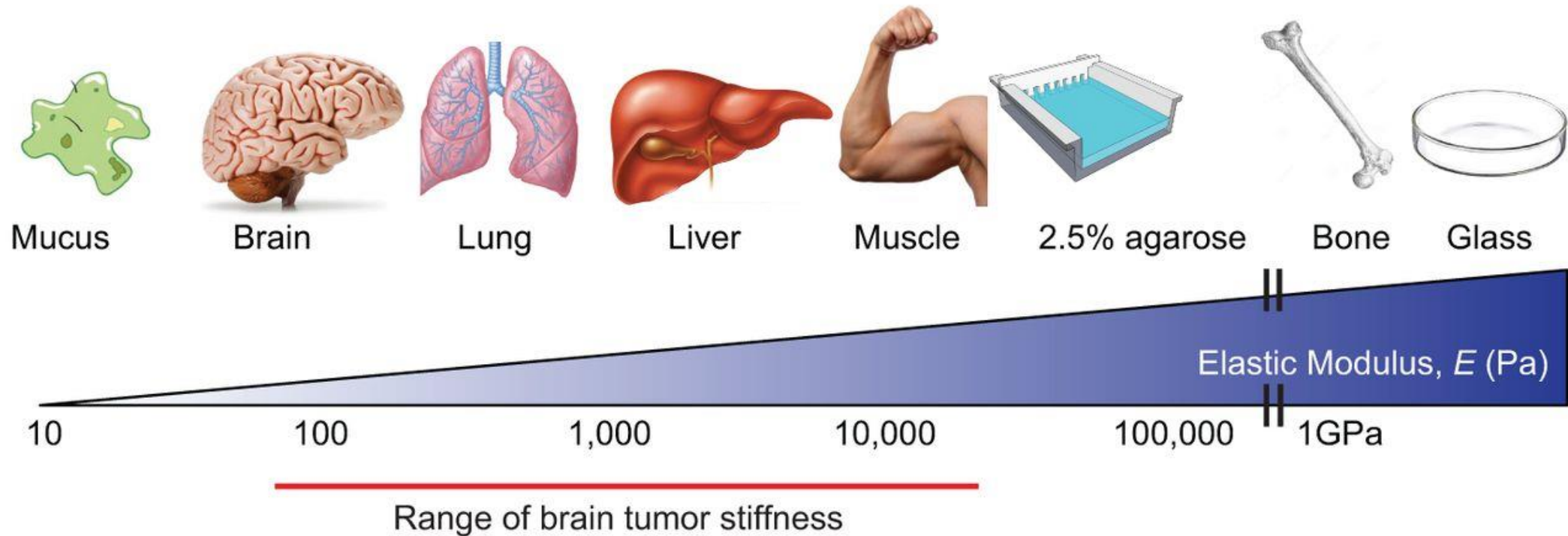
It is possible to «physically» inactivate virus by em excitation if...

M. Mattarelli, M. Vassalli, S.Caponi ACS Photonics (2020)

USING LIGHT TO PROBE AND IMAGE THE MATERIALS PROPERTIES

Mechanics in biology

Tissues and cells shape, size and also mechanical properties strongly depend on their function



NEW SETUP FOR SIMULTANEOUS μ -BRILLOUIN AND μ -RAMAN SPECTROSCOPY

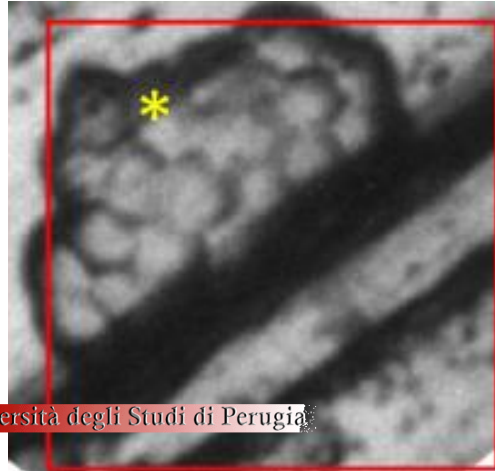
Living cells



Università degli Studi di Perugia



Biofilms



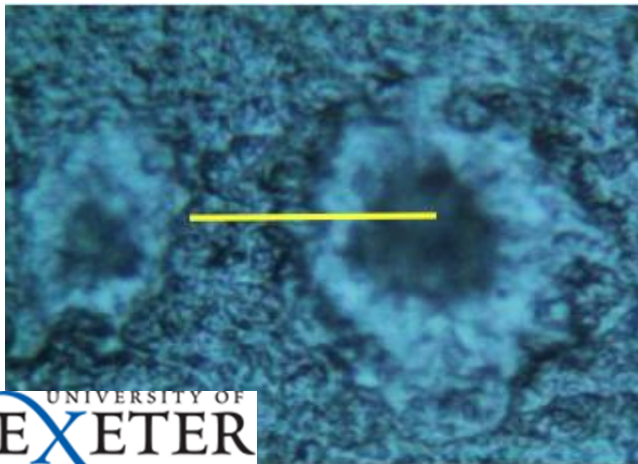
Università degli Studi di Perugia

Biomimetic materials : ECM

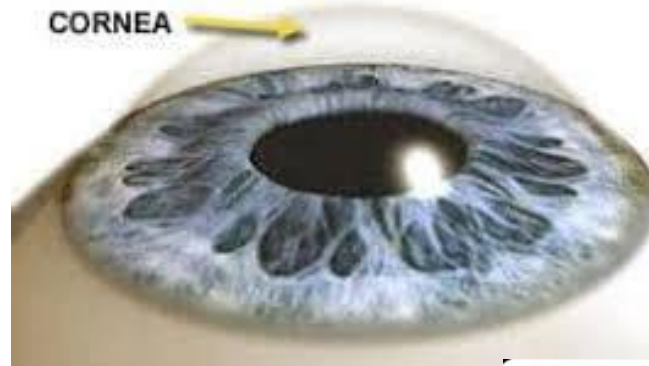


University of Glasgow

Amyloid Plaques in Transgenic Mouse Brain



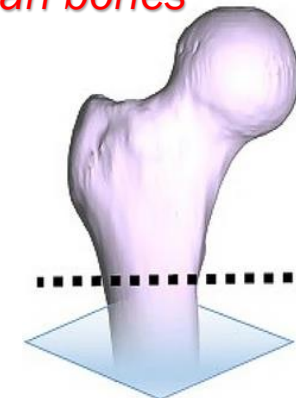
Human cornea



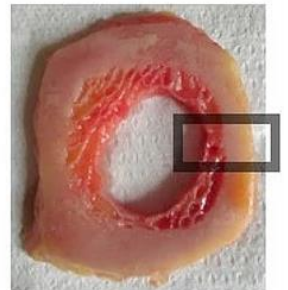
INO-CNR
ISTITUTO
NAZIONALE DI
OTTICA



Human bones

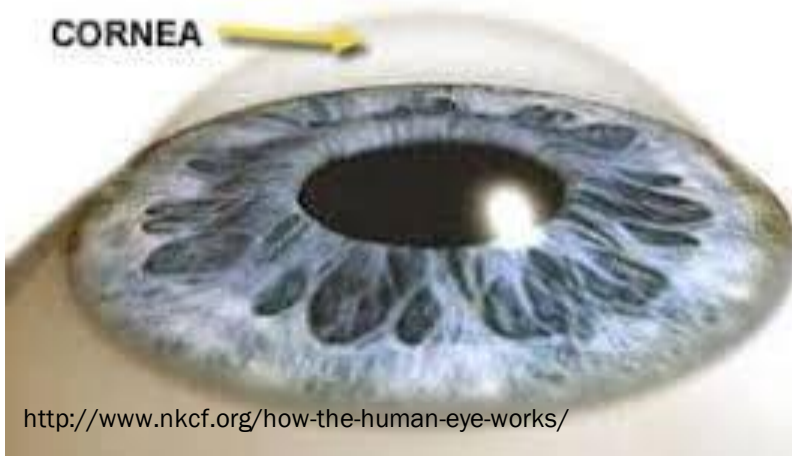


Diaphysis



Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico

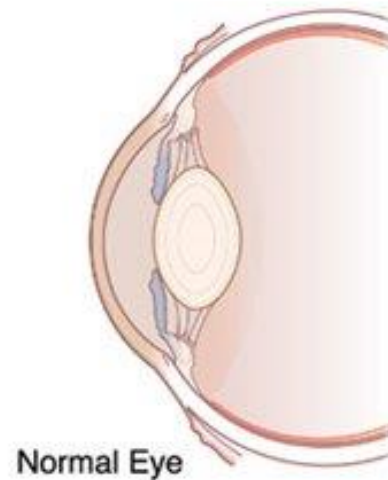
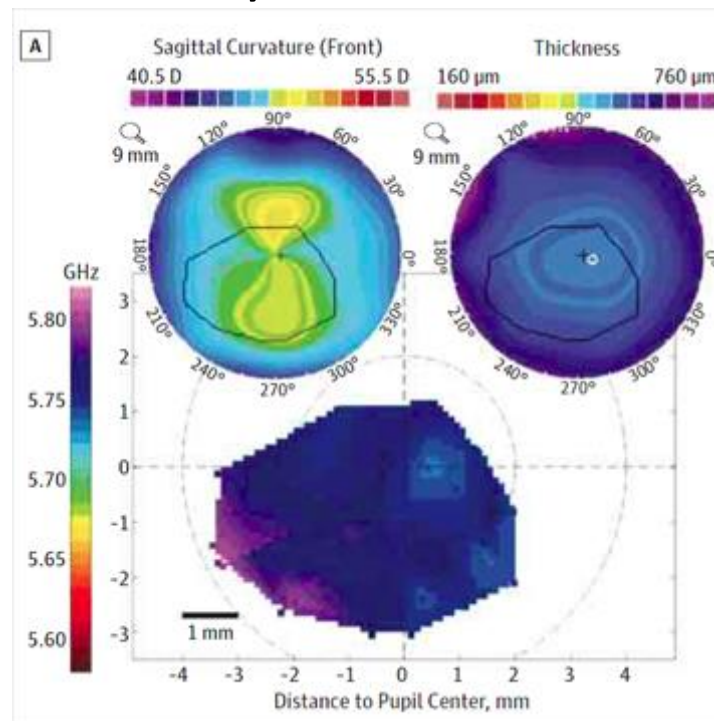
Link between biomechanics and diseases: analysis on human cornea



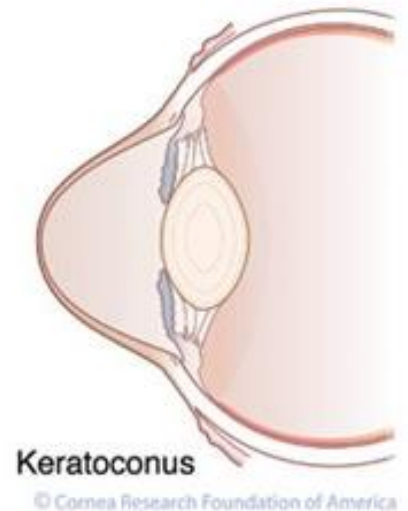
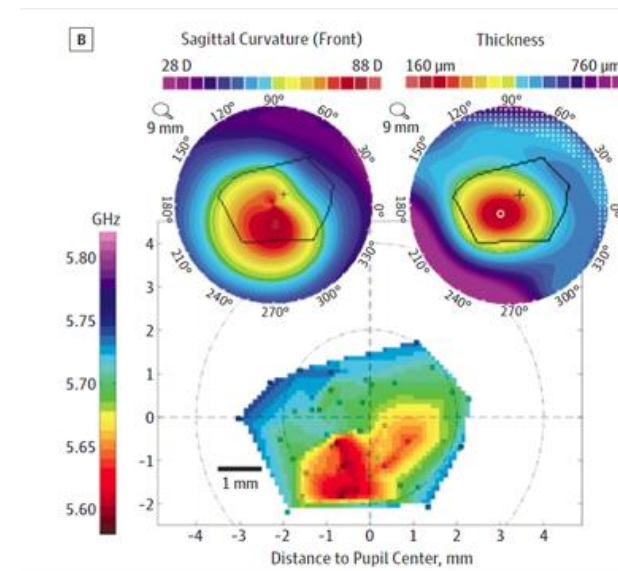
healthy cornea

The first in vivo imaging effort was reported by a team at Harvard Medical School, aiming at evaluating biomechanical properties of cornea and crystalline lens in animal models, and then in human in 2012.

cornea with advanced keratoconus

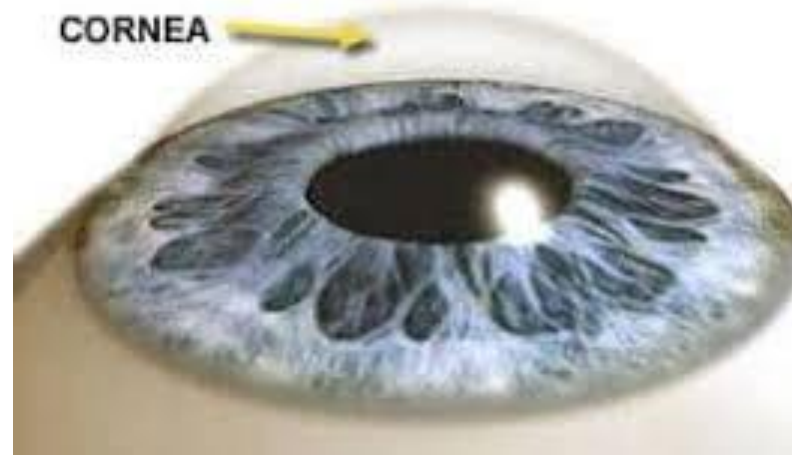
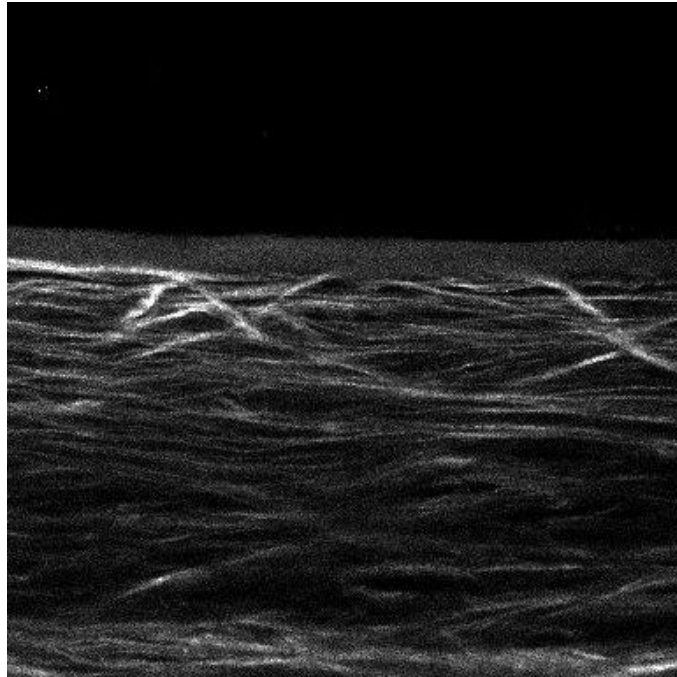


JAMA Ophthalmology April 2015 Volume 133, Number 4

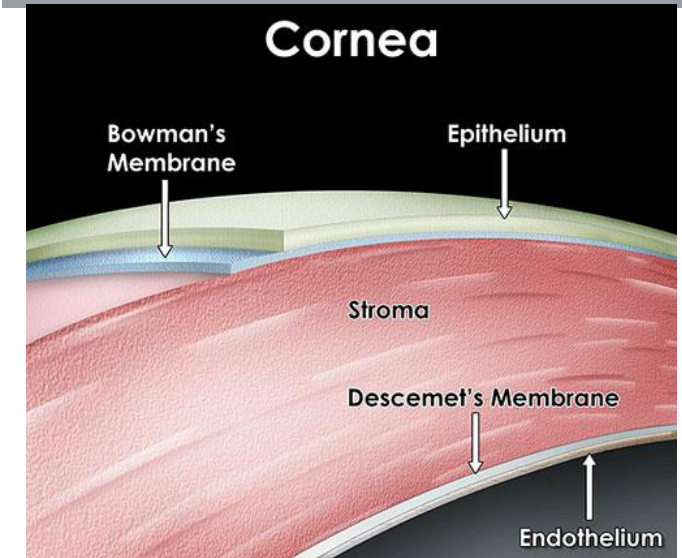


Microstructure drives biomechanics: analysis on human cornea

Healthy Cornea



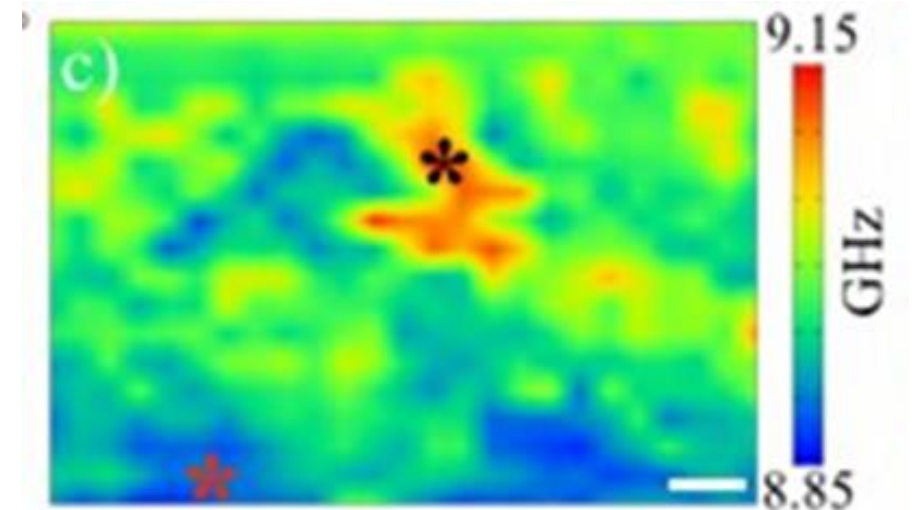
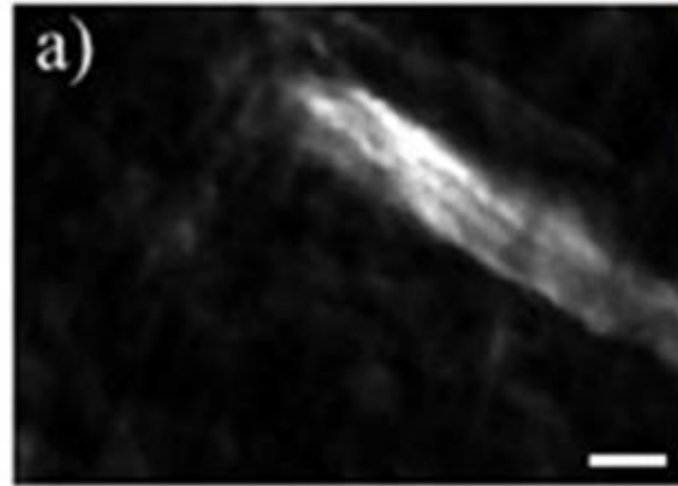
<http://www.nkcf.org/how-the-human-eye-works/>



<http://www.nkcf.org/how-the-human-eye-works/>

SHG-imaging

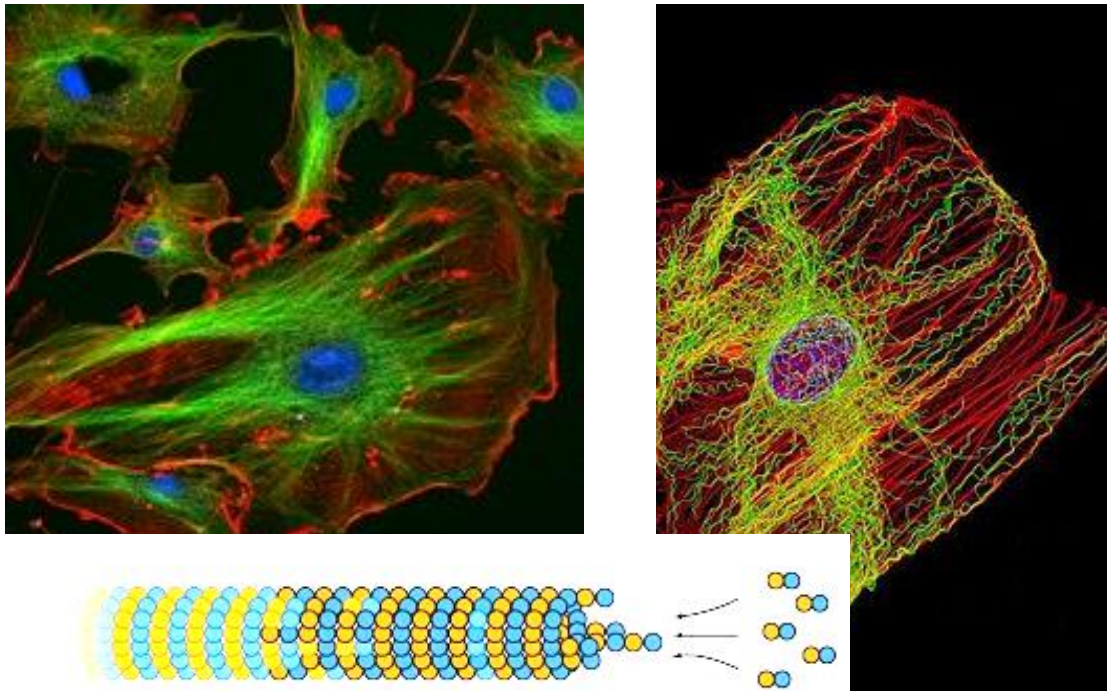
- The **sutural lamellae**, immediately I membrane



The use of a correlative approach was key to achieve a deeper morphological interpretation of the mechanical properties, otherwise inaccessible to ordinary methods based on a single mechanism of optical contrast.

Mercatelli et al. 10.1038/s42003-019-0357-y

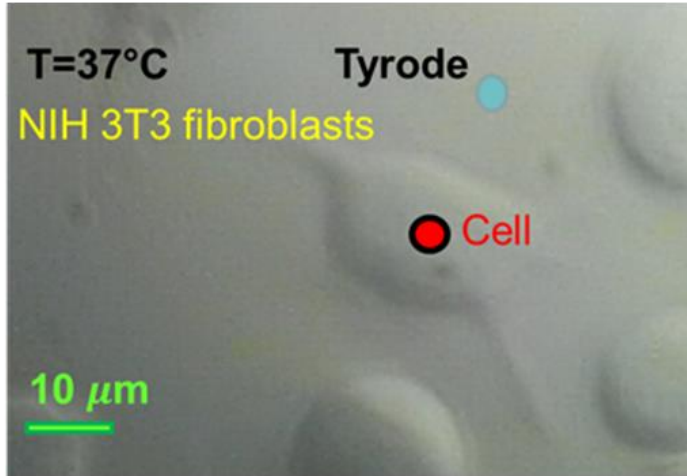
CELLS ARE MECHANICAL OBJECTS...



The mechanical properties of cells influence their cellular and subcellular functions, including

- cell adhesion,
 - migration,
 - differentiation,
 - organelle organization
 - trafficking inside the cytoplasm.
-
- Biomechanics are crucial in the onset of several diseases (cancer, Atherosclerosis, glaucoma, amyotrophic lateral sclerosis...)

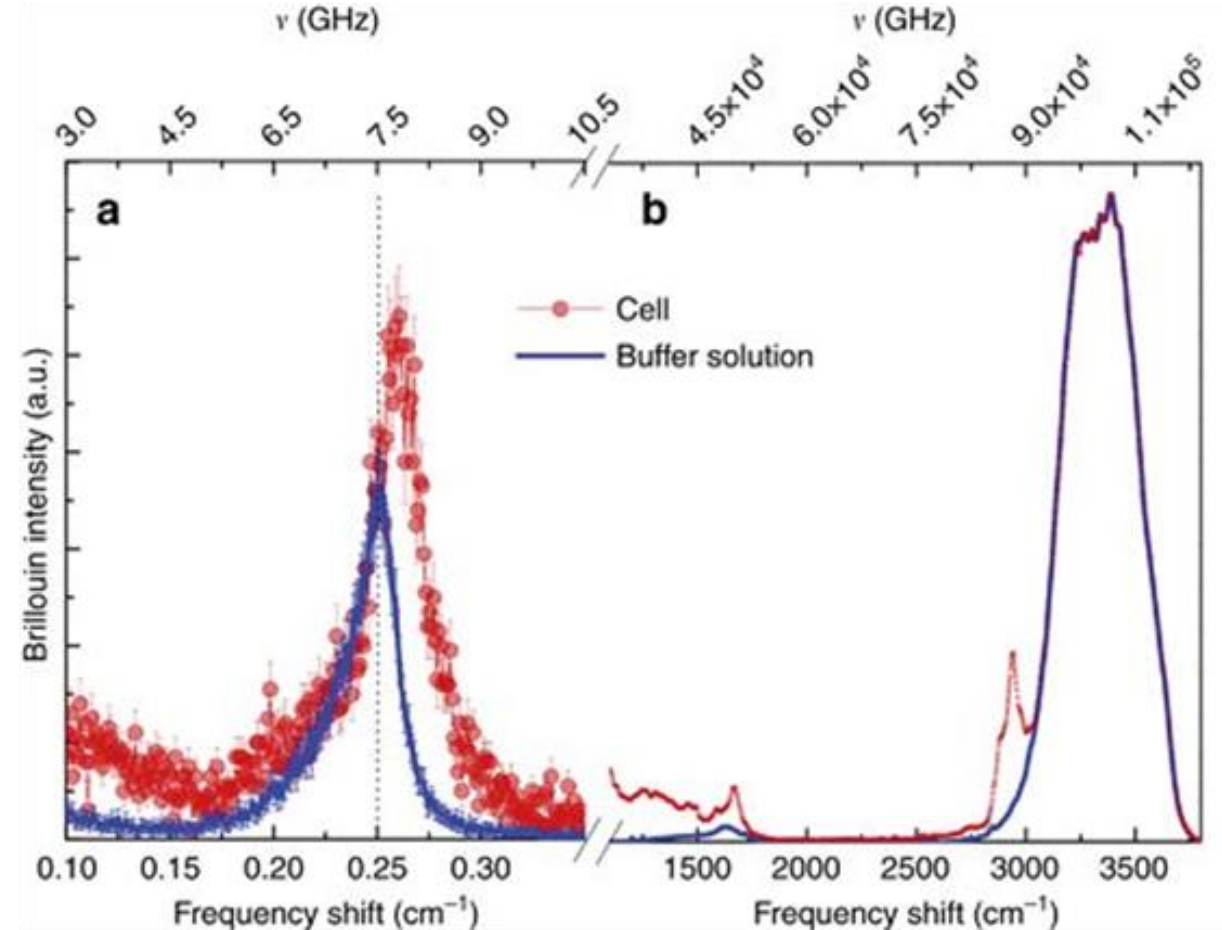
MECHANICAL AND CHEMICAL ANALYSIS OF SINGLE LIVING CELLS



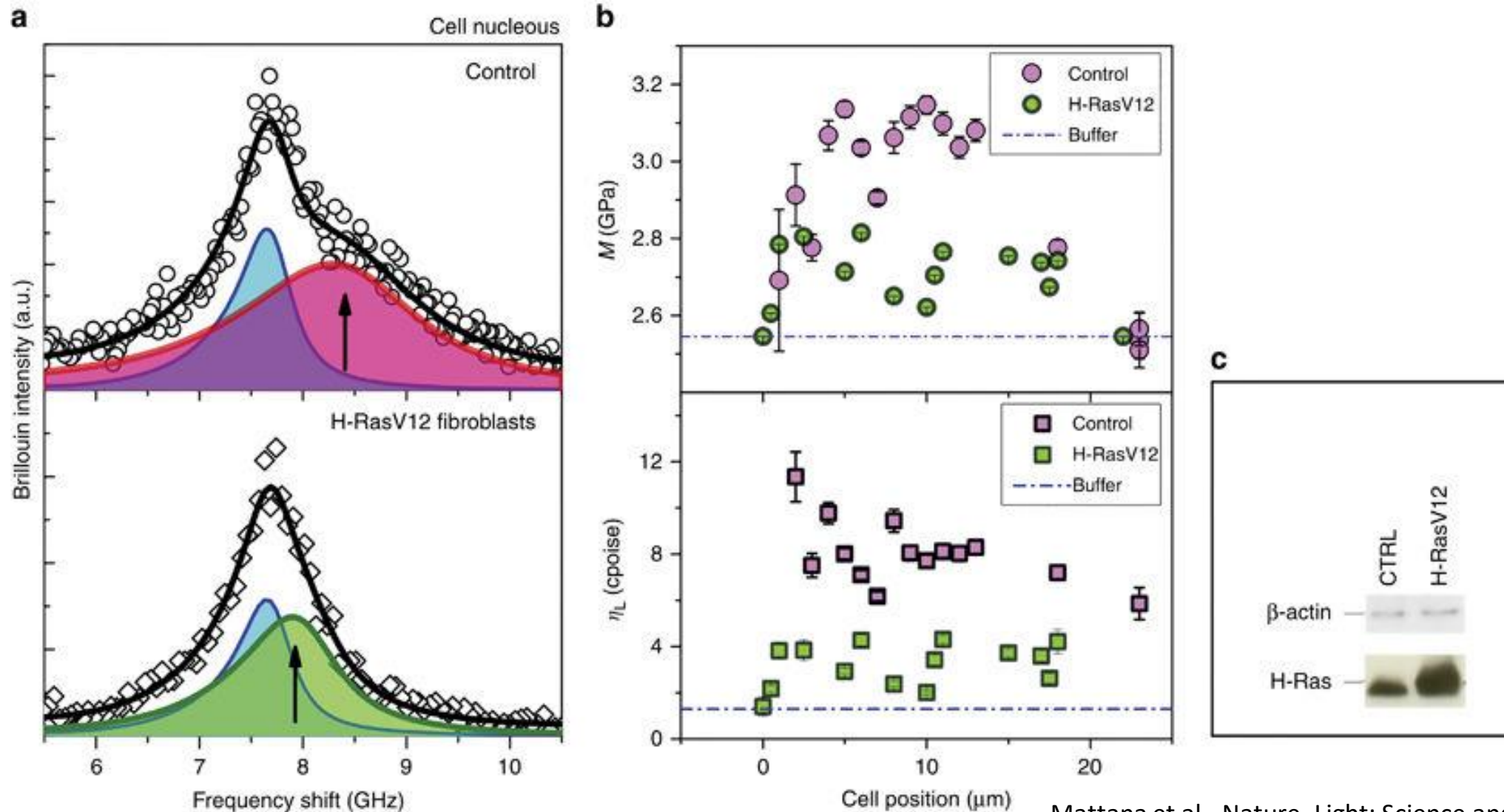
MULTISCALE ANALYSIS

(from molecular to collective dynamics)

- Label free
- Non contact
- In physiological condition
- Sub-micrometric resolution



EVIDENCE OF DIFFERENT ELASTIC PROPERTIES IN TRANSFECTED TUMORAL CELLS

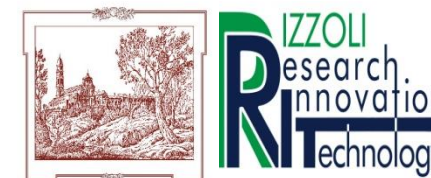


THE COMMUNITY



- WG1 Medical & Clinical Applications*
- WG2 Phenotyping & Novel Applications*
- WG3 Instrument Design & Data Analysis*
- WG4 Correlative & Comparative Methods*

Different partners for specific tasks:
biology, medicine, optics, instrumentation,
fundamental issues.



THE TABLE STABLE LTD.

*Vibration Isolation and
JRS Optical Instruments*

TESI

- Caratterizzazione meccanica e chimica di sistemi biomimetici
(Dep. Biomedical Engineering Università di Glasgow)
Progetto Royal Society (UK)
- Analisi di singole cellule in condizioni fisiologiche e patologiche
(Dipartimento Di Chimica, Biologia e Biotecnologie)
- Ottimizzazione e sviluppo di strumentazione innovativa
per analisi spettroscopica di materiali biologici



CONCLUSIONE

- Utilizzare la luce per investigare la materia biologica (attraverso le vibrazioni): un ampio spettro di attività.
- Tesi, Curiosità → maurizio.mattarelli@unipg.it
daniele.fioretto@unipg.it
silvia.caponi@cnr.it

