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109° CONGRESSO NAZIONALE

Società Italiana di Fisica

Morpho-mechanics of collagen superstructures revealed by Brillouin-Raman microspectroscopy

Silvia Caponi[1], Maurizio Mattarelli [1,2], Daniele Fioretto

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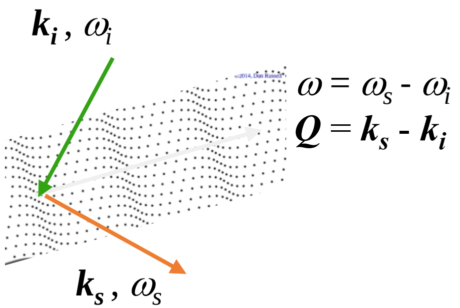
[2)] Department of Physics and Geology, University of Perugia, 06123 Perugia - Italy

Brillouin

thermally activated acoustic waves

Mechanical properties



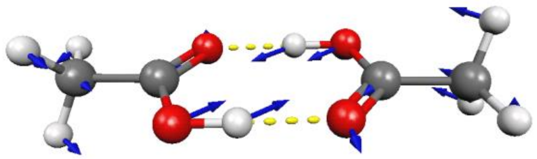
$$M = r \frac{w^2}{Q^2}$$

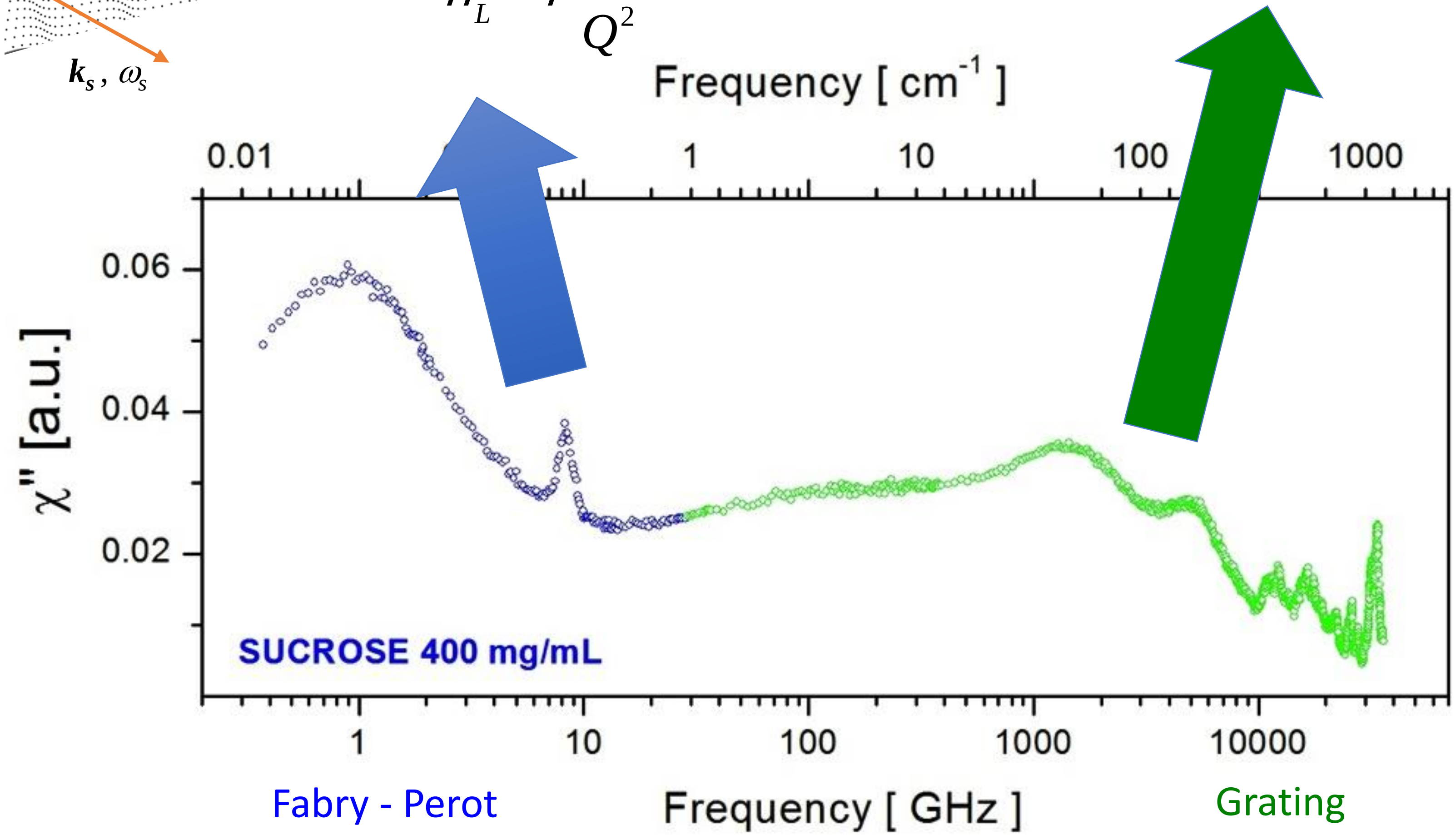
$$h_L = r \frac{G}{Q^2}$$

Raman

vibrational modes of molecules

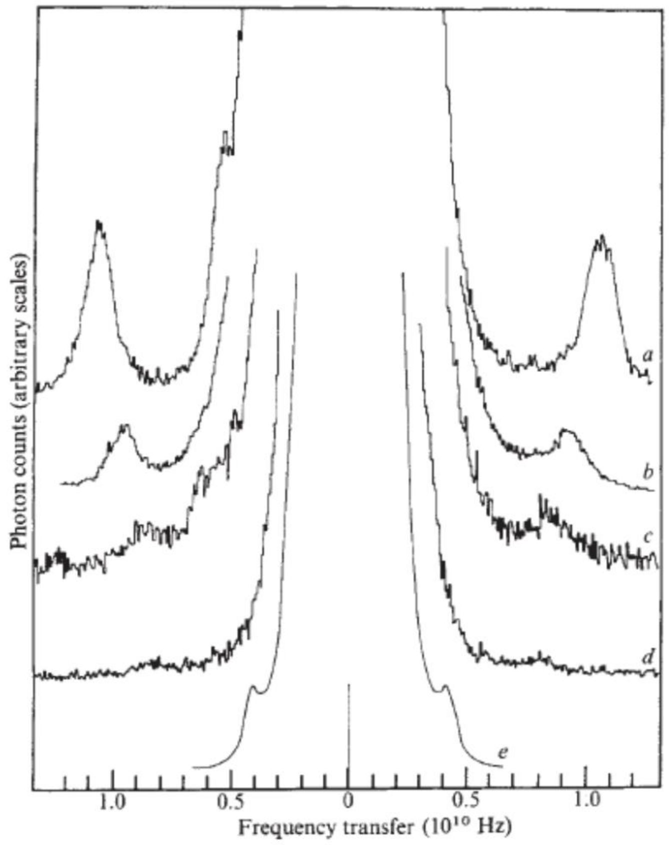
Chemical properties - composition, structure



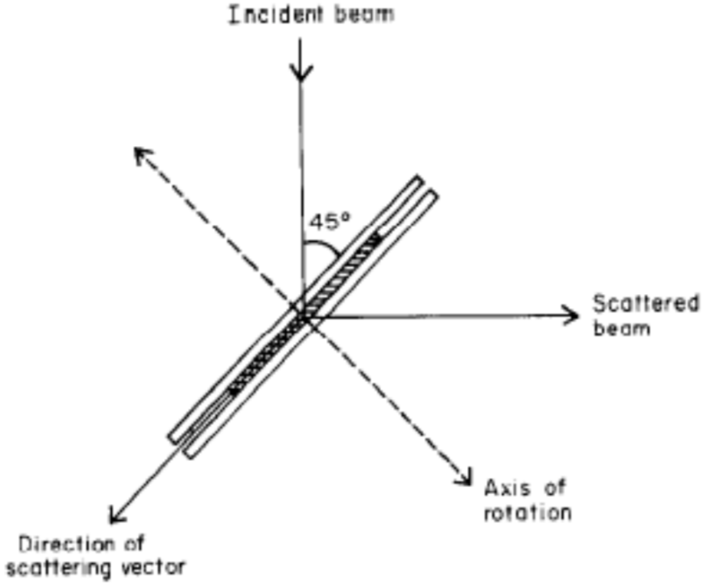


First biological applications of Brillouin scattering

Rat tail tendon collagen fibres



R Harley et al. Nature 1977



Elastic constant	Dry collagen (density 1·35 g/cm³)	Native collagen (density 1·12 g/cm³)
c_{33}	17·9 GN m^{-2}	7·8 GN m^{-2}
c_{11}	11·7 GN m^{-2}	4·0 GN m^{-2}
c_{44}	3·3 GN m^{-2}	—
c_{12}	5·1 GN m^{-2}	—
c_{13}	7·1 GN m^{-2}	—

$$E_{\parallel} = 11{\cdot}9 \text{ GN m}^{-2} \quad \sigma_{13} = 0{\cdot}42$$

$$E_{\perp} = 8{\cdot}3 \text{ GN m}^{-2} \quad \sigma_{12} = 0{\cdot}26$$

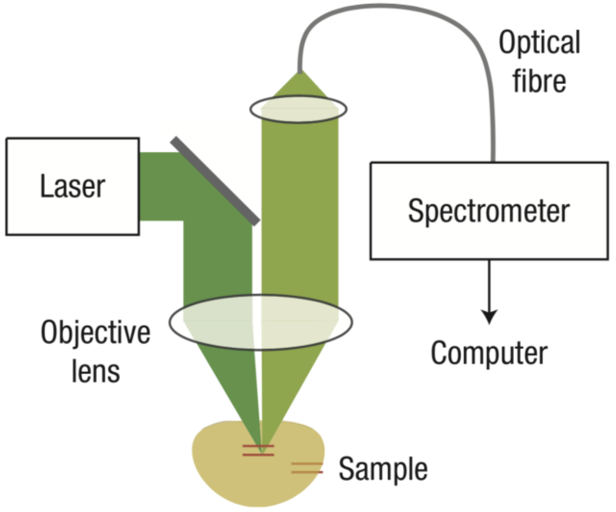
$$G = 3{\cdot}3 \text{ GN m}^{-2}$$

S Cusack & A Miller J. Mol. Biol. 1979

EMBL Grenoble

Brillouin meets Confocal Microscopy: micro-Brillouin imaging.

G. Scarcelli, S.H. Yun, Nature Photonics, 2, 39 (2007)



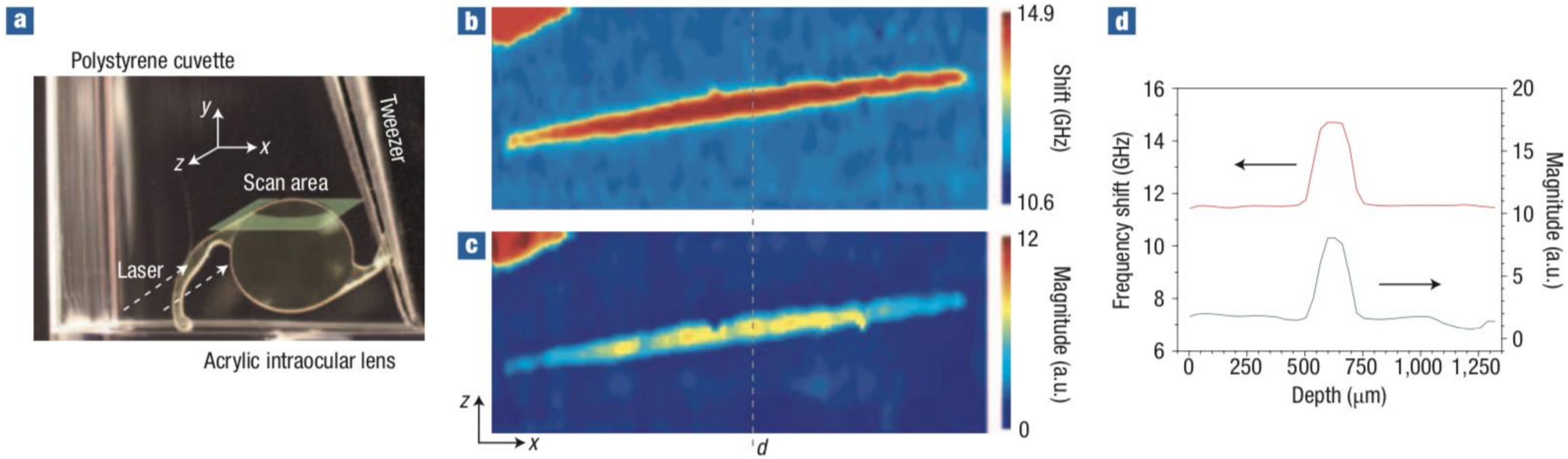
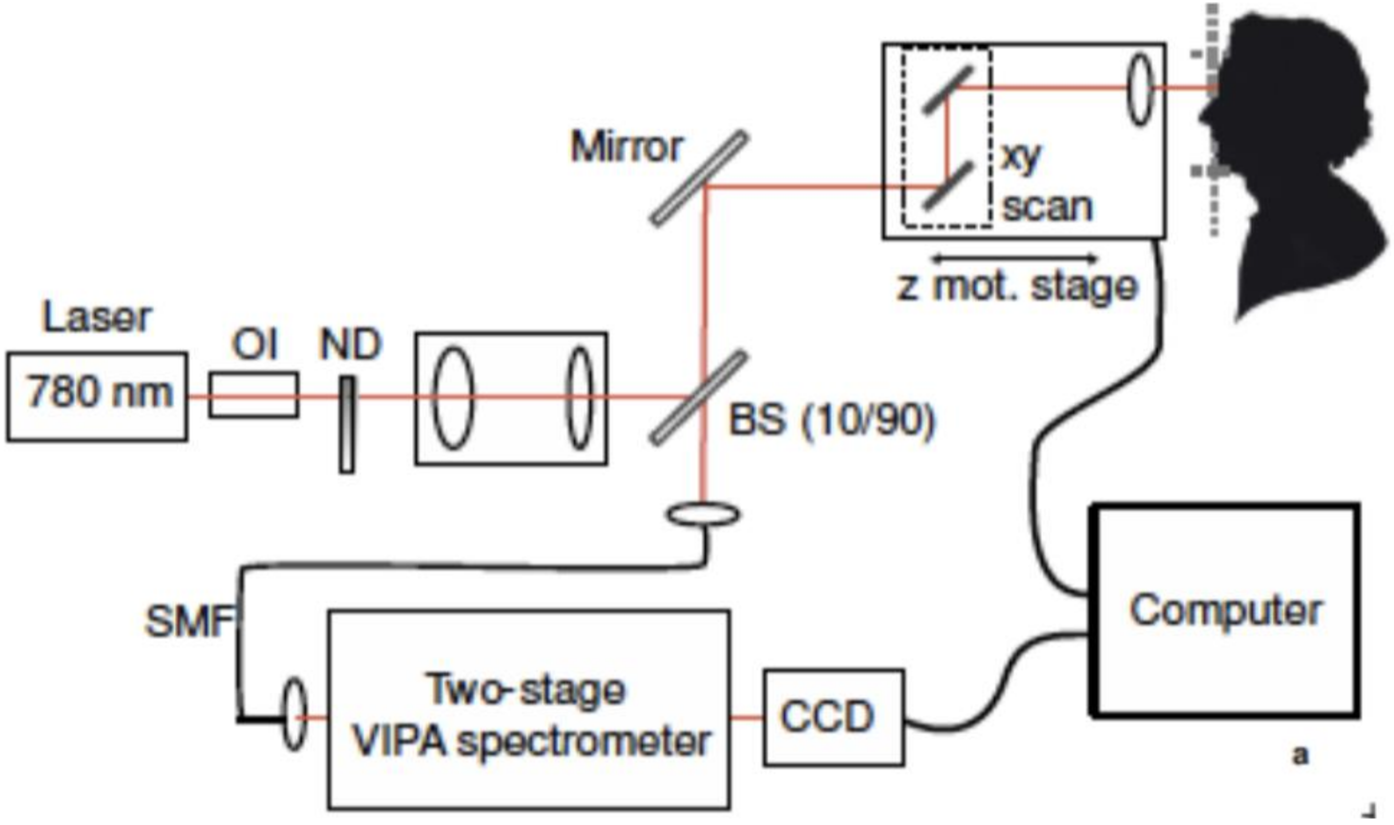


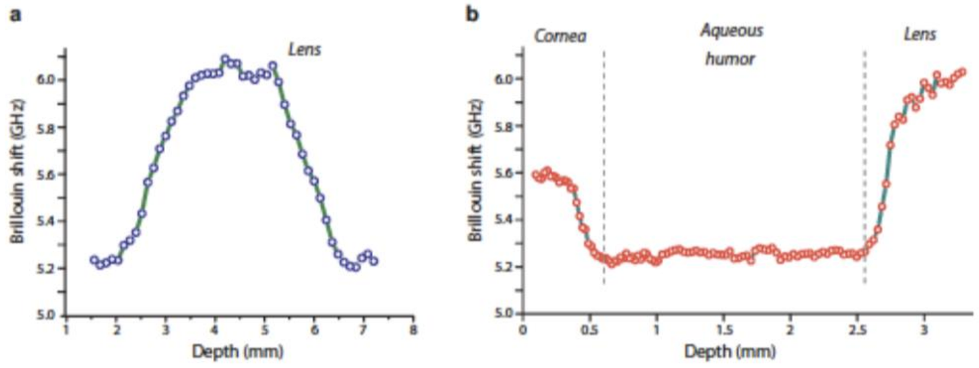
Figure 3 Cross-sectional Brillouin image of an intraocular lens. a, Picture of the sample used. To visualize the outline of the lens clearly, the picture was taken before filling the cuvette with index-matching viscous polymer. The imaged area is 3.2 mm (*x*) × 1.3 mm (*z*). **b**, Image based on measured frequency shifts, corresponding to a cross-sectional map of elastic modulus. **c**, Image created by using Brillouin scattering magnitudes as contrast. **d**, Representative cross-sectional line profiles taken along the dotted line in **b** and **c**. Arrows indicate which *y* axis scale applies.

Brillouin meets Confocal Microscopy: micro-Brillouin imaging

First *in vivo* application to human eye

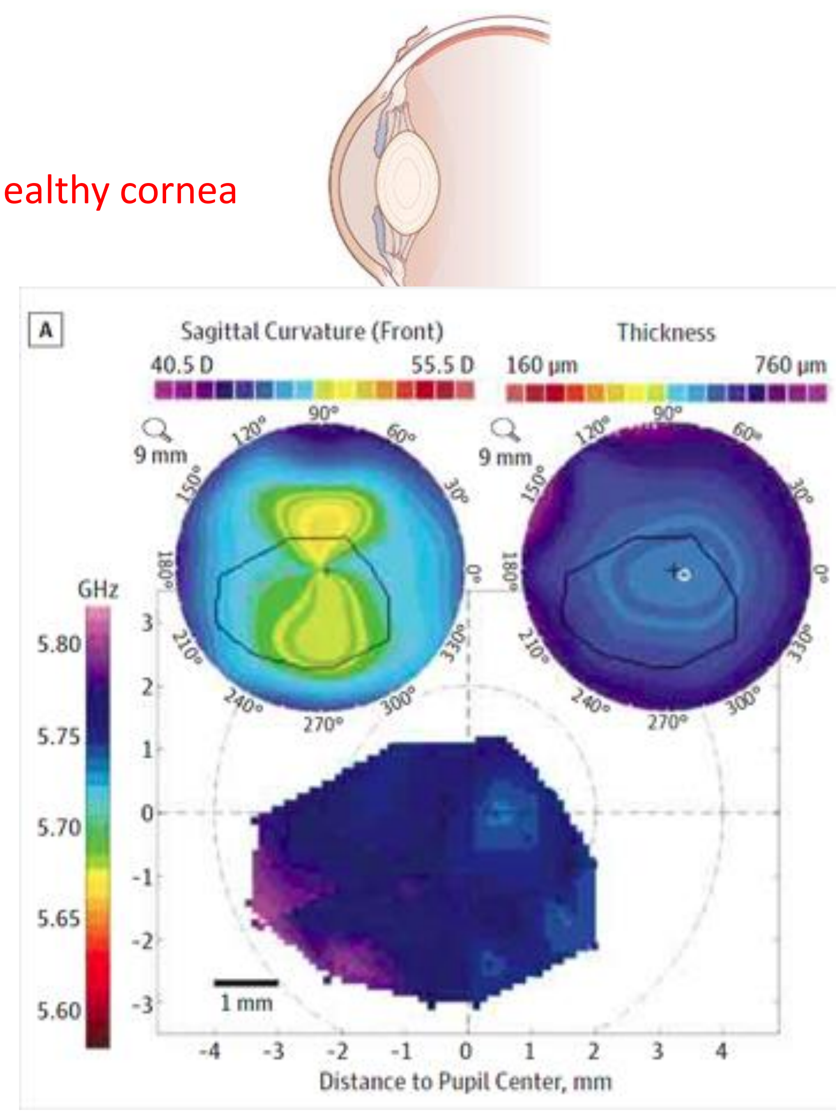
G. Scarcelli, S.H. Yun, Opt. Express, 2012



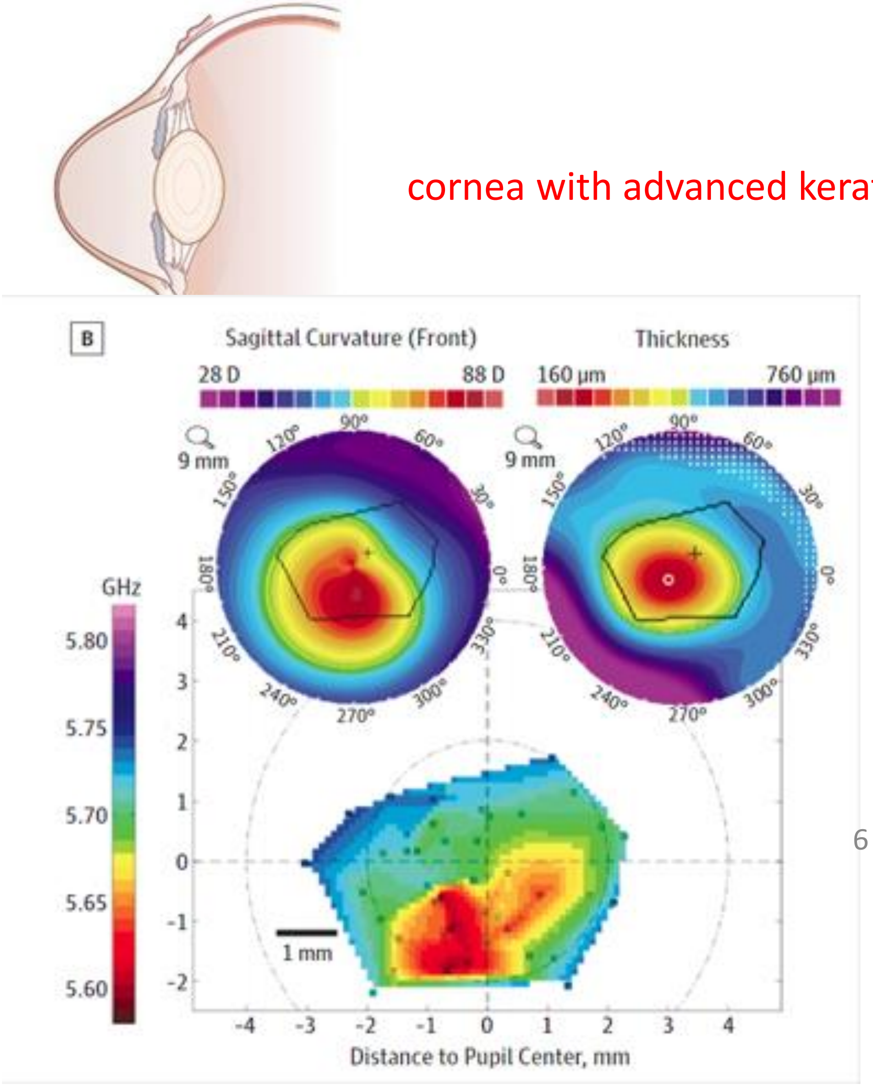


Elastography of human cornea

healthy cornea



cornea with advanced keratoconus



JAMA Ophthalmology April 2015 Volume 133, Number 4

CHEMICAL REVIEWS

Chem. Rev. 2019, 119, 7833–7847

ACS AuthorChoice

Review

Cite This: *Chem. Rev.* 2019, 119, 7833–7847

pubs.acs.org/CR

Brillouin Light Scattering: Applications in Biomedical Sciences

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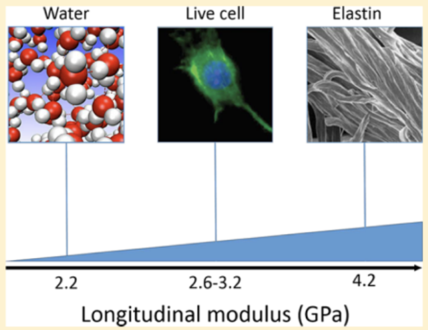
ABSTRACT: Brillouin spectroscopy and imaging are emerging techniques in analytical science, biophotonics, and biomedicine. They are based on Brillouin light scattering from acoustic waves or *phonons* in the GHz range, providing a nondestructive contactless probe of the mechanics on a microscale. Novel approaches and applications of these techniques to the field of biomedical sciences are discussed, highlighting the theoretical foundations and experimental methods that have been developed to date. Acknowledging that this is a fast moving field, a comprehensive account of the relevant literature is critically assessed here.



nature methods

REVIEW ARTICLE

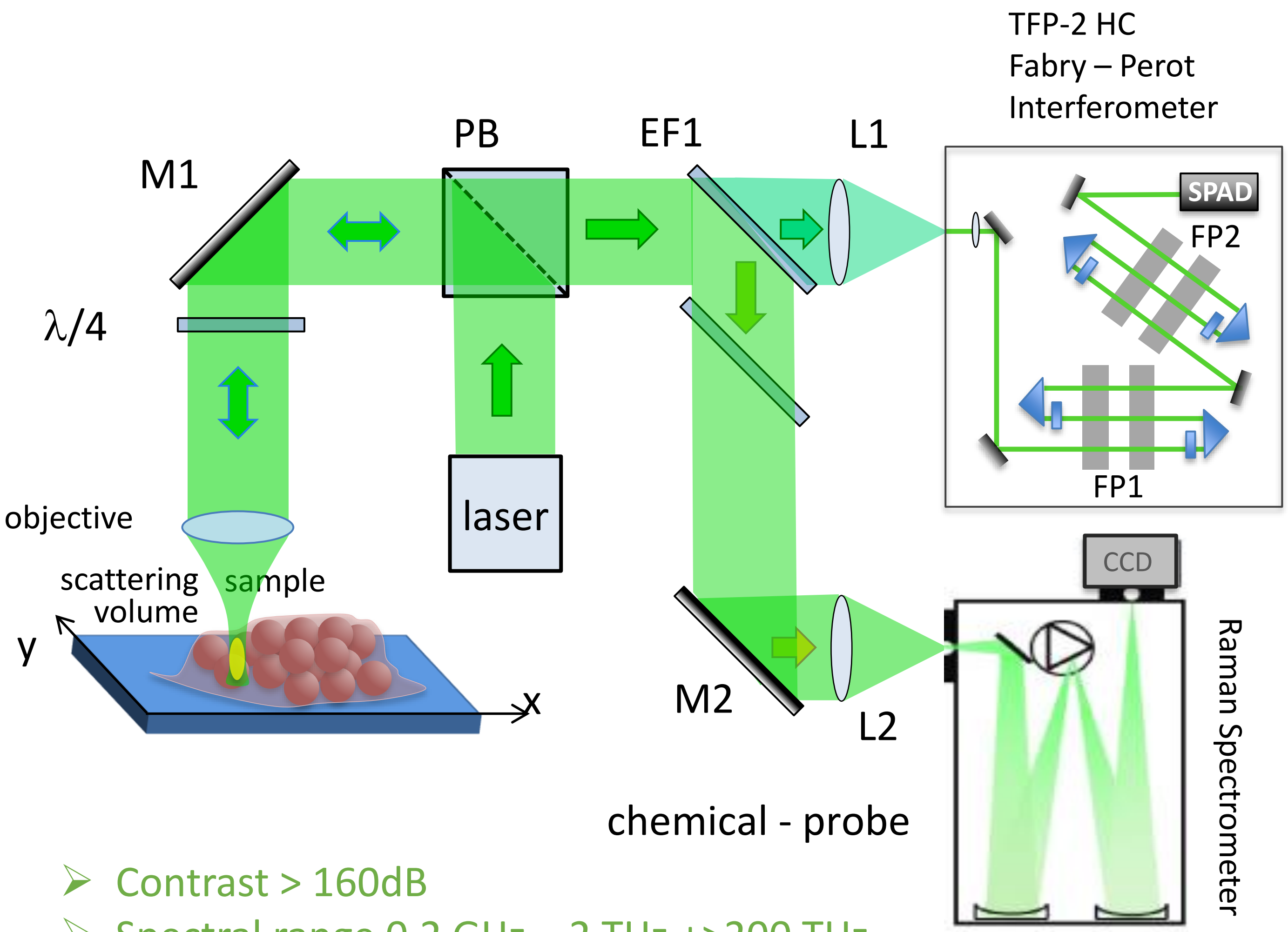
https://doi.org/10.1038/s41592-019-0543-3

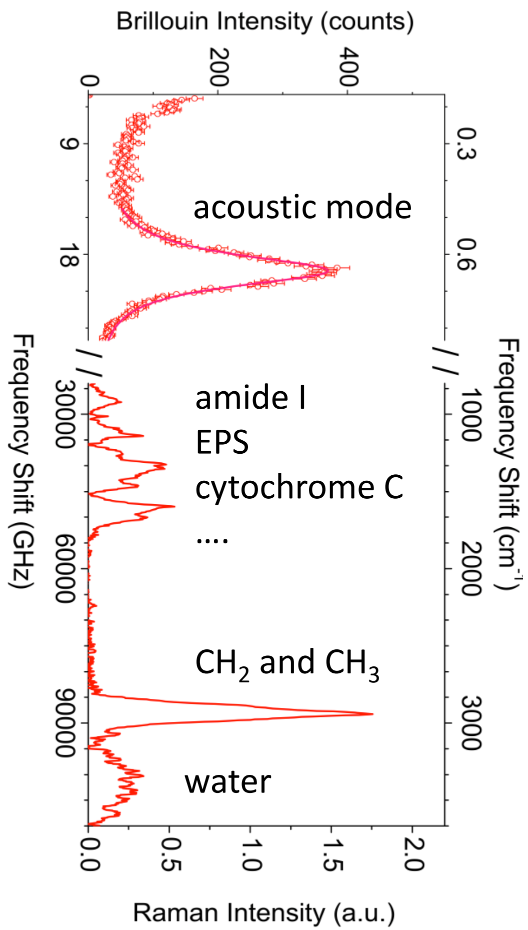
Brillouin microscopy: an emerging tool for mechanobiology

Robert Prevedel[1,2,3]*, Alba Diz-Muñoz[1]*, Giancarlo Ruocco[4,5] and Giuseppe Antonacci[4,6]

Micro Brillouin & Micro Raman @ Perugia

PHYSICAL REVIEW X **7**, 031015 (2017)

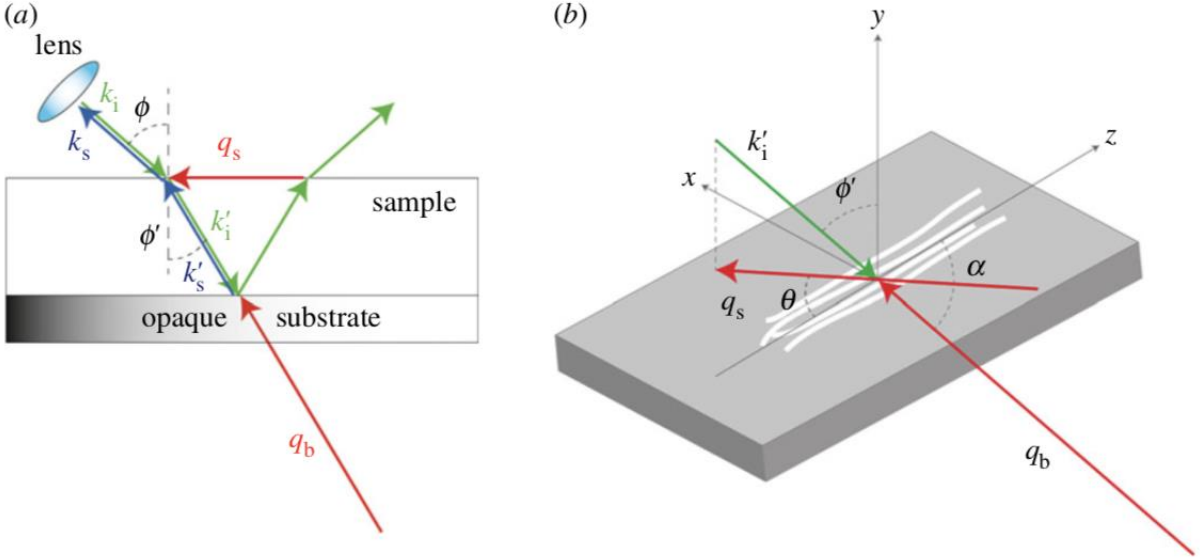


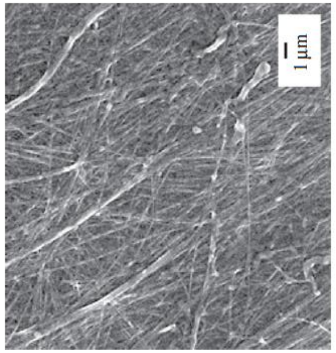


- Contrast > 160dB
- Spectral range 0.2 GHz – 2 THz +>200 THz
- Resolution better than 0.2 GHz
- Time for a measurement 0.5s - minutes

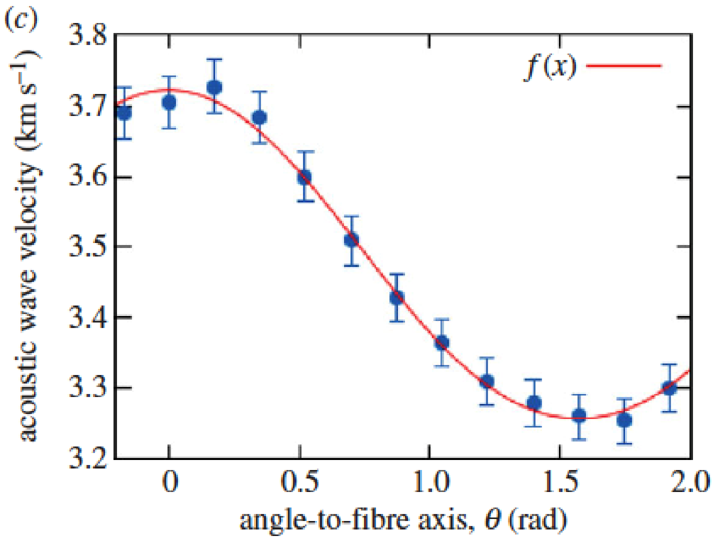
Biomedical applications: Tissues

Anisotropic samples: fibrous proteins of the extracellular matrix





type I collagen in rat tail tendon



$$V_T^2(\theta) = \frac{1}{\rho}\left(\frac{(c_{11}-c_{12})}{2}\sin^2\theta + c_{44}\cos^2\theta\right)$$

$$V_L^2(\theta) = \frac{1}{2\rho}\Big\{c_{11}\sin^2\theta + c_{33}\cos^2\theta + c_{44} + \{[(c_{11}-c_{44})\sin^2\theta + (c_{44}-c_{33})\cos^2\theta]^2 + 4(c_{13}+c_{44})^2\sin^2\theta\cos^2\theta\}^{1/2}\Big\}$$

F. Palombo et al., Journal of The Royal Society Interface 2014, **11**, 20140739

Biomedical applications: Tissues

Anisotropic samples: fibrous proteins of the extracellular matrix

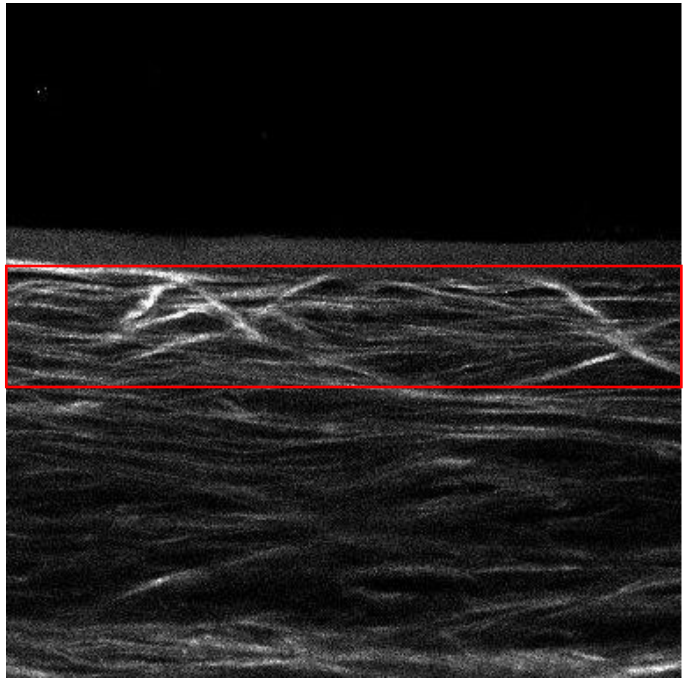
sample	elastic coefficients (GPa)		elastic moduli (GPa)		Poisson's ratios		$\tan(\delta)$
collagen	c_{33}	18.6 ± 0.1	$E_{\|\|}$	10.2 ± 0.3	σ_{13}	0.43 ± 0.01	0.063 ± 0.01
	c_{11}	14.3 ± 0.1	$E_{\perp}$	8.3 ± 0.3	σ_{12}	0.32 ± 0.02	
	c_{44}	3.2 ± 0.1	G	3.2 ± 0.1			
	c_{12}	8.0 ± 0.1	K	10.9 ± 0.5			
	c_{13}	9.7 ± 0.2					
elastin	c_{33}	11.5 ± 0.2	$E_{\|\|}$	6.1 ± 0.4	σ_{13}	0.40 ± 0.02	0.062 ± 0.01
	c_{11}	10.4 ± 0.1	$E_{\perp}$	5.3 ± 0.6	σ_{12}	0.40 ± 0.06	
	c_{44}	1.9 ± 0.2	G	1.9 ± 0.2			
	c_{12}	6.6 ± 0.2	K	8 ± 1			
	c_{13}	6.8 ± 0.3					
ligament	c_{33}	10.8 ± 0.1					0.059 ± 0.01
	c_{11}	10.8 ± 0.1					
cartilage	c_{33}	13.4 ± 0.2					0.080 ± 0.01
	c_{11}	13.4 ± 0.2					

F. Palombo et al., Journal of The Royal Society Interface 2014, **11**, 20140739

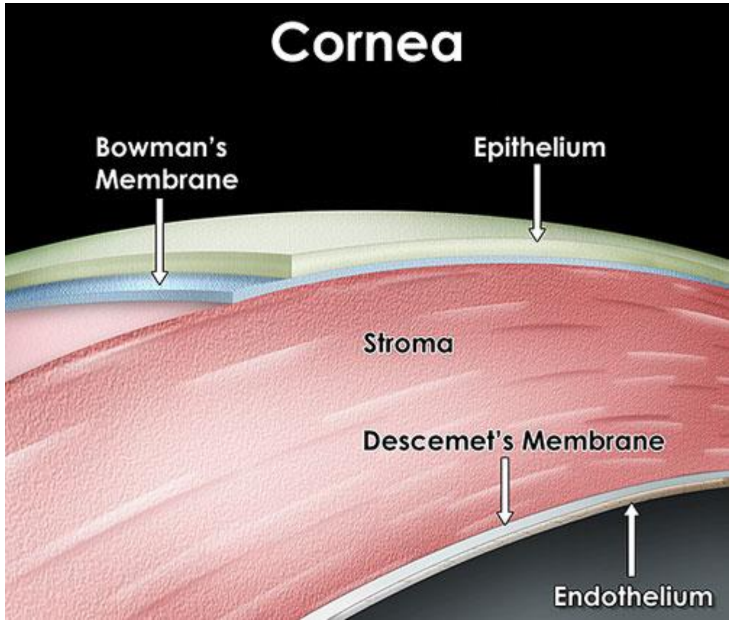
Elastography of human cornea

Morphological information on collagenous tissues by SHG microscopy

SHG-imaging Healthy Cornea



Excitation wav.: 840 nm
Detection wav: 420 nm
Detection type: F-SHG
Pixel dwell time: 20 μs
FOV: 300 μm
Sectioning: Sagittal



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

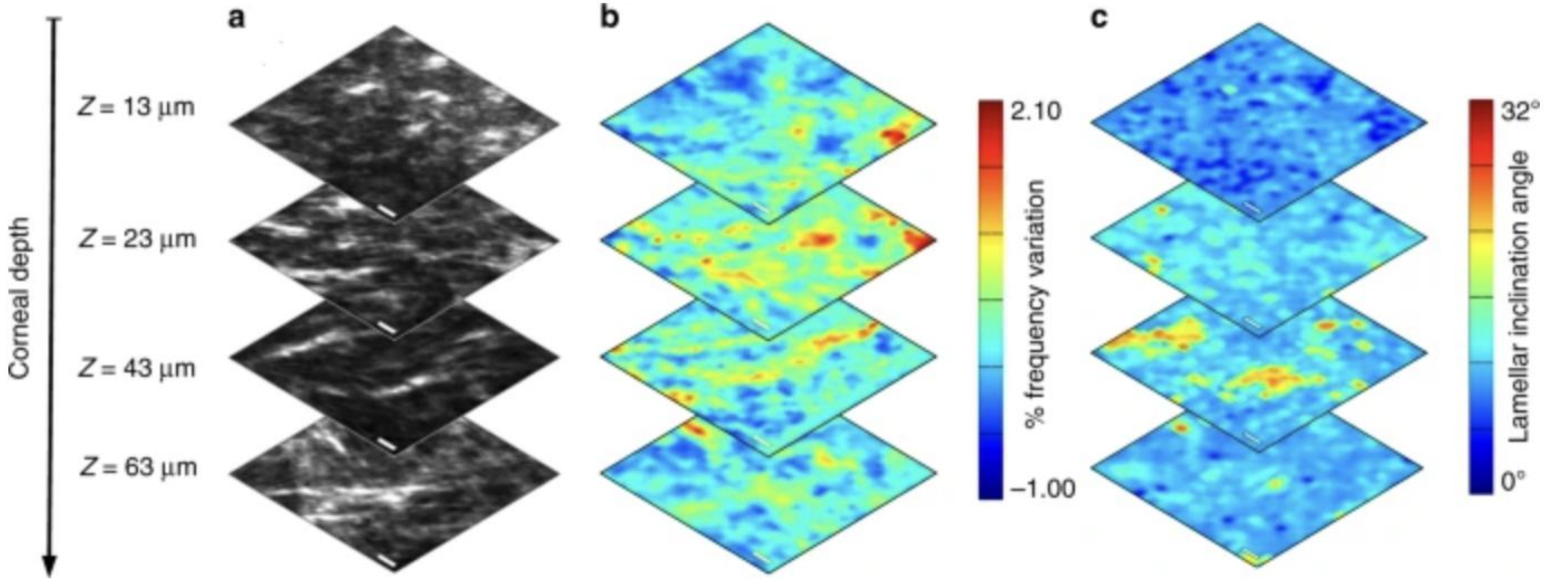
•The **sutural lamellae**, immediately below Bowman's membrane

R. Mercatelli et al. J. Biophoton. 10, 75-83 (2017)

Elastography of human cornea

Collagen morpho-mechanics: correlative SHG & Brillouin microscopy

Fig. 3



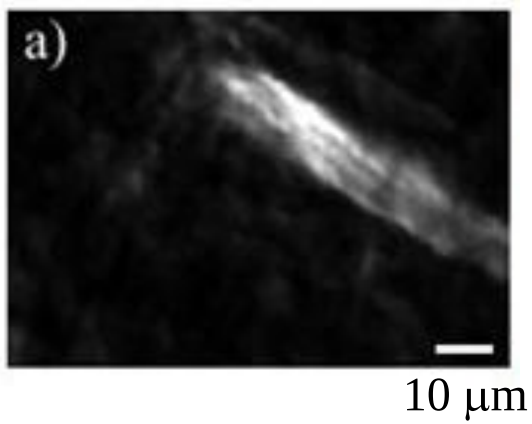
Correlative SHG-Brillouin axial optical sectioning analysis on corneal stroma. Comparison between (**a**) SHG images, (**b**) relative variations of Brillouin frequency and (**c**) lamellar inclination maps, acquired at the specified depths below Bowman's membrane. Scale bars: 10 µm

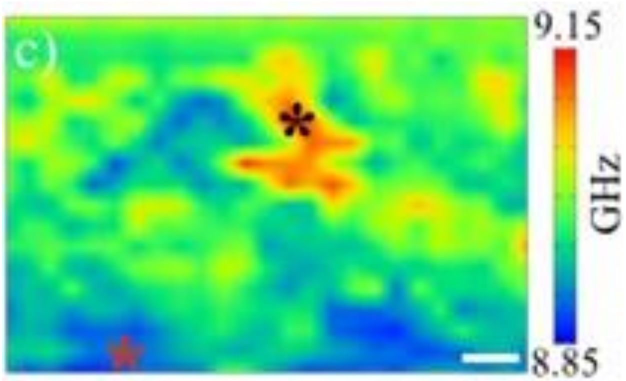
COMMUNICATIONS BIOLOGY

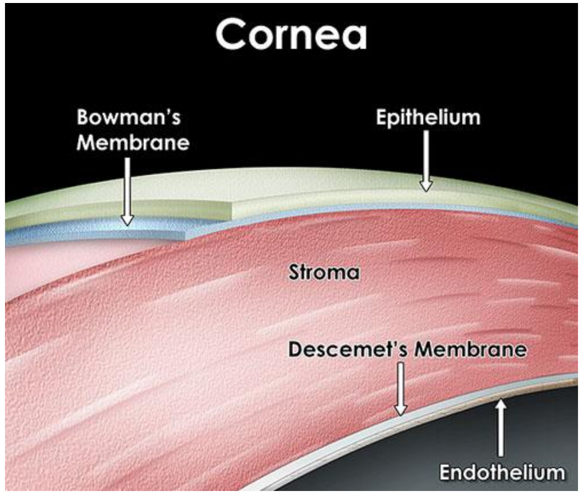
R. Mertcatelli et al., Communications biology **2**, 117 (2019)

Elastography of human cornea

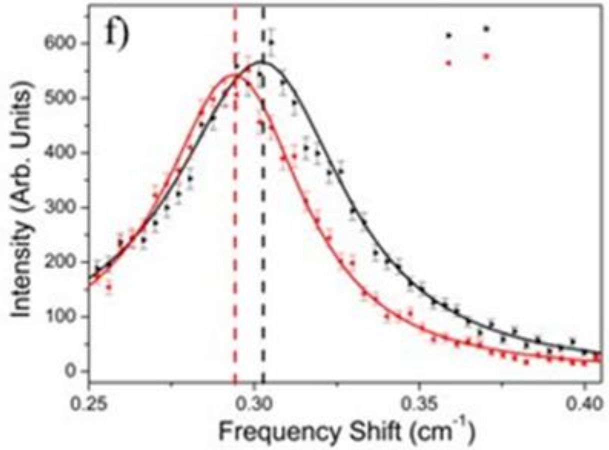
Collagen morpho-mechanics: correlative SHG & Brillouin microscopy







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



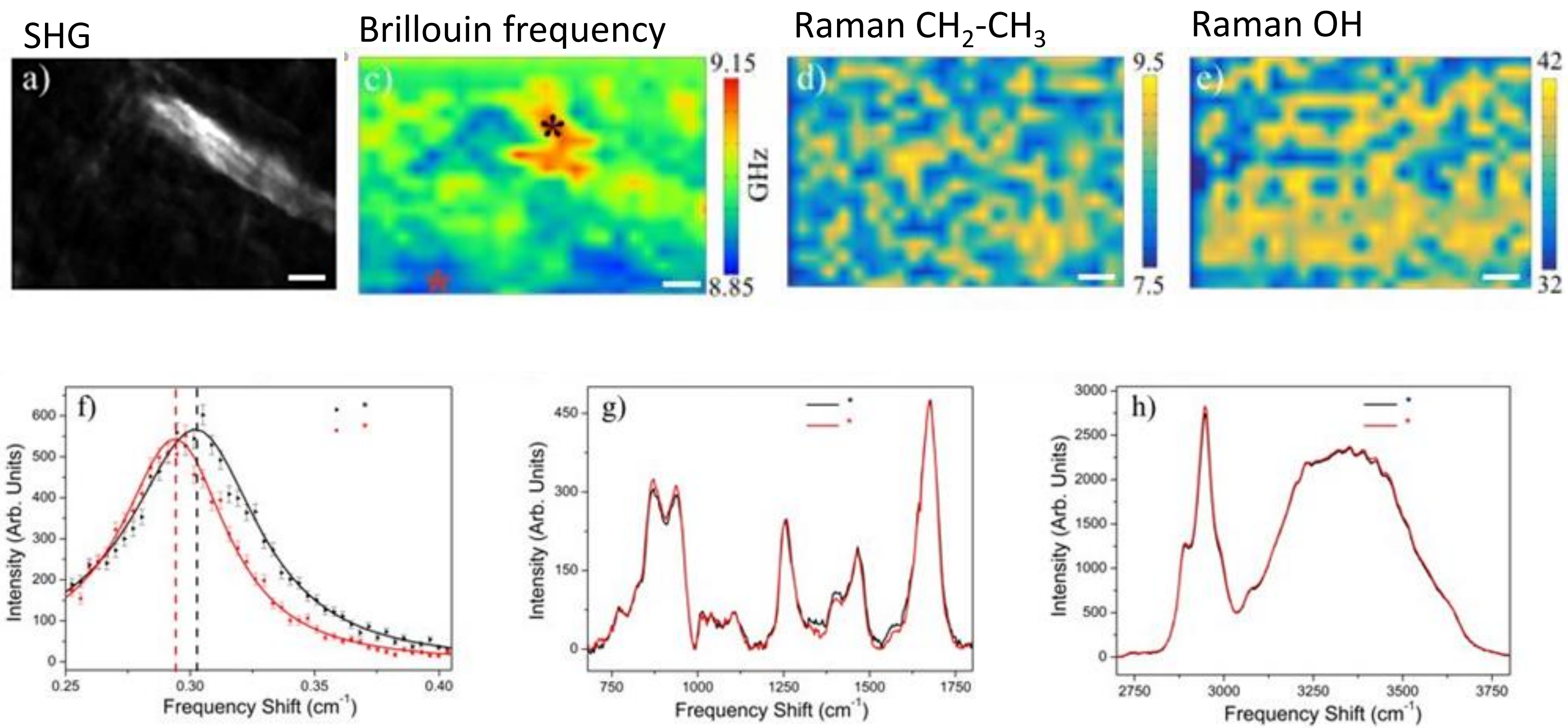
The **heterogeneity in the stiffness** displays a striking conformity with the SHG signal highlighting the ability of Brillouin spectroscopy to detect individual sutural lamellae as an elastic modulation inside the tissue.

COMMUNICATIONS BIOLOGY

R. Mertcatelli et al., Communications biology **2**, 117 (2019)

Elastography of human cornea

Collagen morpho-mechanics: correlative SHG & Brillouin microscopy



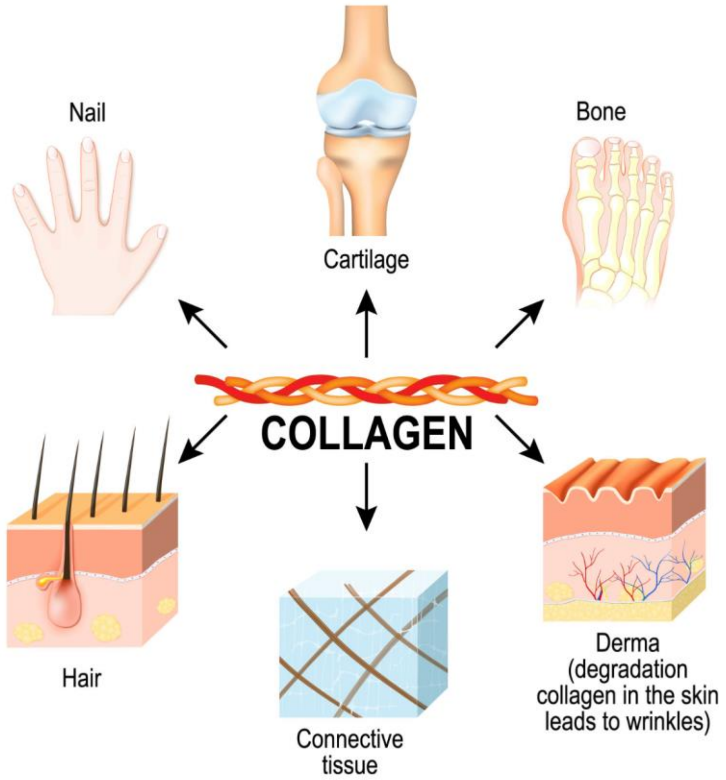
The enhancement of the elastic modulus found in sutural lamellae has to do with morphological rather than biochemical factors.

COMMUNICATIONS BIOLOGY

R. Mertcatelli et al., Communications biology **2**, 117 (2019)

Basic questions: soft vs. hard, a matter of time

Gelatin (animal glue) as a model system in which the macroscopic physical properties can be manipulated to mimic all the relevant biological states of matter, ranging from the liquid to the gel and the glassy phase



The most ubiquitous structural protein

SCIENCE ADVANCES | RESEARCH ARTICLE 2020; **6** : eabc1937

BIOPHYSICS

Viscoelastic properties of biopolymer hydrogels determined by Brillouin spectroscopy: A probe of tissue micromechanics

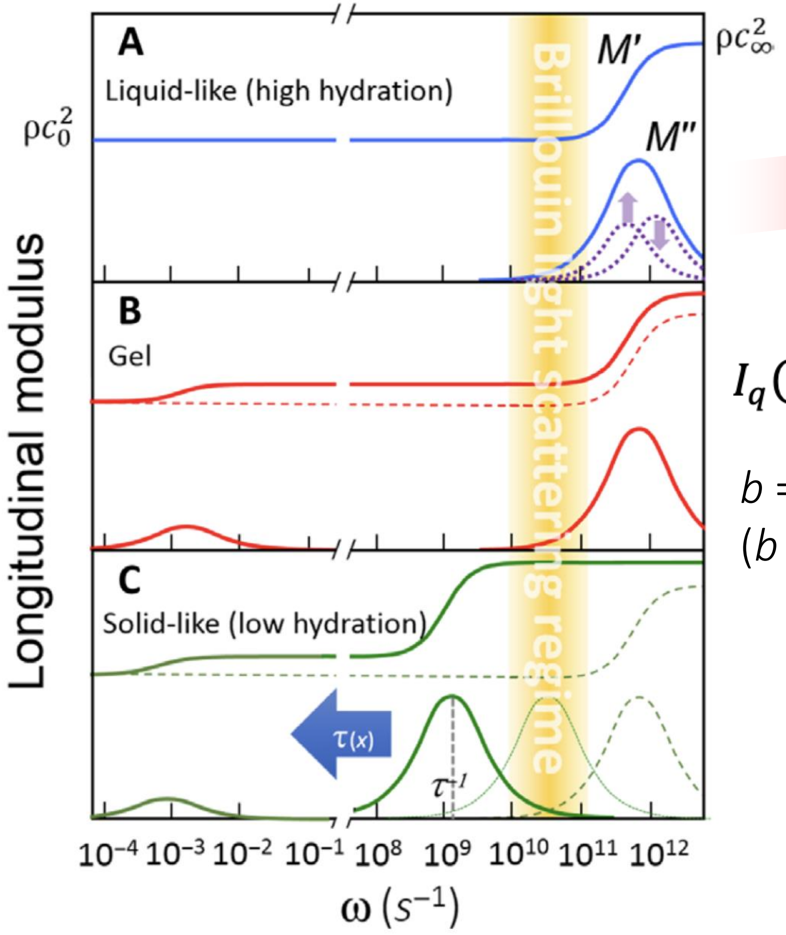
Michelle Bailey[1], Martina Alunni-Cardinali[2], Noemi Correa[1], Silvia Caponi[3], Timothy Holsgrove[4], Hugh Barr[5], Nick Stone[1], C. Peter Winlove[1], Daniele Fioretto[2]*, Francesca Palombo[1]*

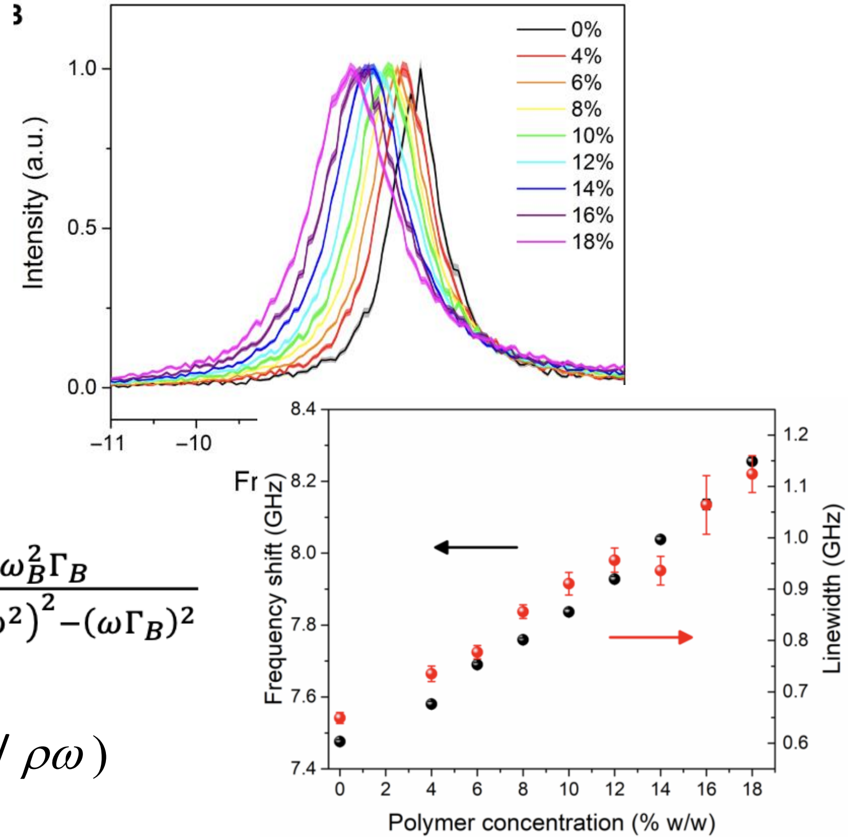
Colla animale in granuli

Denaturated Type I Collagen

Basic questions: soft vs. hard, a matter of time

M. Bailey et al., Science Advances **6**, eabc1937 (2020)





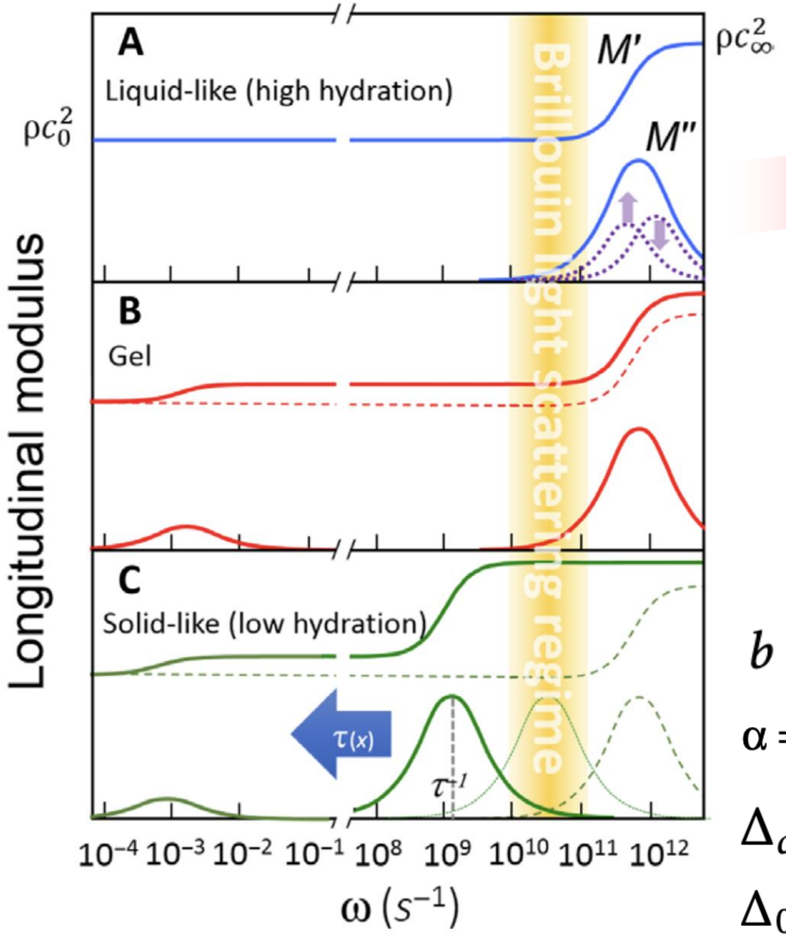
$$I_q(\omega) = I_0 \frac{\omega_B^2 \Gamma_B}{\left(\omega_B^2 - \omega^2\right)^2 - (\omega \Gamma_B)^2}$$

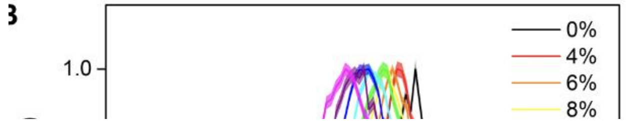
$b = \Gamma / q^2$

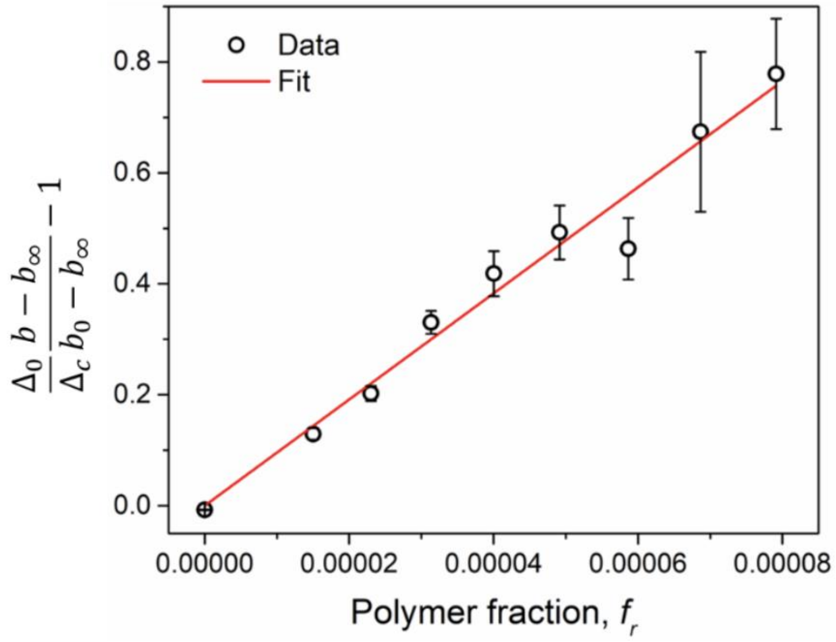
$(b = \eta_L / \rho = M'' / \rho\omega)$

Basic questions: soft vs. hard, a matter of time

M. Bailey et al., Science Advances **6**, eabc1937 (2020)







$$b = \Delta_c[\alpha\tau_h + (1-\alpha)\tau_b] + b_\infty$$

$\alpha = f_r N_h$, where f_r is the fraction of polymer-to-water

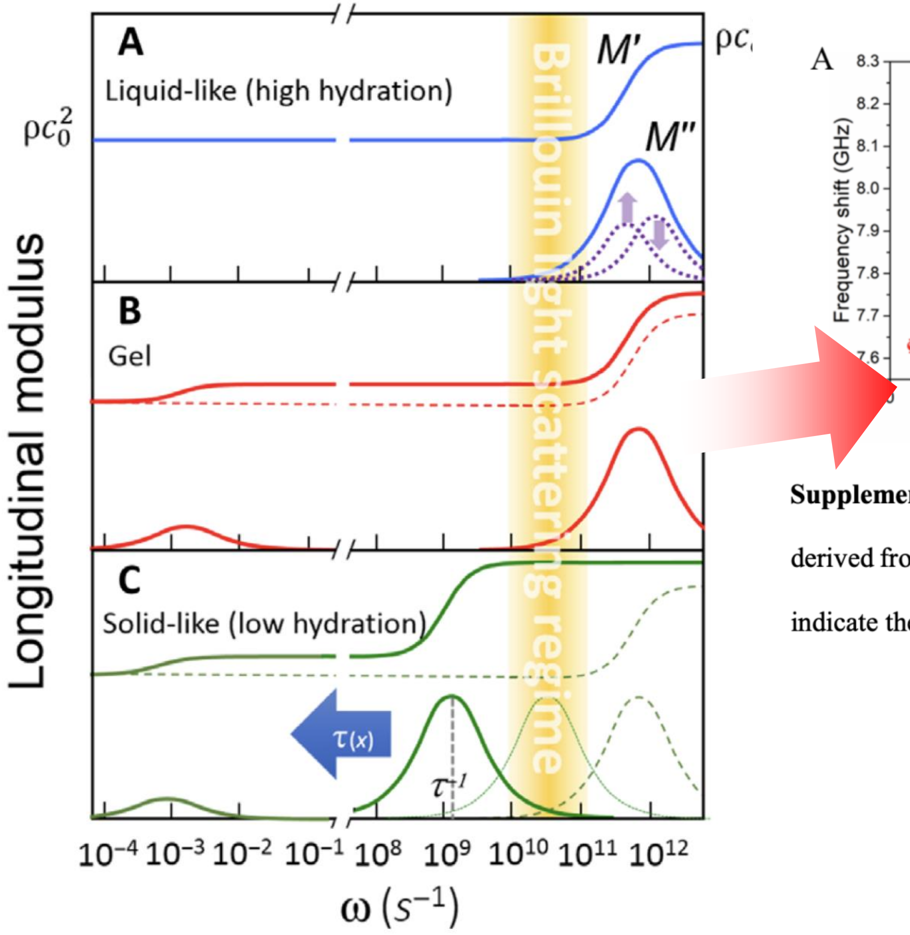
$$\Delta_c = c_\infty^2 - c_0^2$$

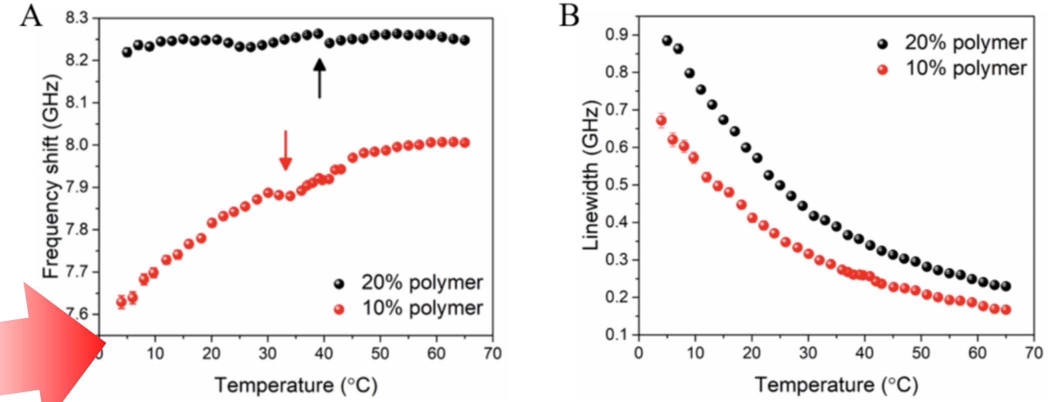
$$\frac{\Delta_0}{\Delta_c}\frac{b-b_\infty}{b_0-b_\infty} - 1 = N_h(\varepsilon - 1)f_r$$

$N_h = 10$ k → retardation parameter $\varepsilon = \tau_h/\tau_b$ =1.9

Basic questions: soft vs. hard, a matter of time

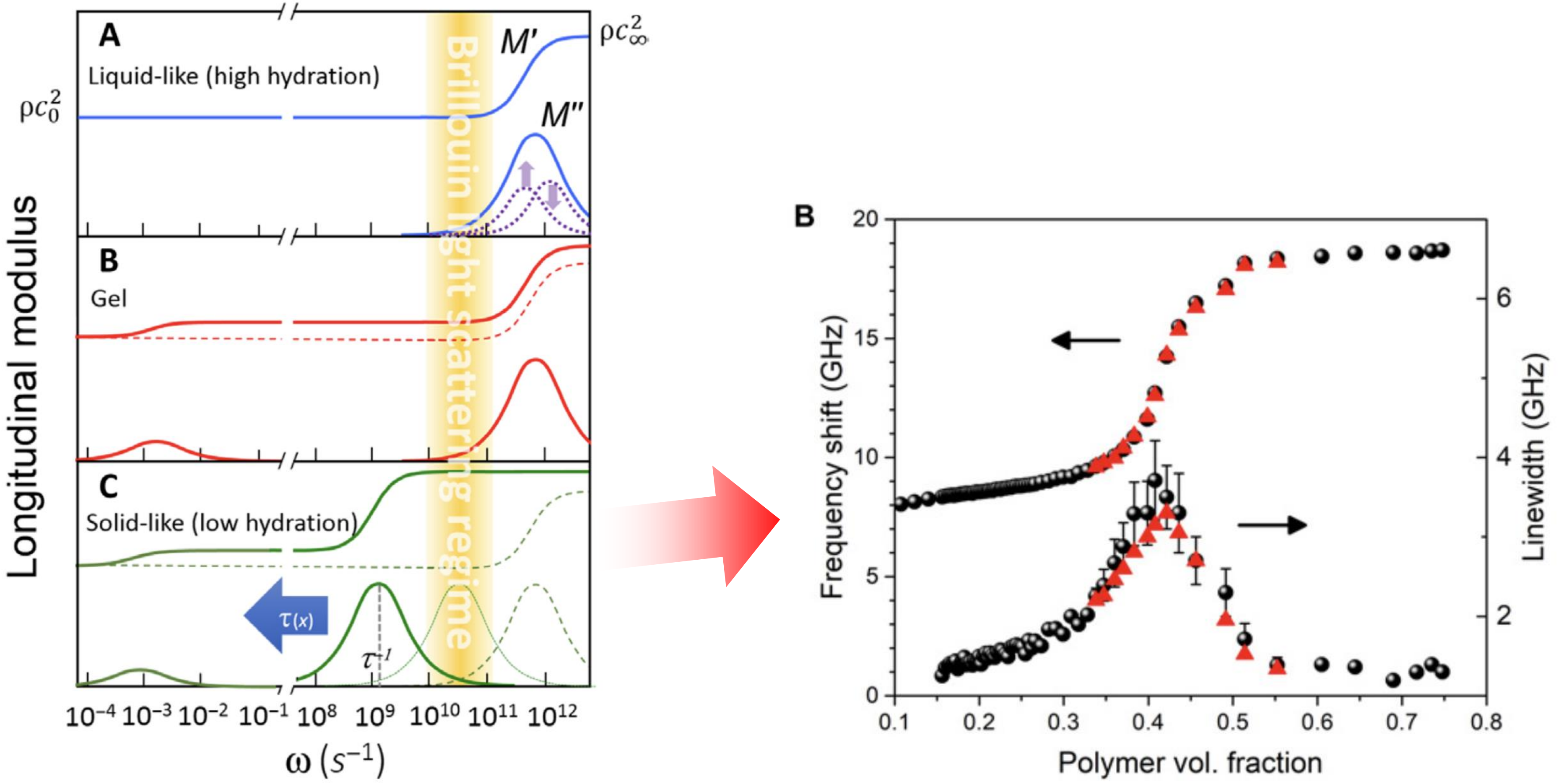
M. Bailey et al., Science Advances **6**, eabc1937 (2020)



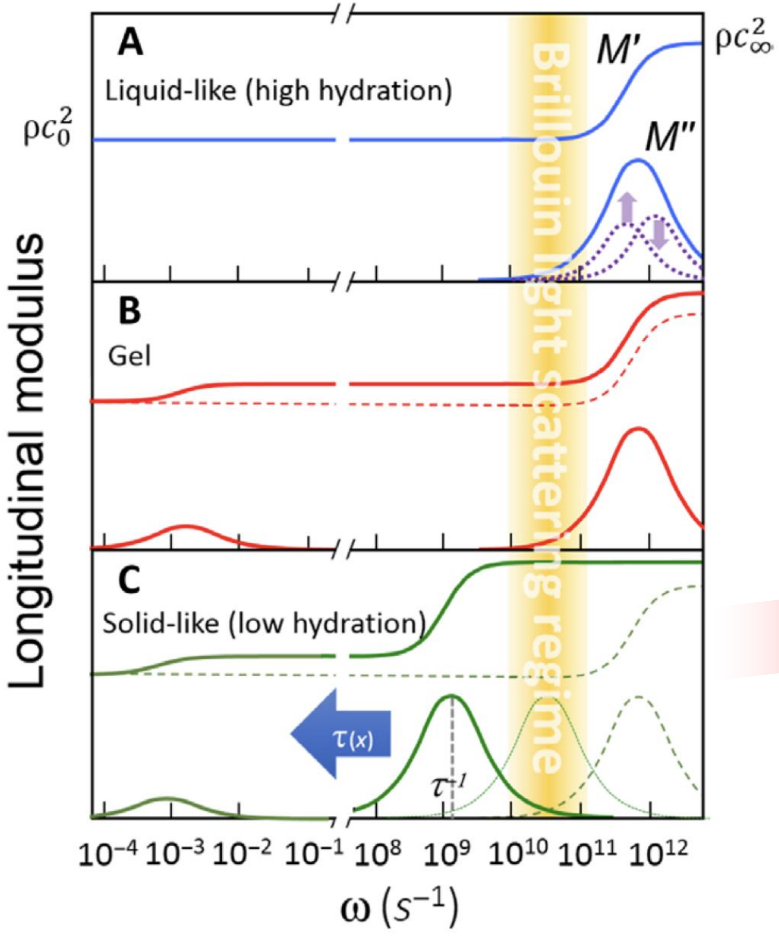


Supplementary Figure s4. Gel transition. Evolution of (A) frequency shift and (B) linewidth derived from Brillouin spectra of hydrogels as the temperature is reduced from 65 to 4-5°C. Arrows indicate the gel transition.

Basic questions: soft vs. hard, a matter of time

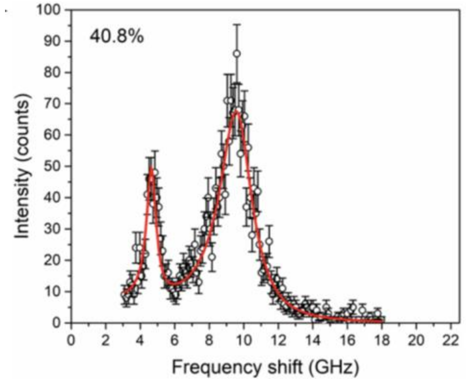


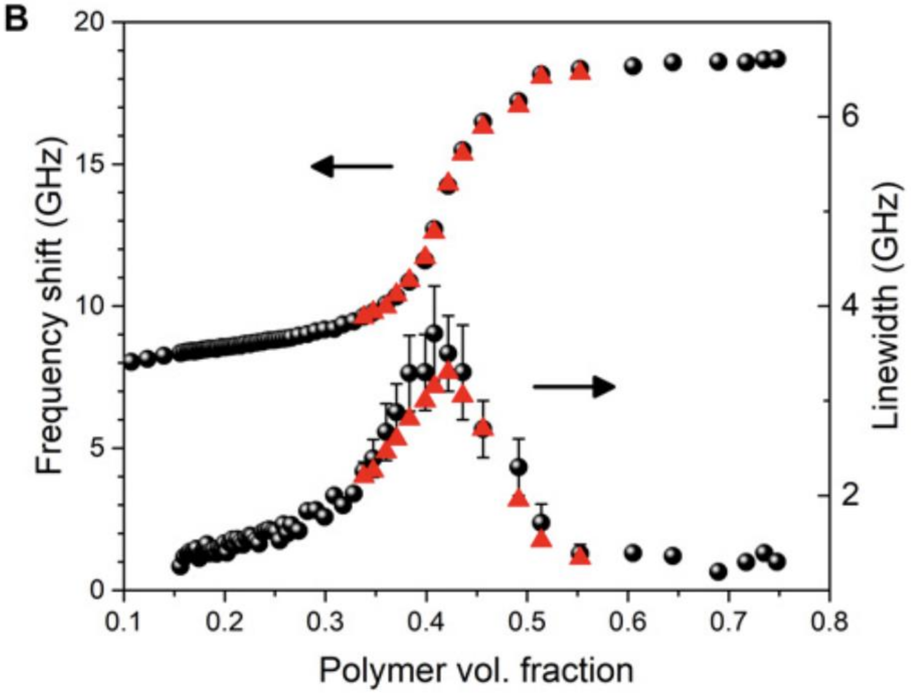
Basic questions: soft vs. hard, a matter of time



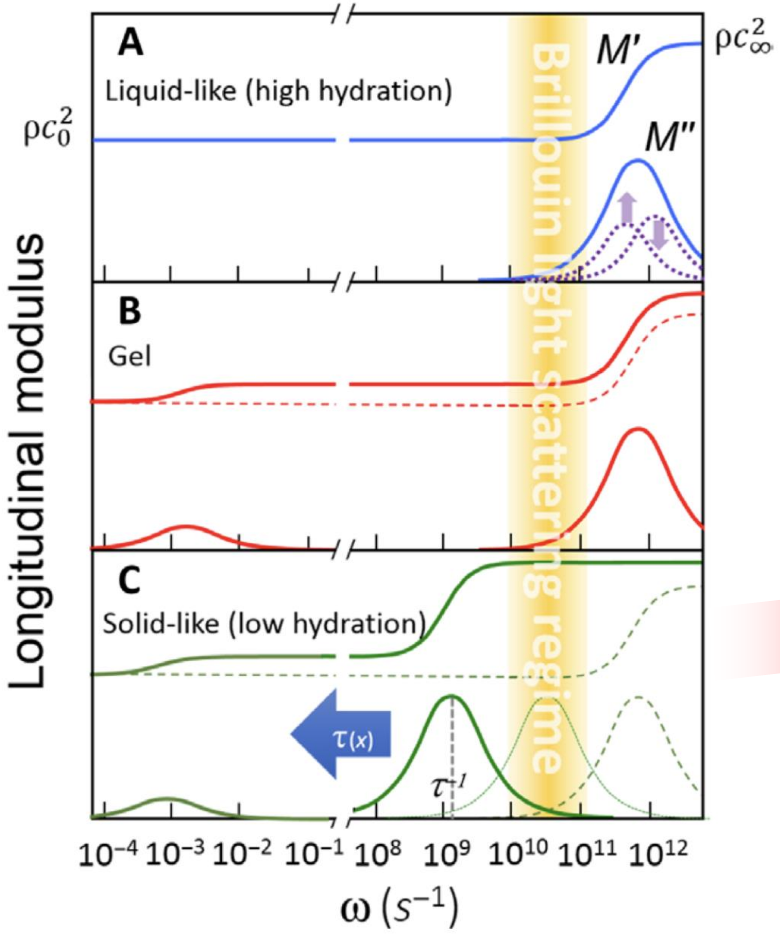
$$I_q(\omega) = \frac{I_0}{\omega}\Im\{[M(\omega)/\rho - \omega^2/q^2]^{-1}\}$$

$$\frac{M(\omega)}{\rho} = c_\infty^2 - \frac{c_\infty^2 - c_0^2}{[1+(i\omega\tau)^a]^b}$$

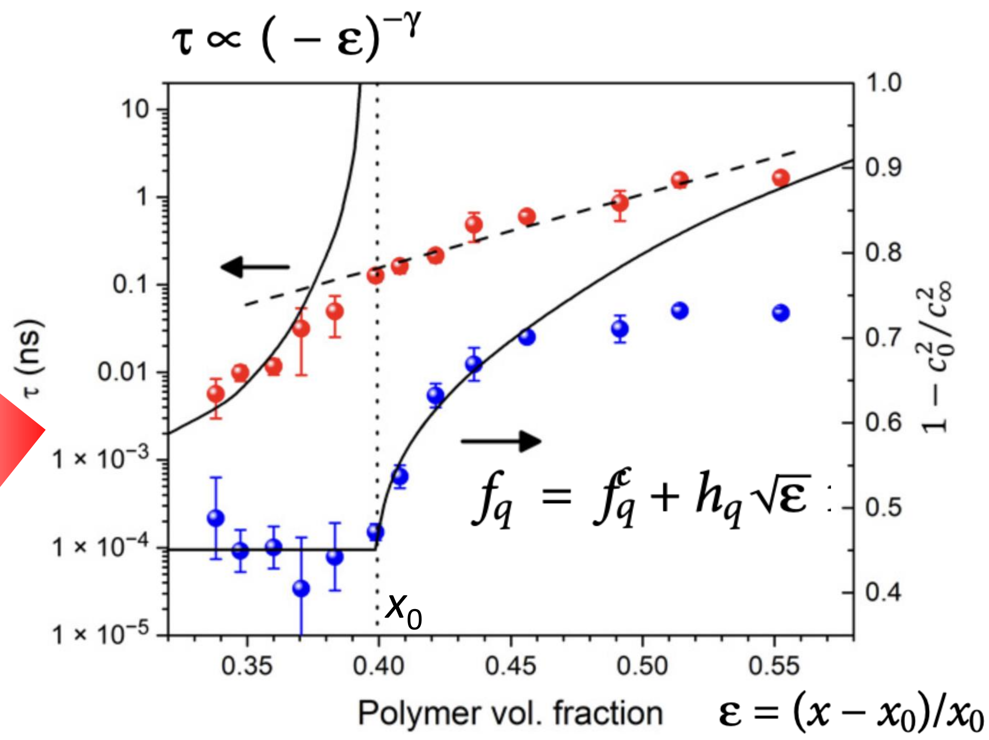




Basic questions: soft vs. hard, a matter of time



Signature of power law divergence of the relaxation time and square root singularity of the non-ergodicity parameter (+ secondary relaxations) @ a critical polymer concentration x_0



MCT is able to capture the early stage structural arrest mechanism, even in animal glue

Summary

Brillouin light scattering is an “ancient” technique that is gaining interest as an optical elastography technique to provide a non-destructive, non-contact probe of the micromechanics of biological matter

Biological matter is a challenging workbench for testing a number of fundamental issues, such as the extent of acoustic modes in disordered matter, the role of heterogeneity in the propagation and attenuation of acoustic modes, the dynamics of hydration water and the nature of the structural arrest and of the glass transition

Thank You!

Martina Alunni Cardinali
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Sara Mattana
Maurizio Mattarelli
Alessandra Anna Passeri

UNIPG

Francesca Palombo
Nick Stone
Peter Winlove

UNIVERSITY OF EXETER

Silvia Caponi
Lucia Comez

iom Istituto Officina dei Materiali

Filippo Scarponi

TABLE STABLE

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

BIOBRILLOUIN

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