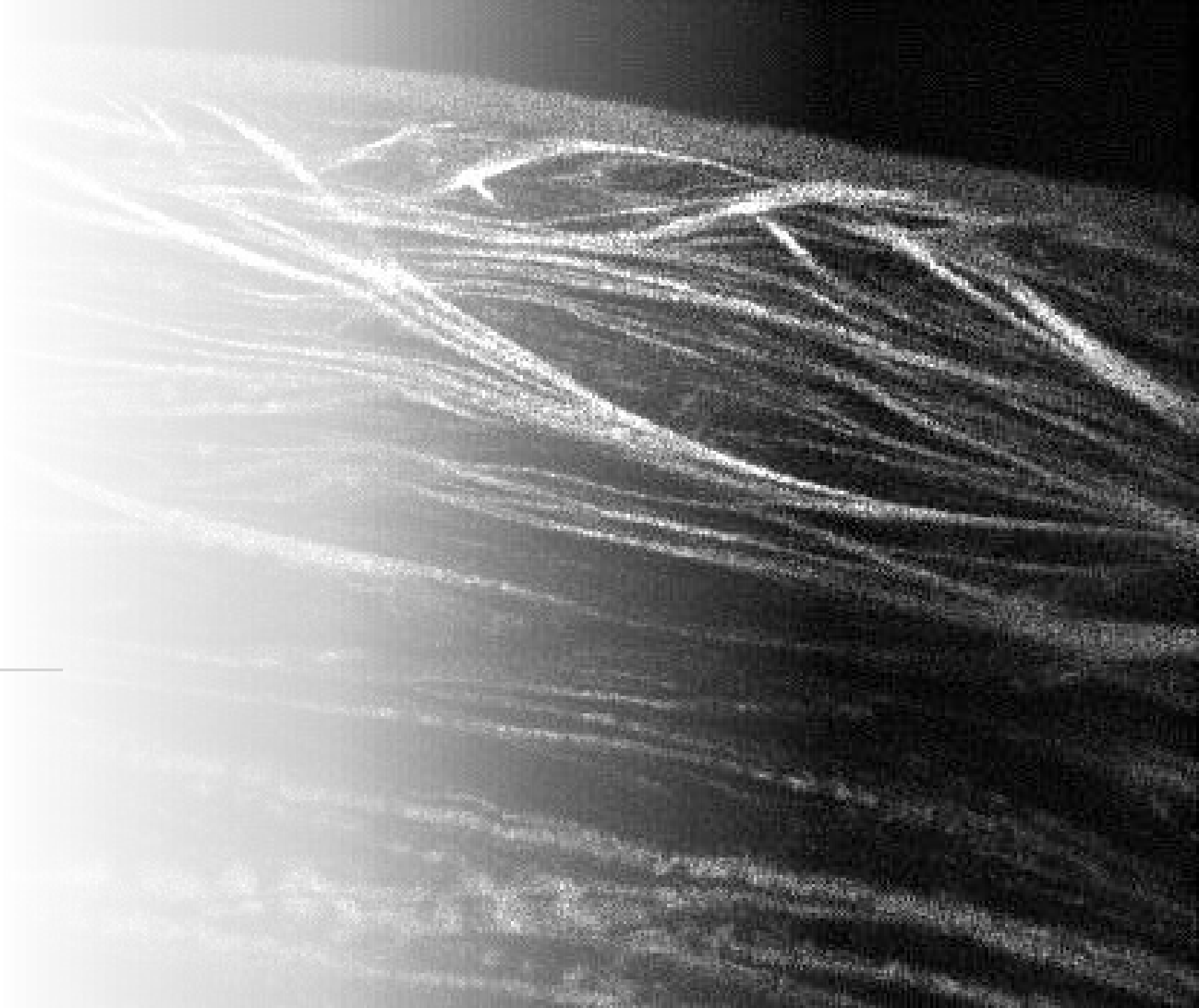
# Microscopie della Cornea



Francesca Rossi f.rossi@ifac.cnr.it

Istituto di Fisica Applicata *Nello Carrara*

Consiglio Nazionale delle Ricerche

Firenze

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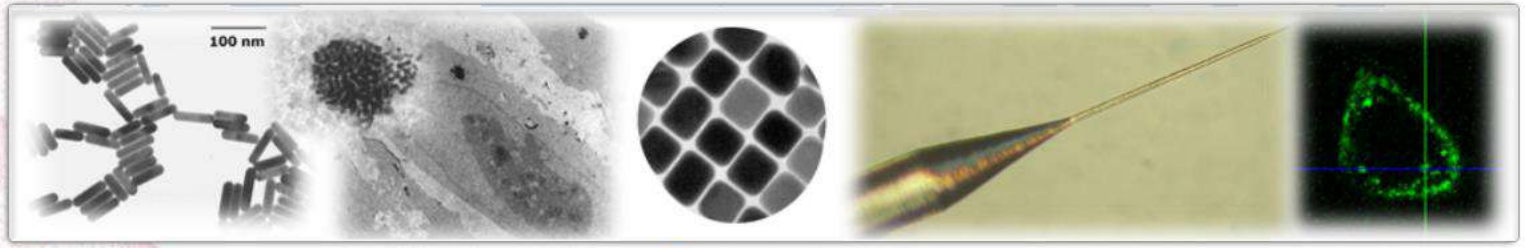


L'Istituto di Fisica Applicata "Nello Carrara" (IFAC) è parte del Consiglio Nazionale delle Ricerche (CNR) il principale ente pubblico che persegue in Italia obiettivi di ricerca ed innovazione.

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***Sette ricercatori di IFAC inclusi nella lista dei World's Top 2% Scientists***
*da "PLOS Biology"*
Giovanni Agati, Bruno Aiazzi, Francesco Baldini, Simonetta Paloscia, Sara Tombelli, Giancarlo Righini e Mauro Bacci, inclusi nella lista dei World's Top 2% Scientists redatta dalla Stanford University

Archivio

Produzione scientifica

## Progetti attivi

Progetti Internazionali: 20
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## Attività editoriali

**Book Series**
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TSRR Volumi precedenti
TSRR Vol.7 (2015)
TSRR Vol.8 (2016)
TSRR Vol.9 (2017)


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INPS Sesto Fiorentino
MPR
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Novotel Firenze Nord Aeroporto
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Bricoman
Intrend
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Firenzecard
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ASSISTENZA AUTORIZZATA...
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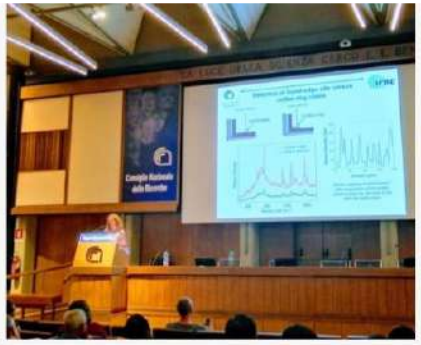
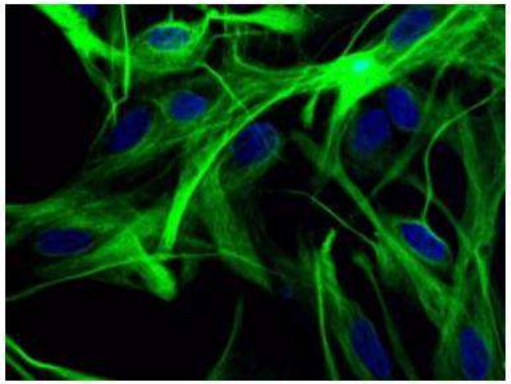
HEALING WOUNDS
WITH LIGHT

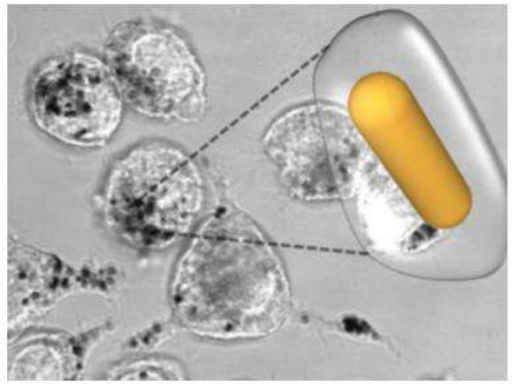

SPIE

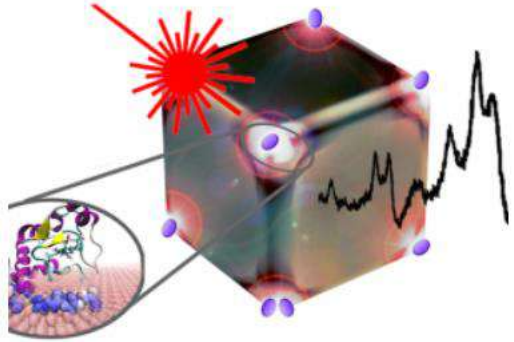




Photonics for Medical Technologies

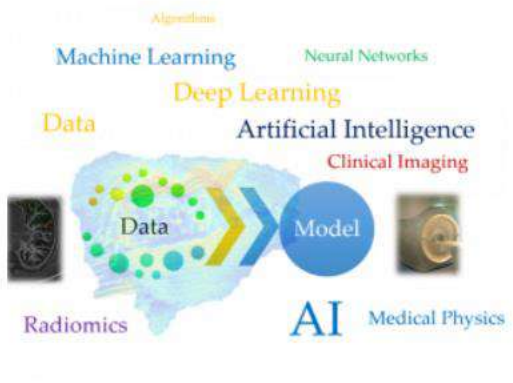


Nanoplasmonics for Medicine and Robotics



Plasmon-Enhanced Spectroscopy and Sensing

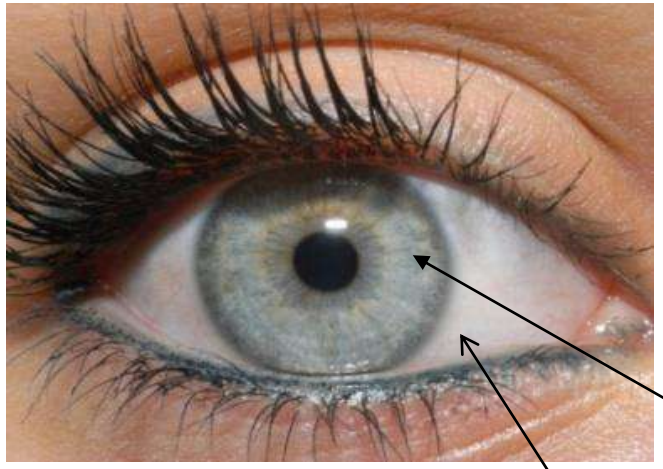
Photonics for Agri-Food

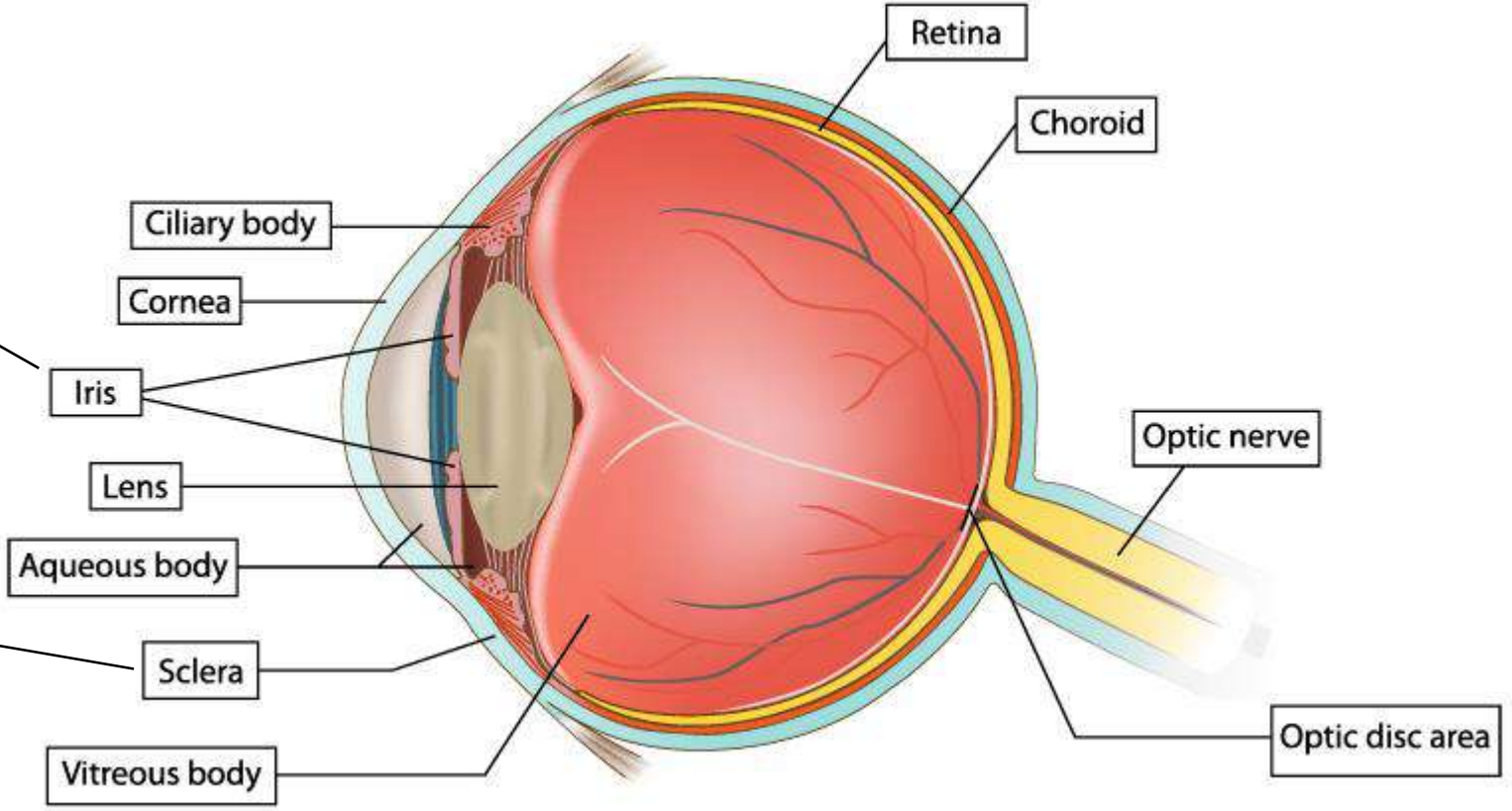



Artificial Intelligence for Health

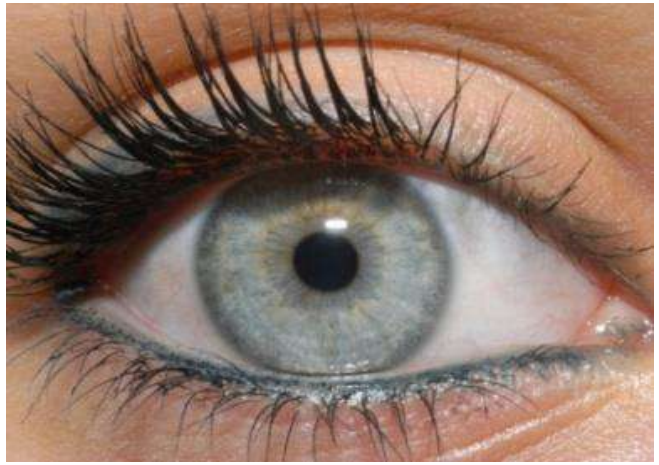
Innovation Transfer and Training

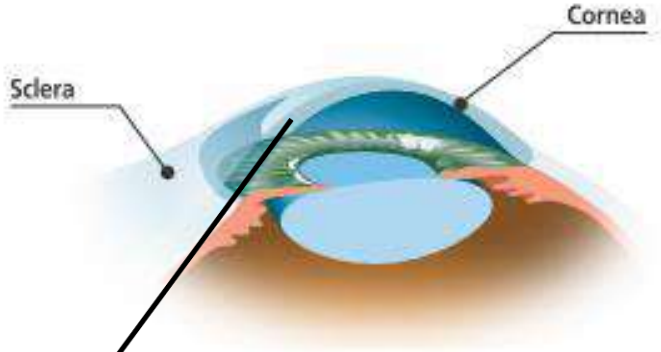
# Background: the cornea morphology



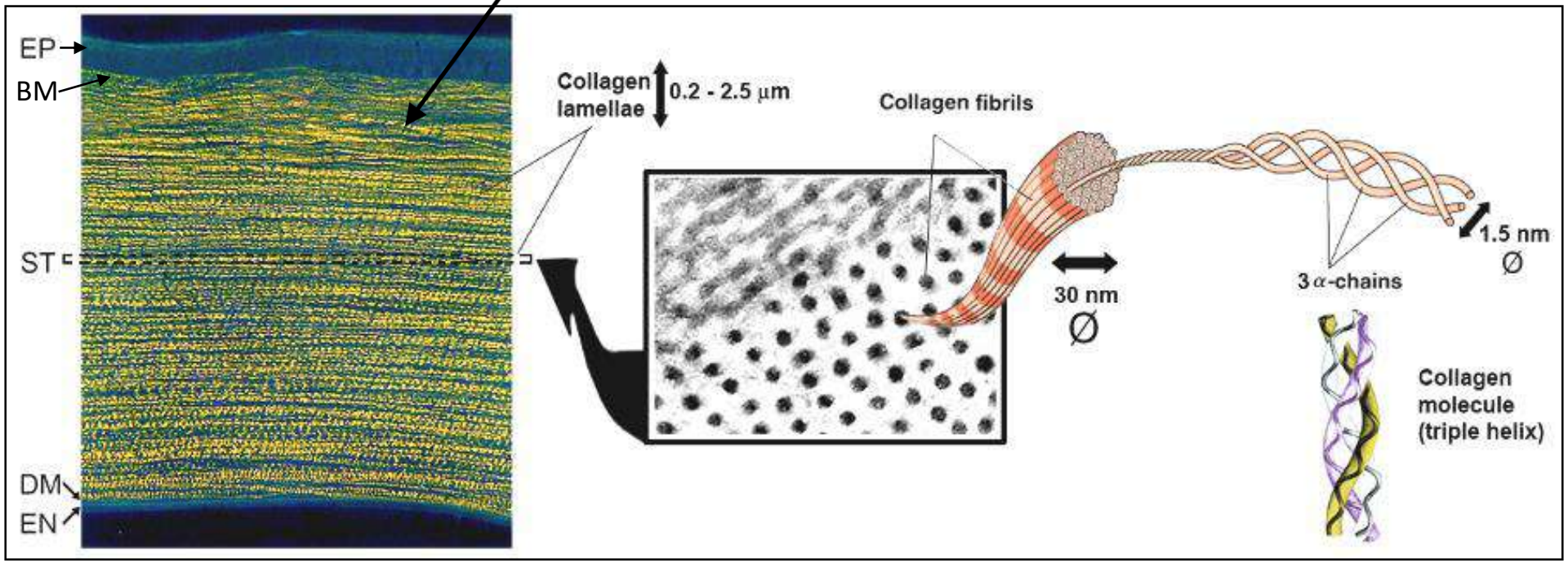


# Background: the cornea morphology






Cornea:
transparent, avascularized tissue



# Microscopia *classica* – Light Microscopy

- Il campione biologico è:
  - Soggetto a deterioramento
  - Senza resistenza meccanica una volta prelevato
  - Trasparente

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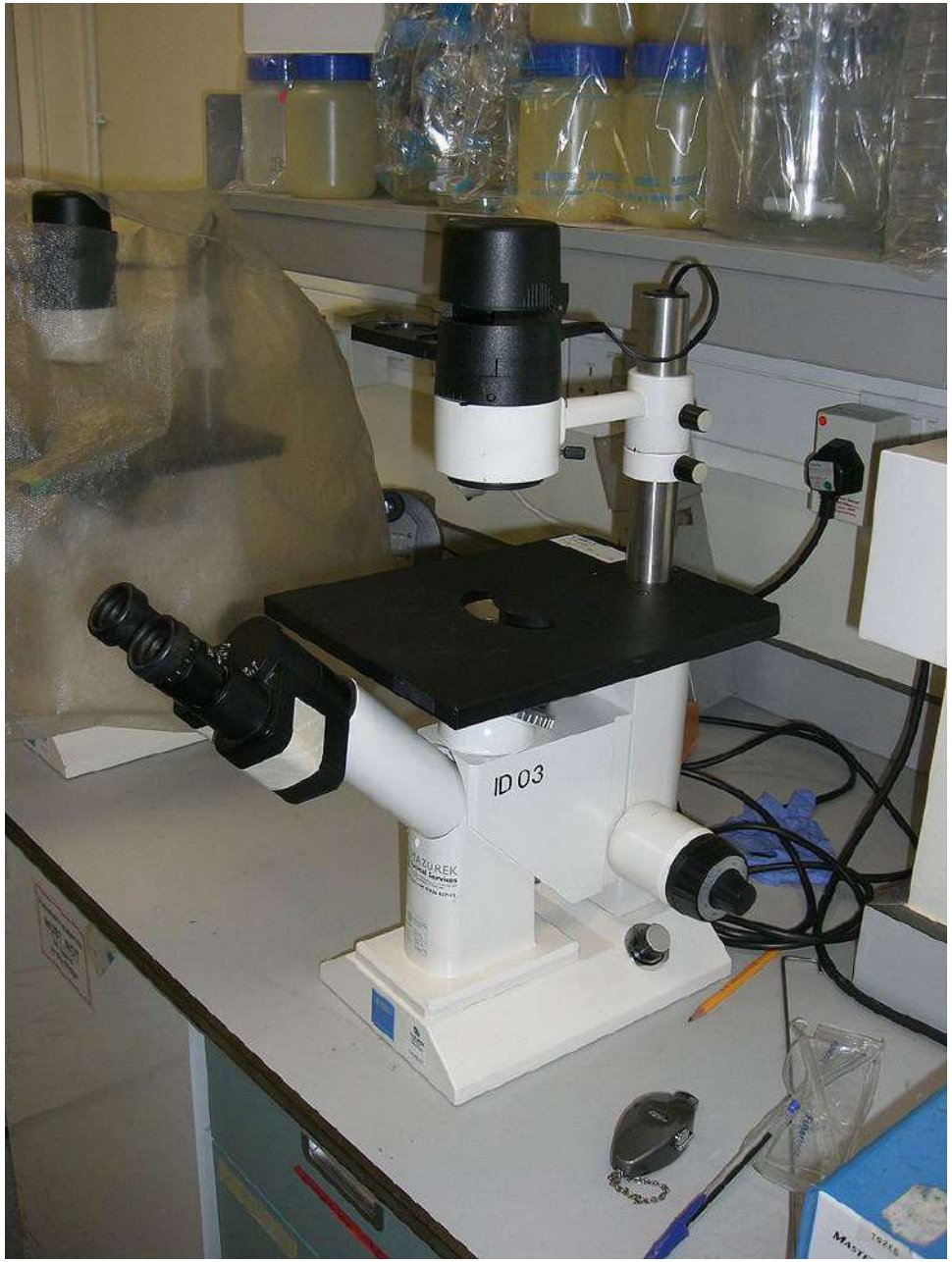
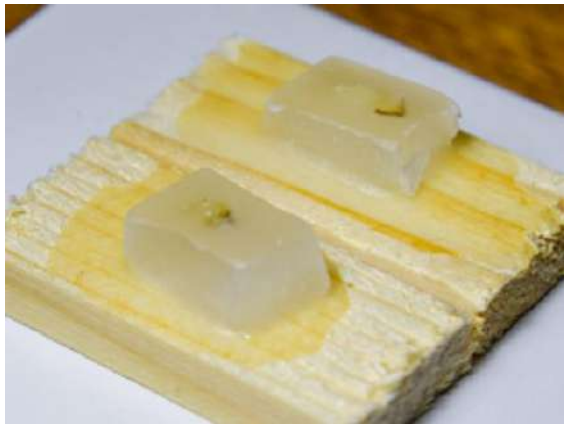


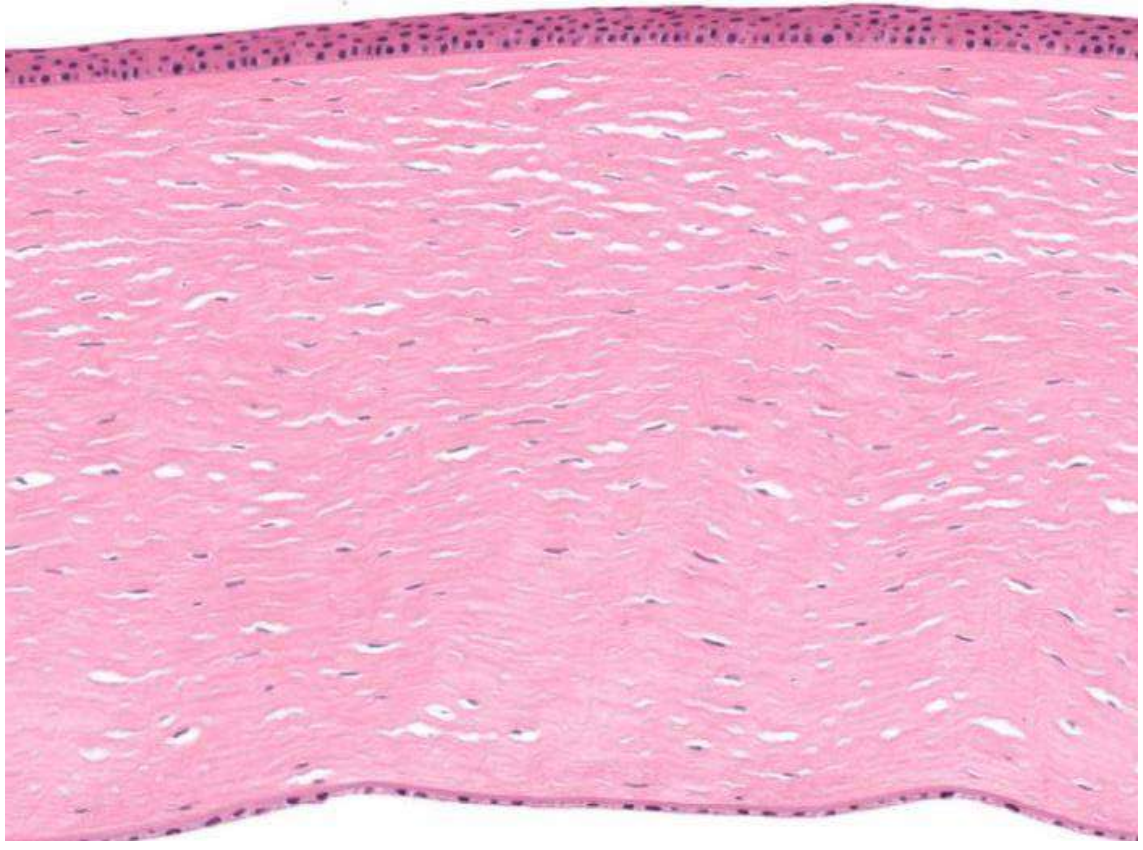
# Microscopia *classica* - Istologia

- Fasi di preparazione del campione:
    - Prelievo
    - Fissazione (paraformaldeide)
    - Inclusione (paraffina)
    - Sezionamento
    - Montaggio
    - Colorazione

# Microscopia *classica* - Istologia

- Colorazione
  - Ematossilina e Eosina (H&E)



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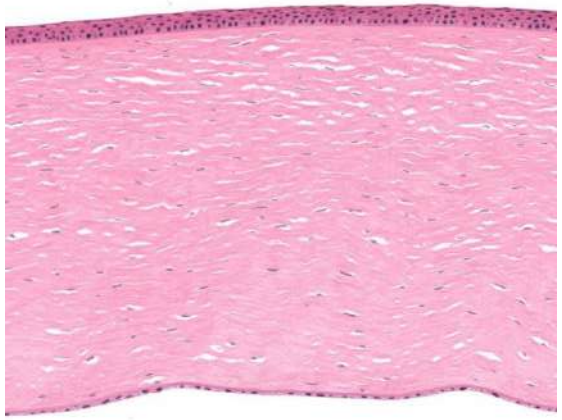

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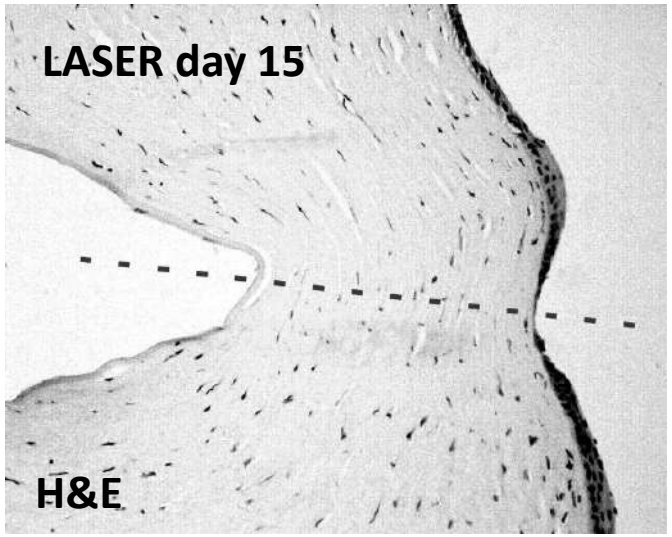
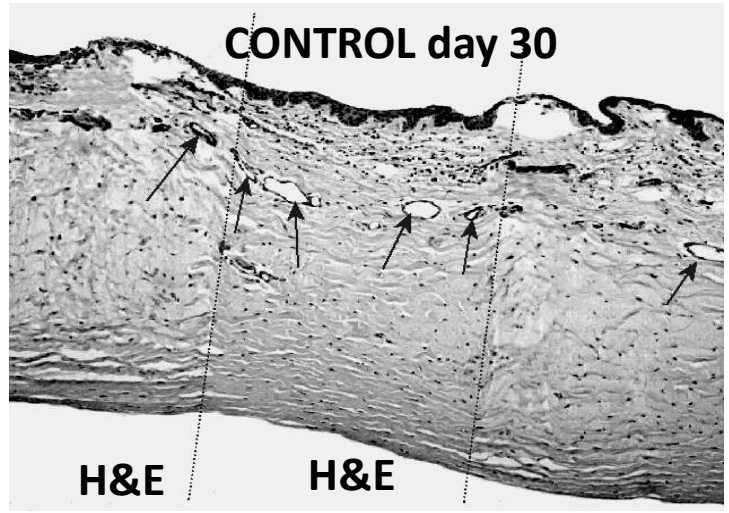


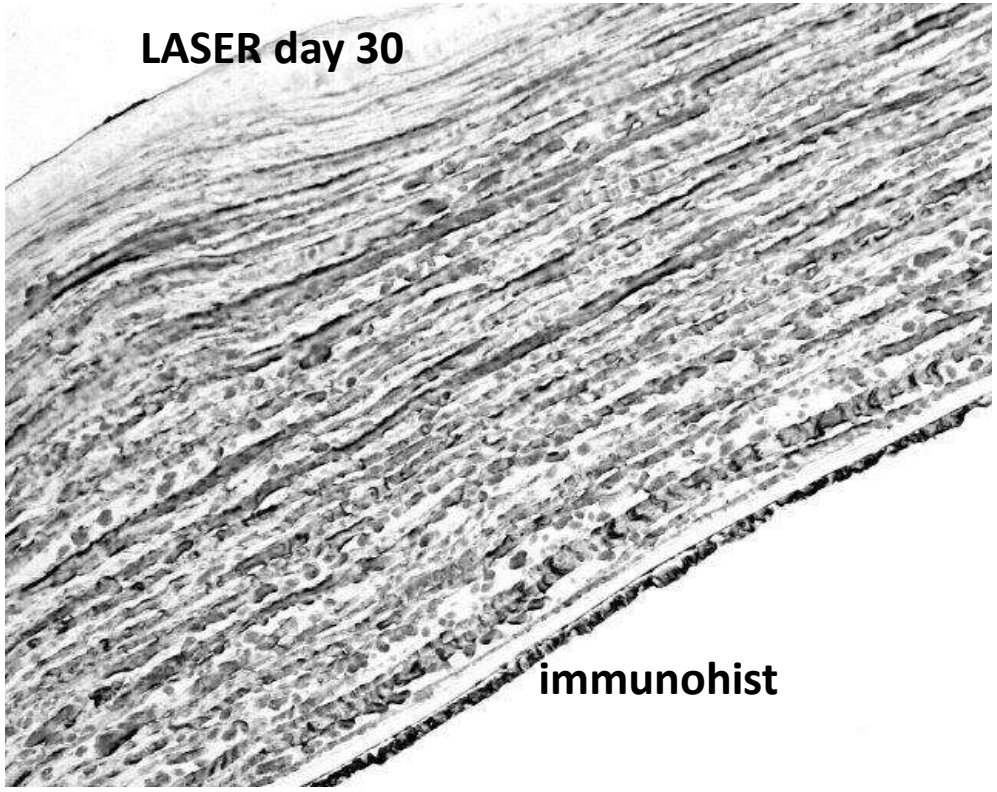


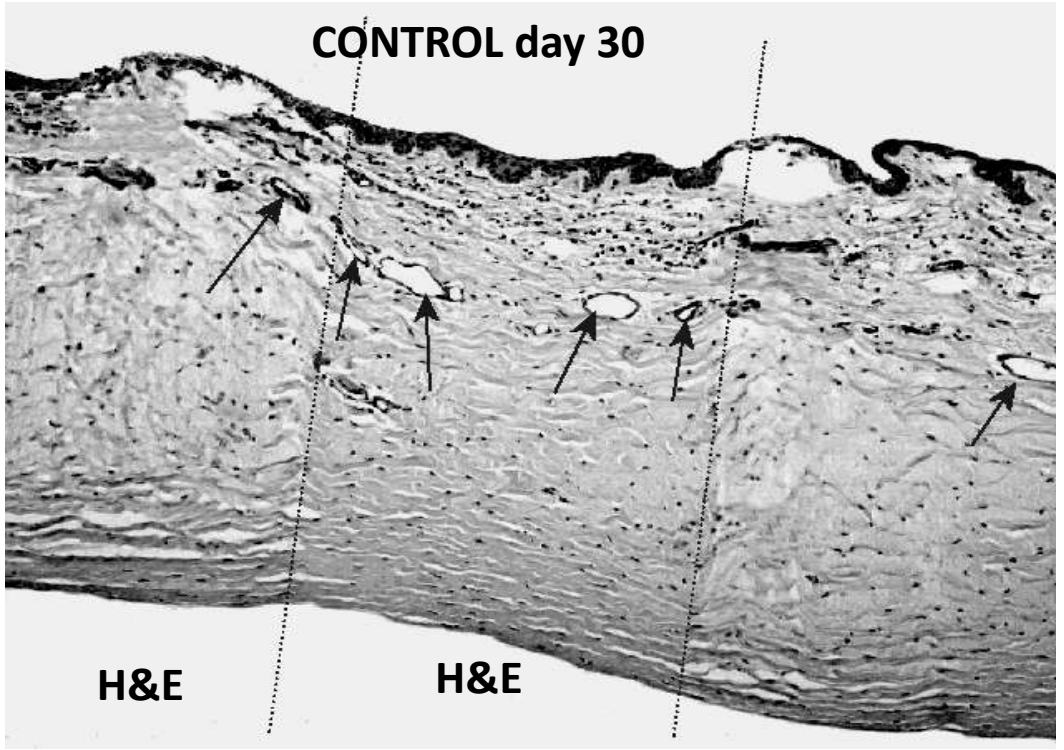



F. Rossi, et al. *Experimental study on the healing process following laser welding of the cornea*, J Biomed Opt., 10(2):024004, doi: 10.1117/1.1900703 (2005).

# Microscopia *classica* – altri coloranti

- Istochimica, Immunoistochimica ed in Immunofluorescenza

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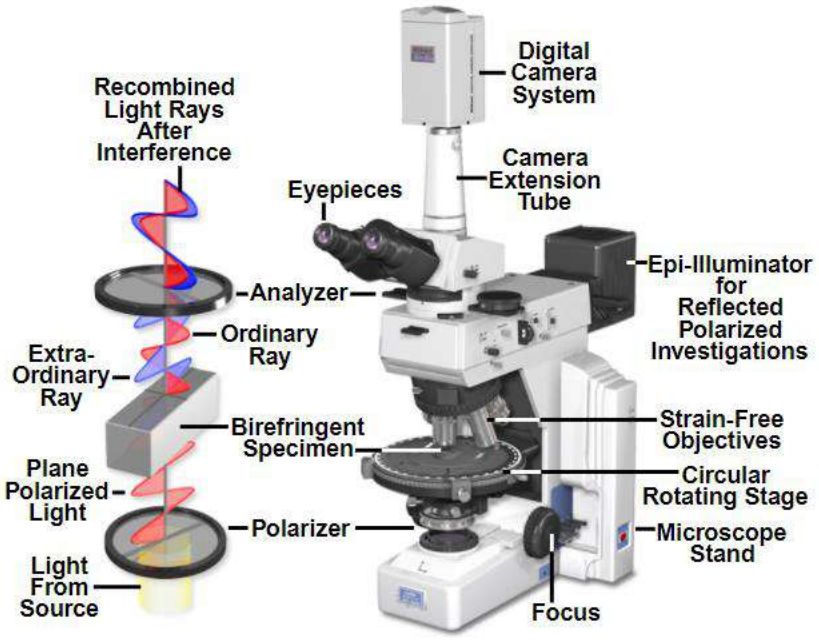
# Microscopia in polarizzazione

- Analizza strutture anisotrope e restituisce informazioni sulla loro struttura

*Figure 1* - *Polarized Light Microscope Configuration*



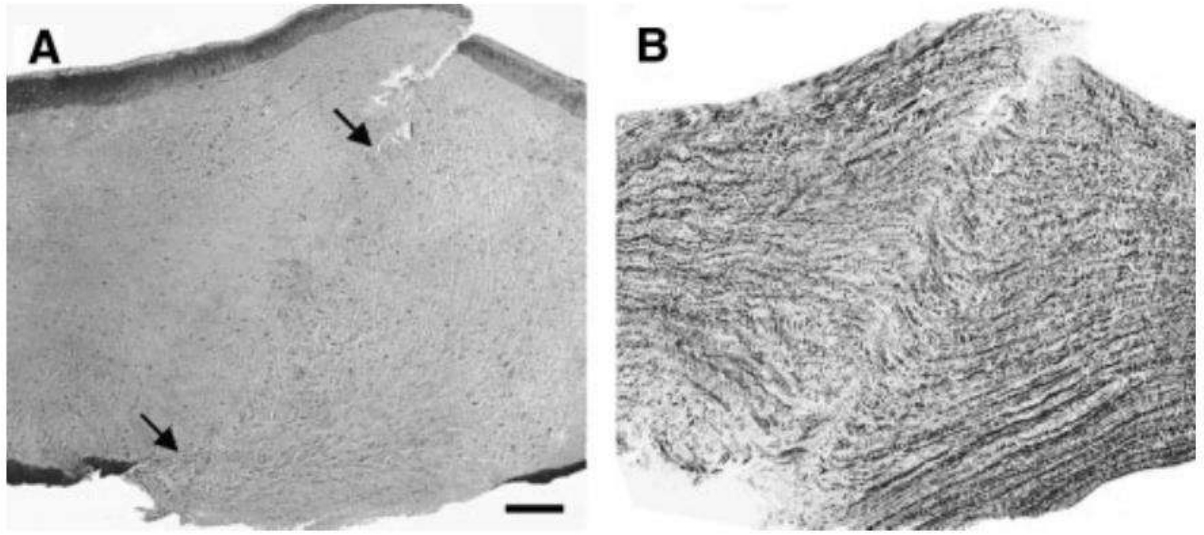



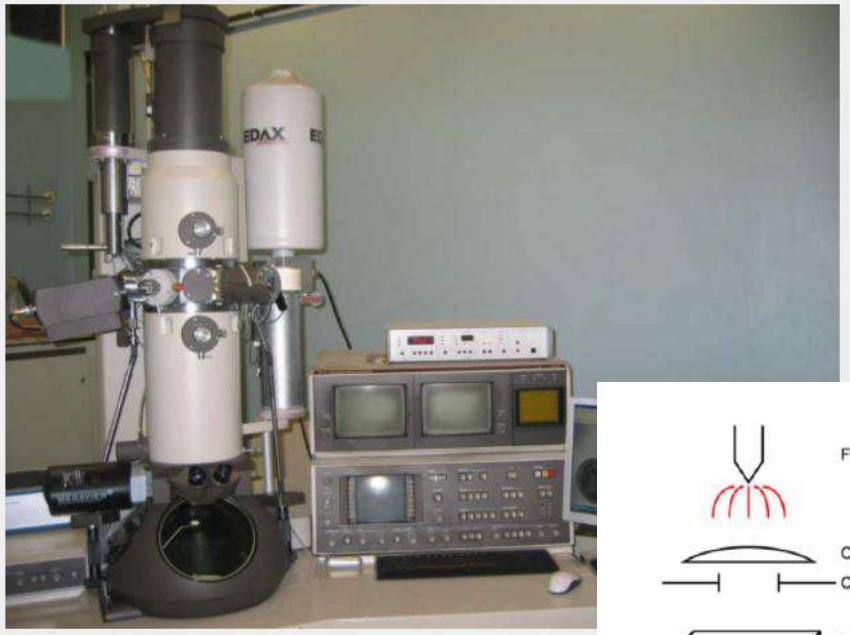

Fig. 1. Microscopic examination of diode laser-welded corneal stroma (bar = 100 μm). **A**: Hematoxylin-eosin stain revealed absence of tissue coagulation or charring at the welded site (between the arrows). **B**: Negative image of the same sample stained with picrosirius red dye shows bridging structures across the wound. The birefringence signal appears more diffused at the weld site but with an intensity comparable to the one of laser-untreated regions.

P. Matteini, et al., *Microscopic Characterization of Collagen Modifications Induced by Low-Temperature Diode-Laser Welding of Corneal Tissue*. Lasers Surg Med, 39, 597-604, doi: 10.1002/lsm.20532 (2007)

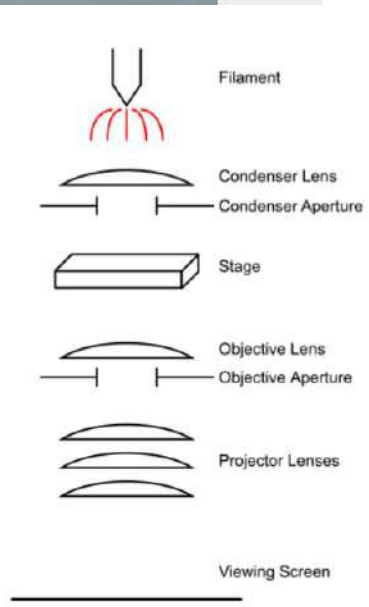
# Transmission Electron Microscopy (TEM) analysis

TEM CM 12 PHILIPS

Consiglio Nazionale delle Ricerche



con tecnologia CRYO-GATAN UHRST 350




## Electron Microscopes

Put simply, electron microscopes use electrons rather than visible light to create an image. To use electrons for microscopy, you need to focus the electron beam and you need a vacuum. The beam is sent onto the sample, bounces off and creates a three-dimensional image of the surface. The wavelength of the electrons is much smaller than the wavelength of light from a bulb or laser, resulting in higher resolution. When you want to use an electron microscope, your sample must be electrically conductive, so the electrons bounce off. That's why samples are often coated in a thin layer of gold or other metal. As you can imagine, this process doesn't fit very well with living biological samples.

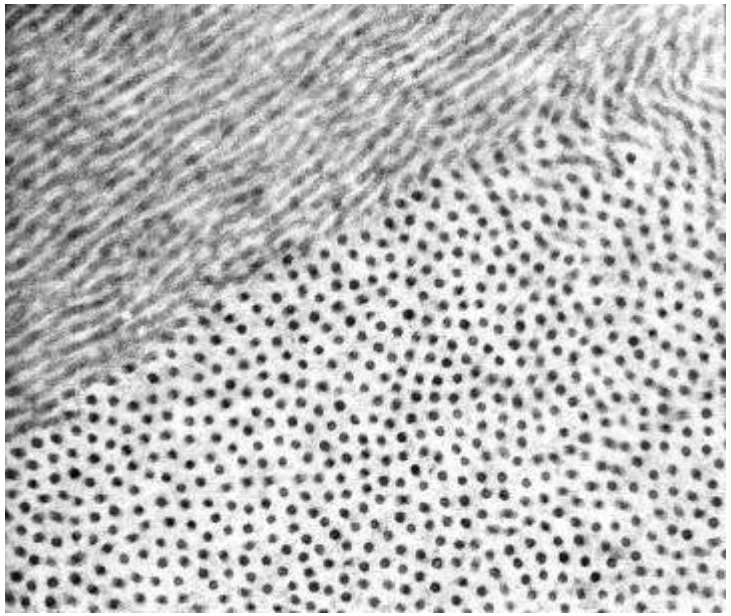
Occorre trattare il campione per poterlo osservare

# Transmission Electron Microscopy (TEM) analysis

- Quantitative evaluation of two morphological parameters to assess fibrillar integrity:

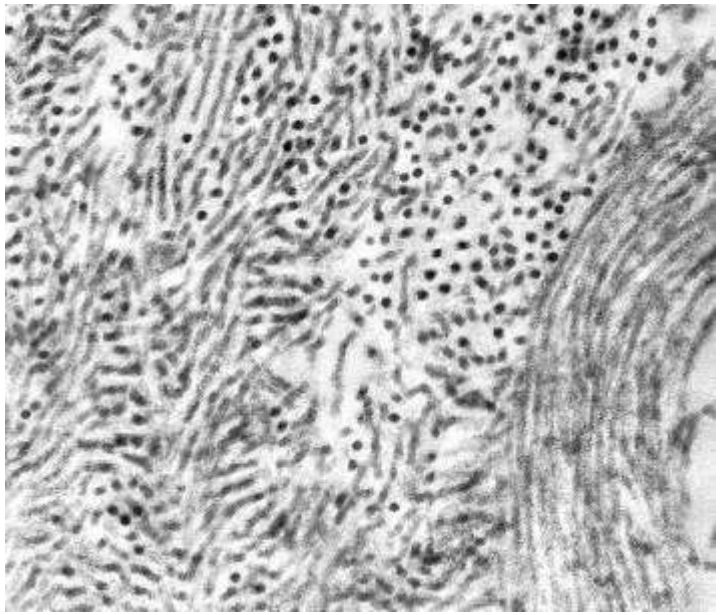
  - Fibril diameter
  - Fibril periodicity

***Control ex vivo poricne eye***



*X 13500*

***Diode laser-welded porcine cornea***



# Transmission Electron Microscopy (TEM) analysis

- Quantitative evaluation of two morphological parameters to assess fibrillar integrity:

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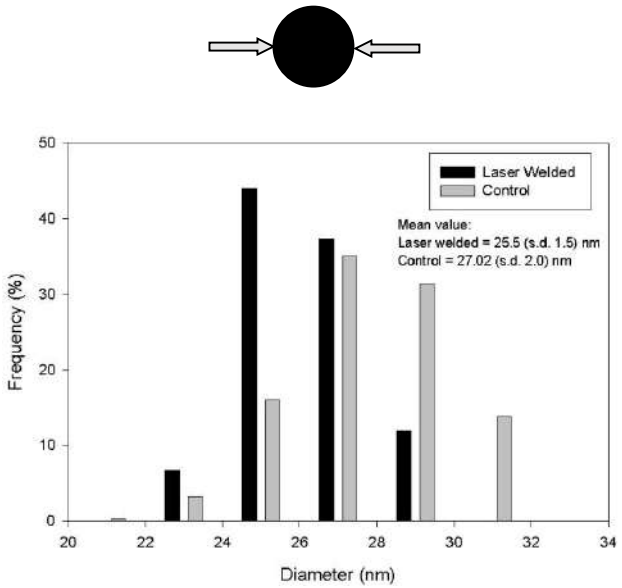


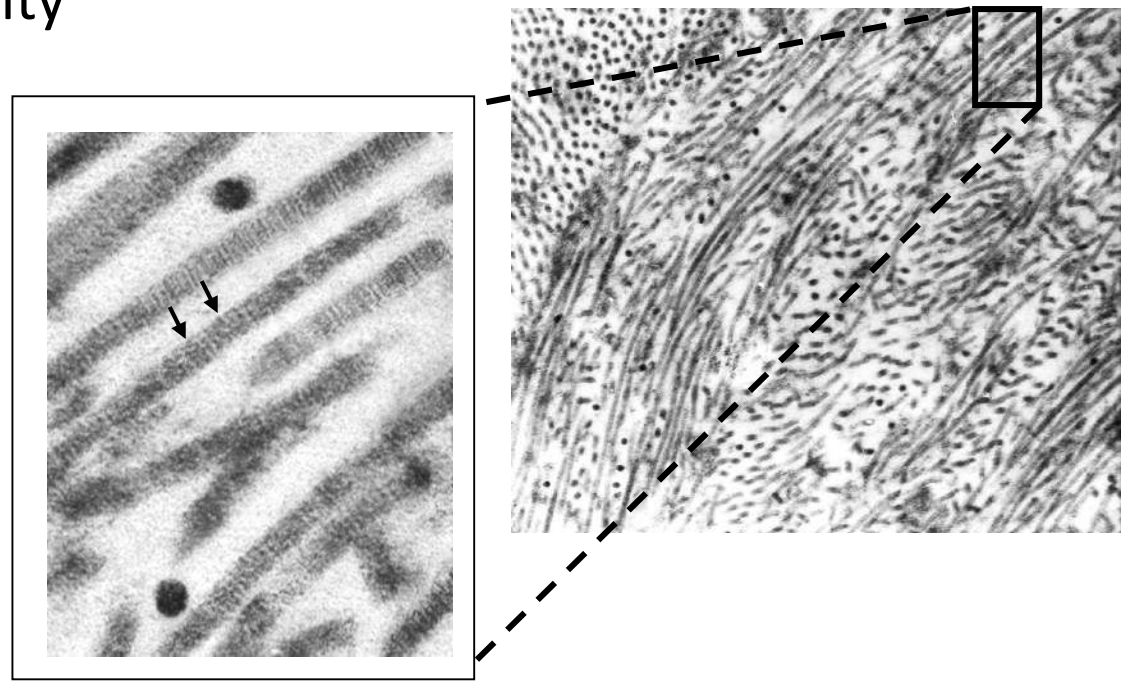

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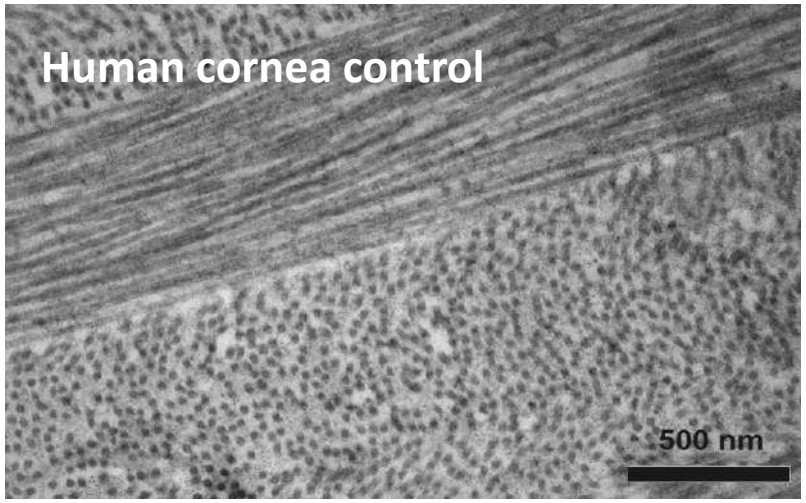
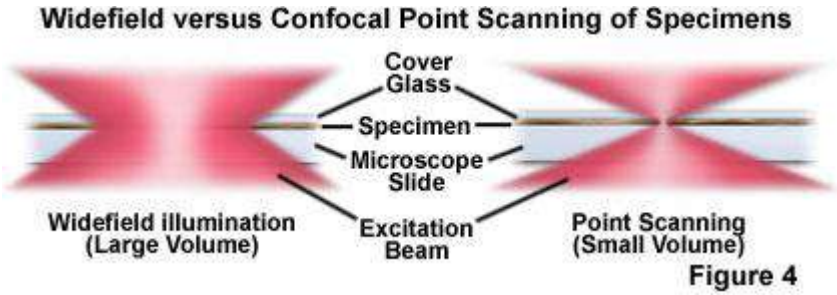



# Microscopia Confocale

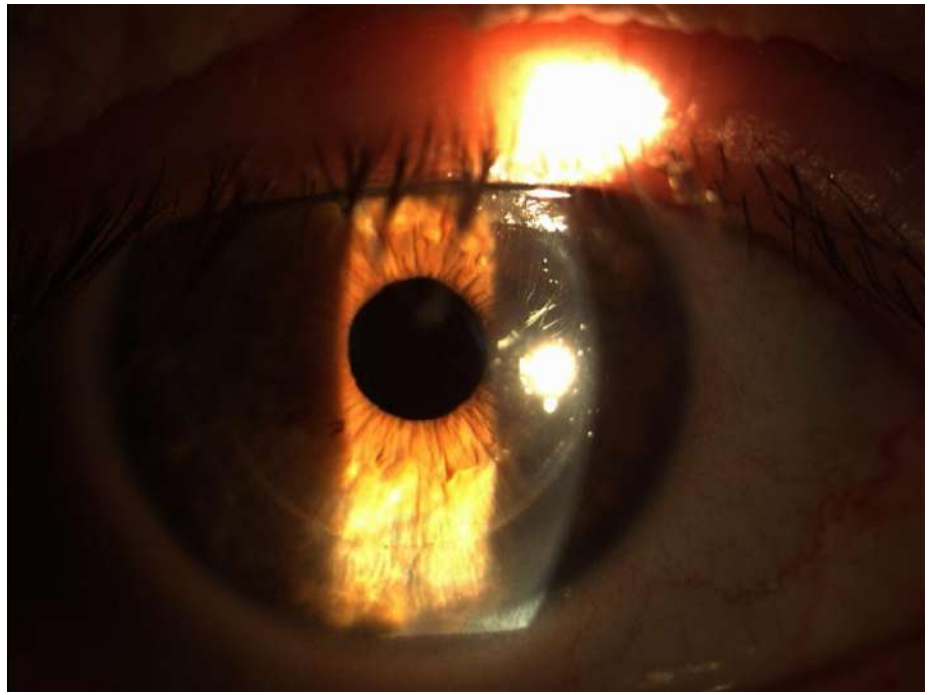

## Confocal Microscopes Deliver Optical Sections

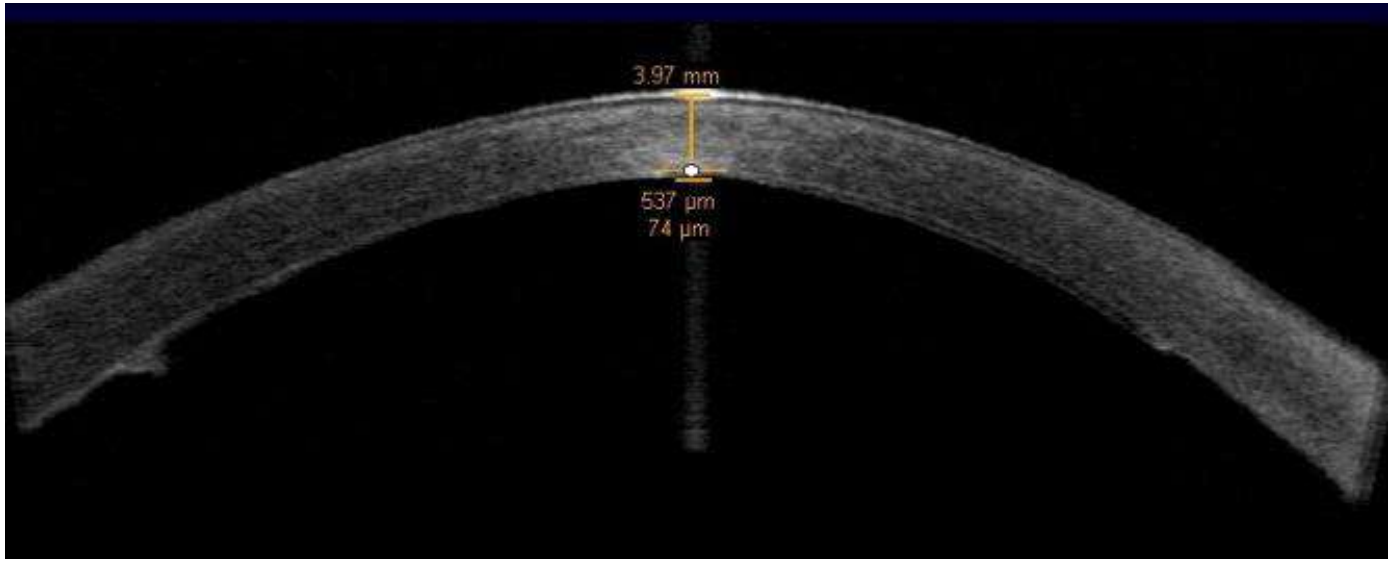
Unlike stereo, zoom and compound microscopes, confocal microscopes use **laser light** as a light source. The laser scans the sample using different patterns, and the image is assembled with a computer. The laser can penetrate the sample deeper than light from a bulb. The result is a three-dimensional image of controlled depth of field. You can examine interior structures of cells, model organisms and tissue by stacking several images from different optical planes.

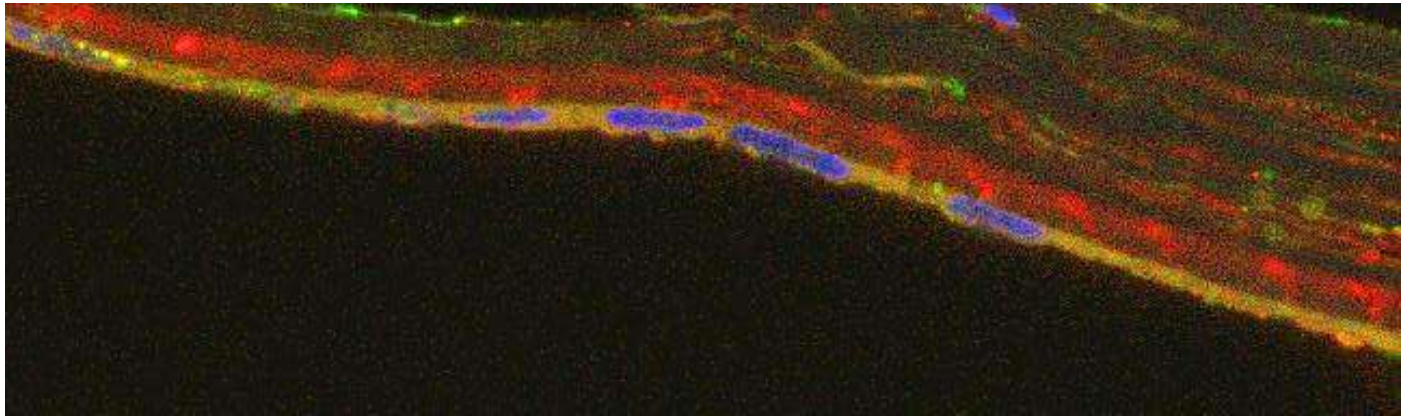
The point light source and the camera are in the same plane, hence the name "confocal."



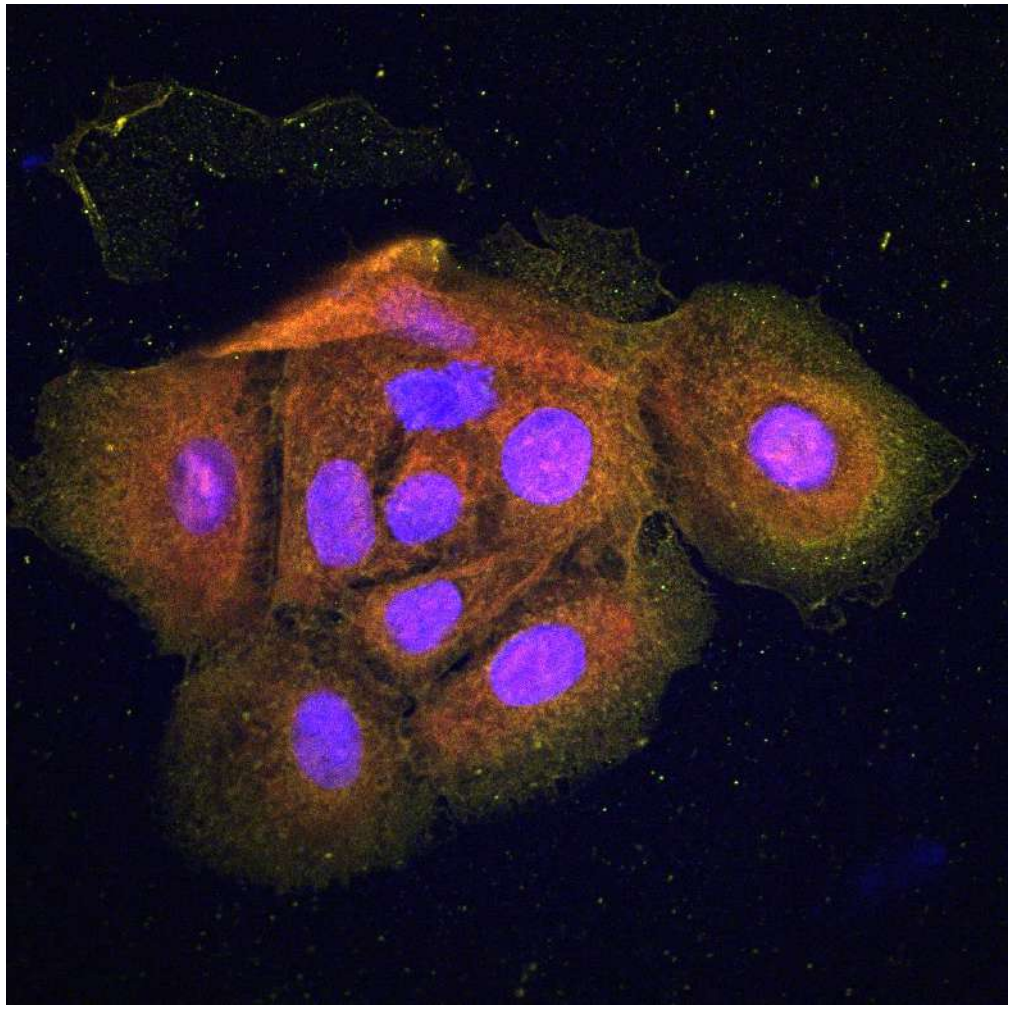
# Background: the human corneal endothelium





# Cellule endoteliali da donatore – 8 giorni



Confocal Microscopy (Leica TCS SP8 )
60x oil immersion objective

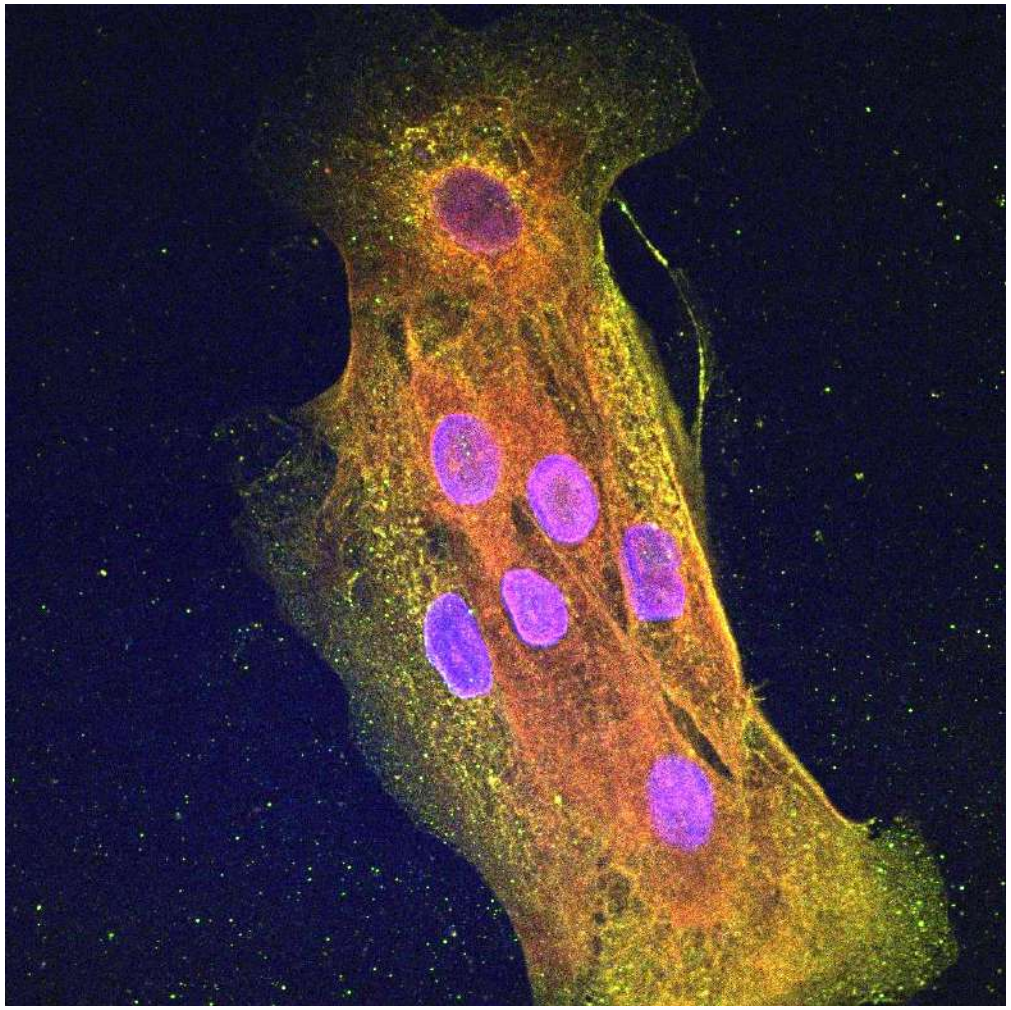
Immunostaining:

**Green** *tight junction* – ZO-1
**Red** Na/K ATPasi
**Yellow** Glypican 4
**Blue** nuclei

| PRIMARY AND SECONDARY ANTIBODIES | | | |
|---|---|---|---|
| **Name** | Company | Type | Dilution Used |
| **Anti- tight junction-ZO1** | AbCam | pAb | 1:200 |
| **Anti-Na/K ATP-asi** | AbCam | pAb | 1:200 |
| **Anti-Glypican 4** | AbCam | mAb | 1:200 |
| **AlexaFluor647 Goat anti-mouse** | AbCam | pAb | 1:400 |
| **AlexaFluor555 Donkey anti-rabbit** | AbCam | pAb | 1:400 |
| **AlexaFluor488 Donkey anti-goat** | AbCam | pAb | 1:400 |

F. Tatini, et al. *Confocal microscopy and electrophysiological study of single patient corneal endothelium cell cultures.* Proc SPIE 9711, 97110G, doi: 10.1117/12.2212636 (2016).

# Cellule endoteliali da donatore - 10 giorni



Confocal Microscopy (Leica TCS SP8 )
60x oil immersion objective

Immunostaining:

**Green** *tight junction* – ZO-1
**Red** Na/K ATPasi
**Yellow** Glypican 4
**Blue** nuclei

| PRIMARY AND SECONDARY ANTIBODIES | | | |
|---|---|---|---|
| **Name** | Company | Type | Dilution Used |
| **Anti- tight junction-ZO1** | AbCam | pAb | 1:200 |
| **Anti-Na/K ATP-asi** | AbCam | pAb | 1:200 |
| **Anti-Glypican 4** | AbCam | mAb | 1:200 |
| **AlexaFluor647 Goat anti-mouse** | AbCam | pAb | 1:400 |
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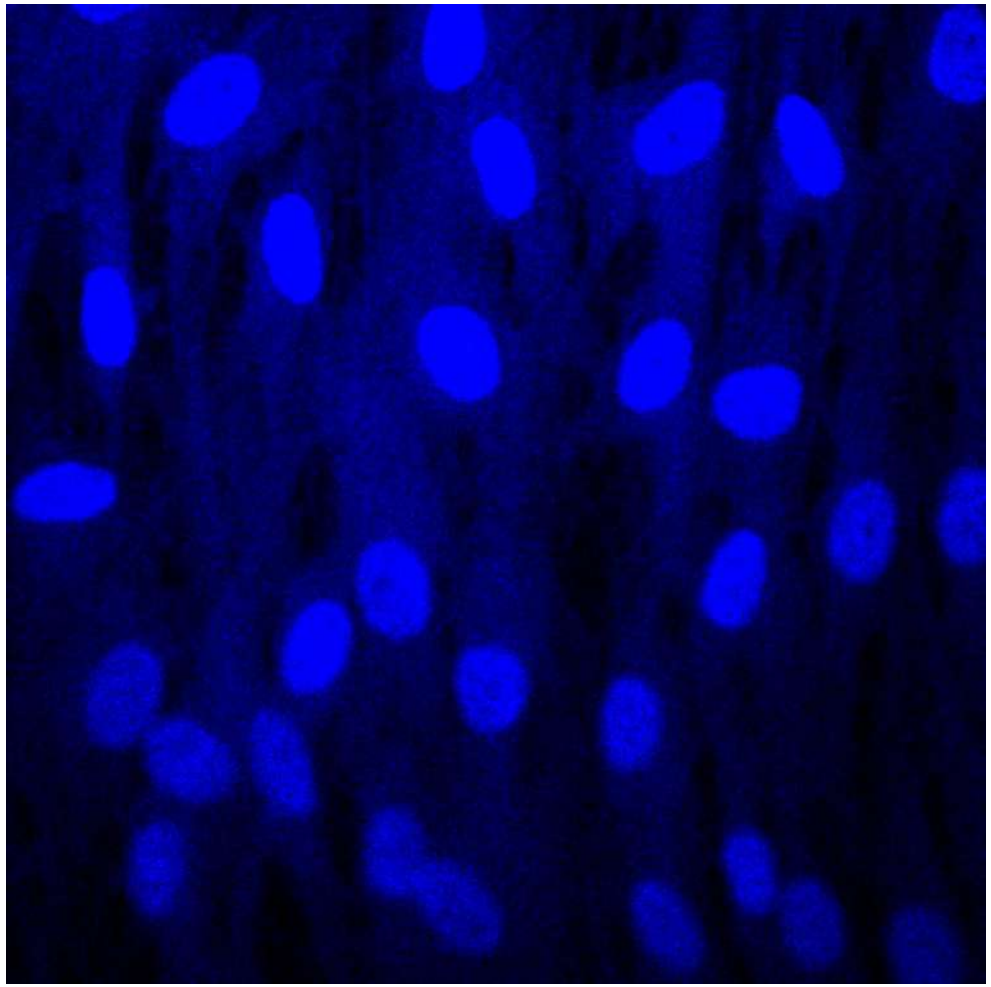
F. Tatini, et al. *Confocal microscopy and electrophysiological study of single patient corneal endothelium cell cultures.* Proc SPIE 9711, 97110G, doi: 10.1117/12.2212636 (2016).

# Cellule endoteliali da donatore - 15 giorni



Confocal Microscopy (Leica TCS SP8 )
60x oil immersion objective

Immunostaining:

**Green** *tight junction* – ZO-1
**Red** Na/K ATPasi
**Yellow** Glypican 4
**Blue** nuclei

| PRIMARY AND SECONDARY ANTIBODIES | | | |
|---|---|---|---|
| **Name** | Company | Type | Dilution Used |
| **Anti- tight junction-ZO1** | AbCam | pAb | 1:200 |
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| **AlexaFluor647 Goat anti-mouse** | AbCam | pAb | 1:400 |
| **AlexaFluor555 Donkey anti-rabbit** | AbCam | pAb | 1:400 |
| **AlexaFluor488 Donkey anti-goat** | AbCam | pAb | 1:400 |

F. Tatini, et al. *Confocal microscopy and electrophysiological study of single patient corneal endothelium cell cultures.* Proc SPIE 9711, 97110G, doi: 10.1117/12.2212636 (2016).

# Microscopia confocale *in vivo*

Corneal Imaging: An Introduction (uiowa.edu)

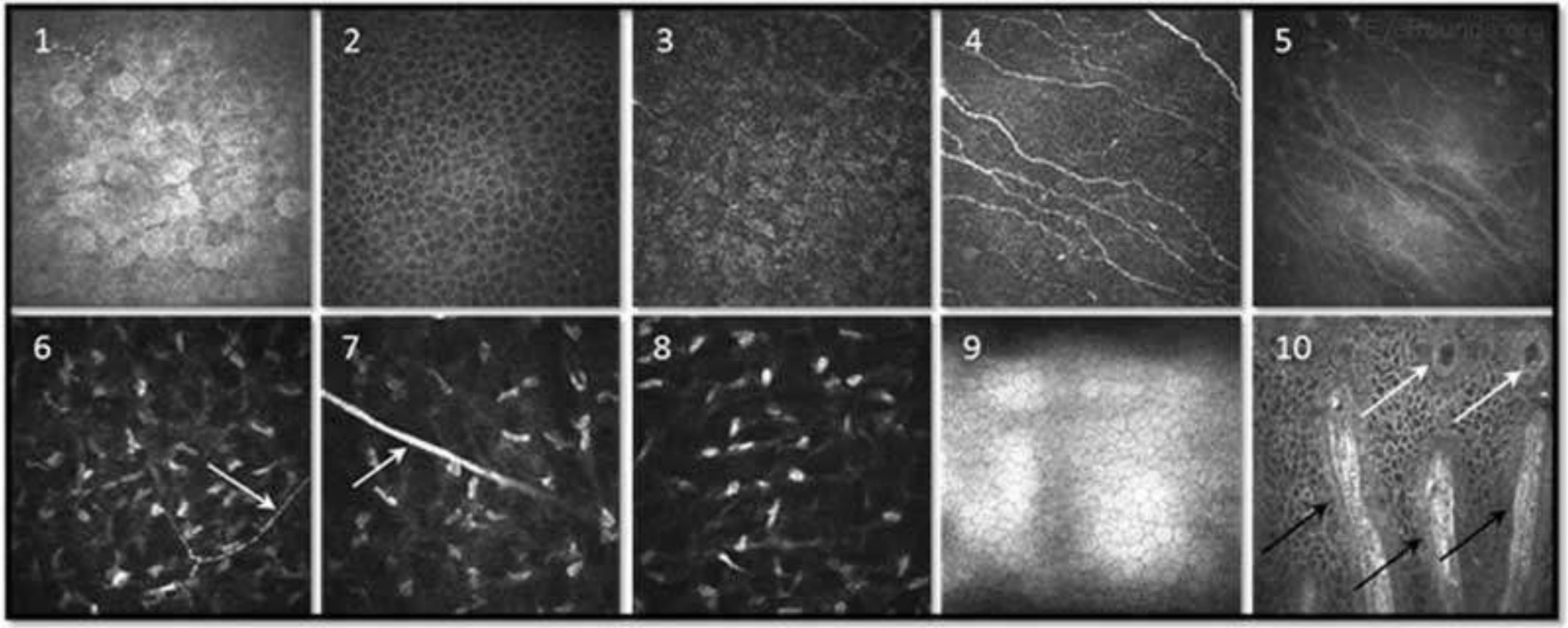



***Figure 14.*** *Confocal microscopy imaging of the various corneal layers using laser-scanning in vivo confocal technology.* ***1-3.*** *Superficial epithelium, epithelial wing cell layer, and basal epithelium;* ***4.*** *Subbasal nerve plexus;* ***5.*** *Bowman's layer;* ***6-8.*** *anterior stroma with nerve (arrow), mid stroma with nerve trunk (arrow), and posterior stroma;* ***9.*** *Endothelium; and* ***10****. Inferior limbal palisade ridges (black arrows) with focal stromal projections (white arrows). Image courtesy of Dr. Neil Lagali (Linköping University, Linköping, Sweden) (11).*

# Microscopia di Seconda Armonica - SHG

E’ un fenomeno non lineare: si illumina il campione con un laser a una frequenza $\omega$ e si osserva un segnale con frequenza doppia $2\omega$

- Non è necessario processare il campione biologico

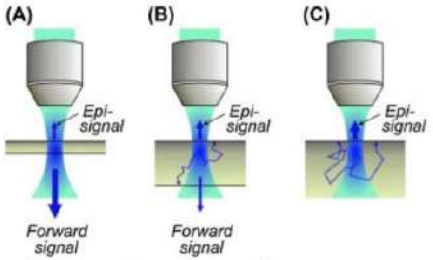



Figure 5.16. Forward- and epi-detection second harmonic generation signal from different tissue thicknesses.

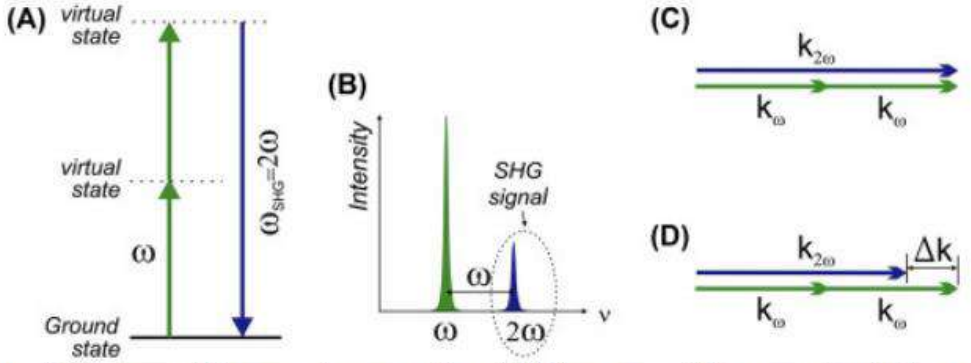



Figure 5.15. Second harmonic generation (SHG) process: (A) energy level diagram, (B) spectral positions of input and output fields, (C) perfect phase matching, and (D) usual wave-vector mismatch in tissue environment, which is compensated by randomness and dispersion.

[Second-Harmonic Generation - an overview (pdf) | ScienceDirect Topics]

Intense laser field induces a nonlinear polarization $P^{2\omega}$ in a noncentrosimmetric molecule resulting in the production of a coherent wave at exactly twice the incident frequency

# Microscopia di Seconda Armonica - SHG

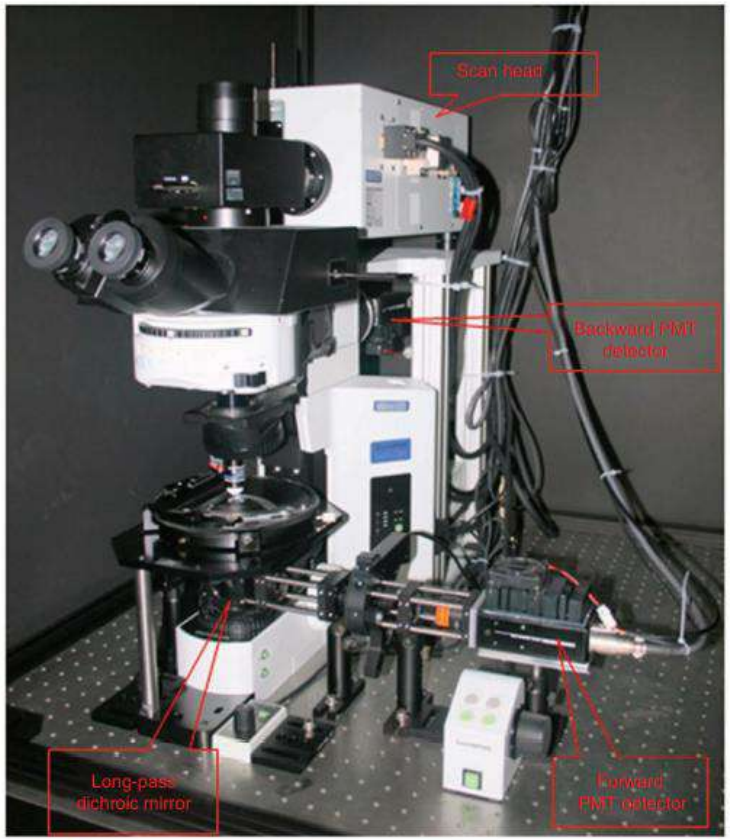



**Figure 4** | Annotated photograph of the upright microscope, detectors and light-tight box.

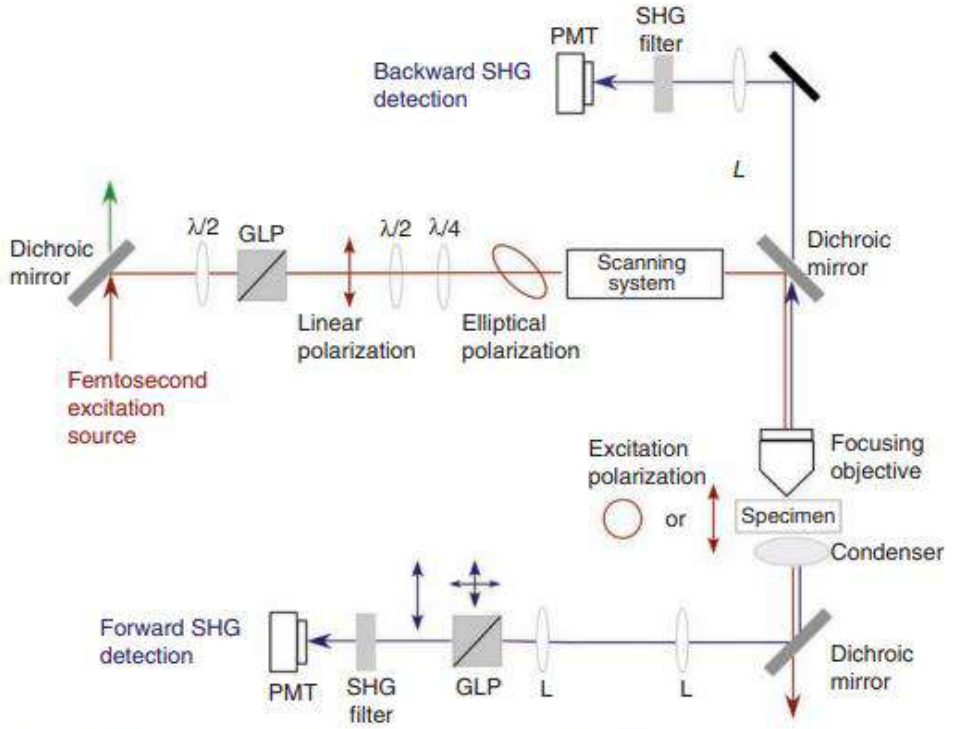
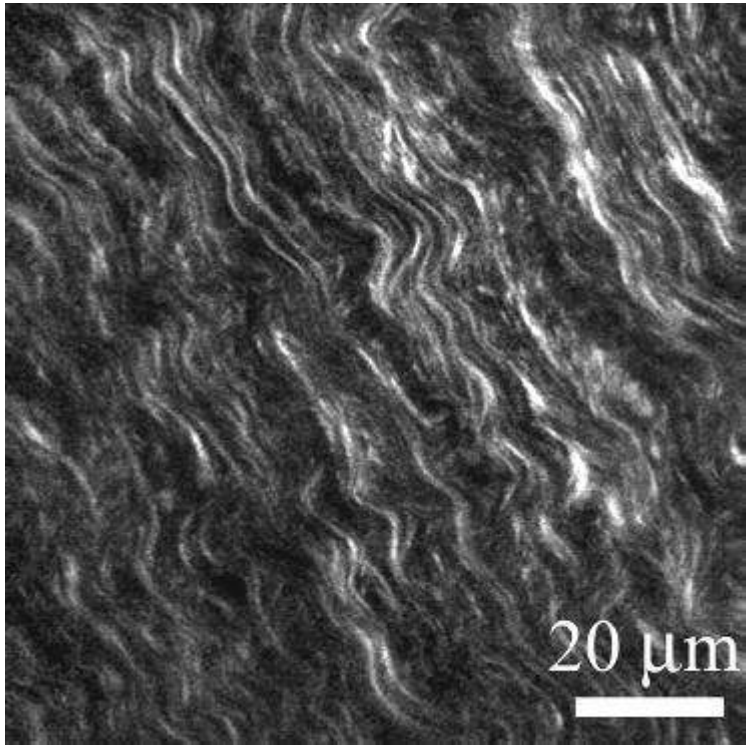



**Figure 2** | Schematic of the optical layout of the SHG microscope, showing the optical components before the scan head and the detection pathways. L, lens; λ/2 and λ/4 are half- and quarter-wave plates, respectively.

nprot.2012.009.pdf

# Microscopia di Seconda Armonica - SHG

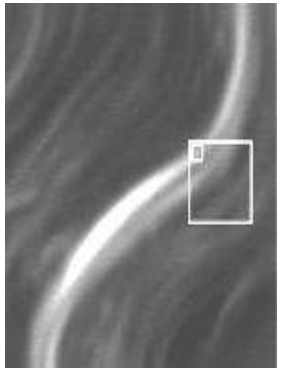
**Collagen is a strong generator of SH signal (high specificity and contrast!)**

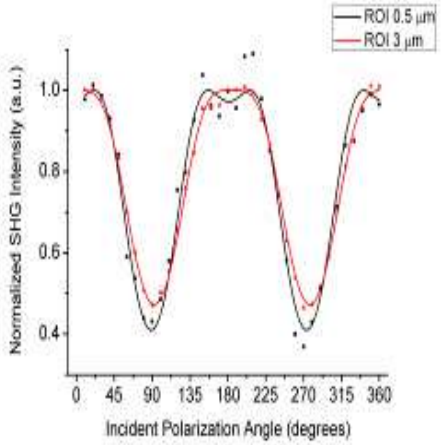


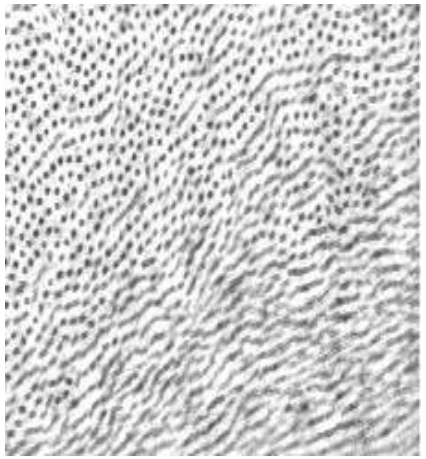

Circularly-polarized SHG images of corneal stroma revealed ~0.5 µm thick fiber-like structures, which actually consisted of many collagen fibrils (only 30 nm thick), organized in lamellar domains.

P. Matteini, et al.. *Photothermally-induced disordered patterns of corneal collagen revealed by SHG imaging*. Optics Express, 17(6), 4868-4878 (2009). 10.1364/OE.17.004868

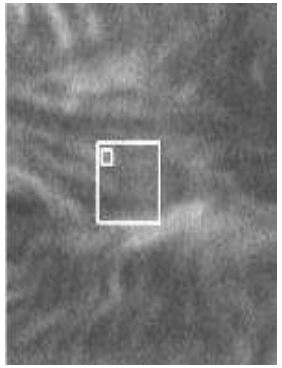
# Microscopia di Seconda Armonica - SHG

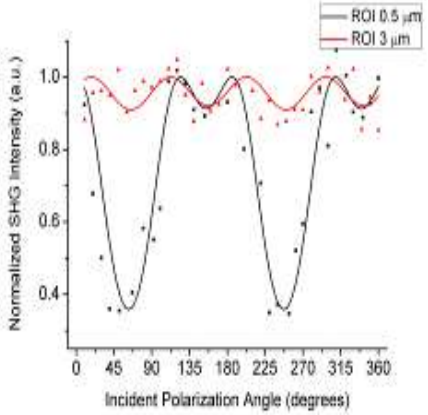


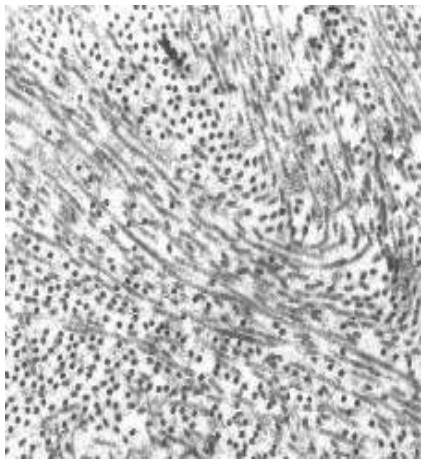


**<u>Control site</u> ⇒ a similar anisotropy was seen between small and large ROIs indicating high alignment among fibrils (intact lamellar domains as detected by TEM)**





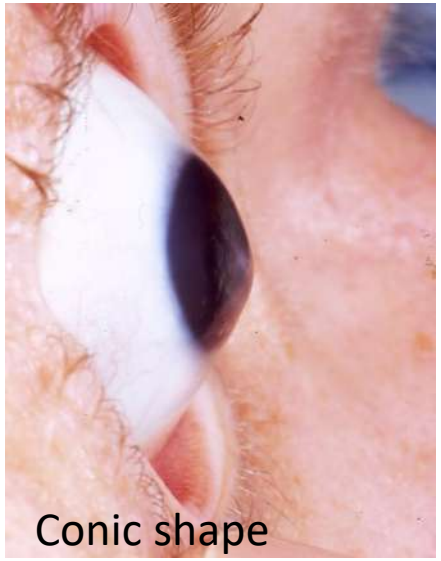
**<u>Welded site</u> ⇒ while small ROI indicated a certain preservation of fibrillar order, in larger areas normal anisotropy profile was lost (randomization of lamellar domains as detected by TEM)**

**SHG** may be applied to study several genetic, pathologic, accidental or surgical-induced **disorder states** of **corneal tissues**

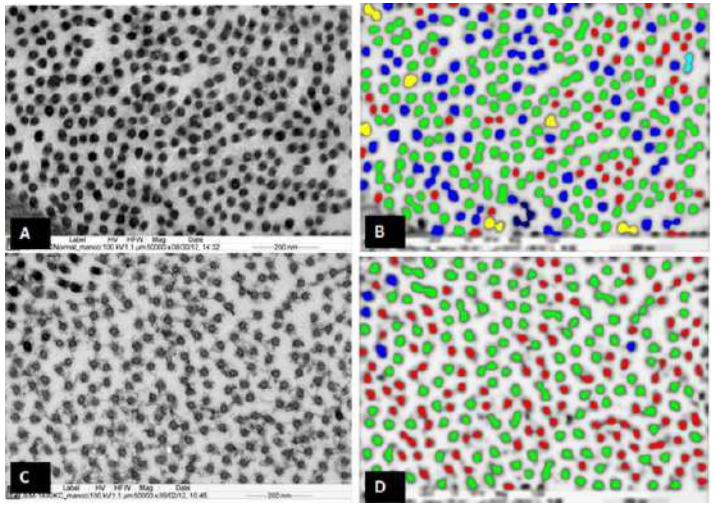
P. Matteini, et al.. *Photothermally-induced disordered patterns of corneal collagen revealed by SHG imaging*. Optics Express, 17(6), 4868-4878 (2009). 10.1364/OE.17.004868

# Pathologies: Keratoconus

- A non-inflammatory, bilateral rare disease
- Structural changes of lamellar planes: cornea thinning and conical shape (distortion in vision and excessive thinning)



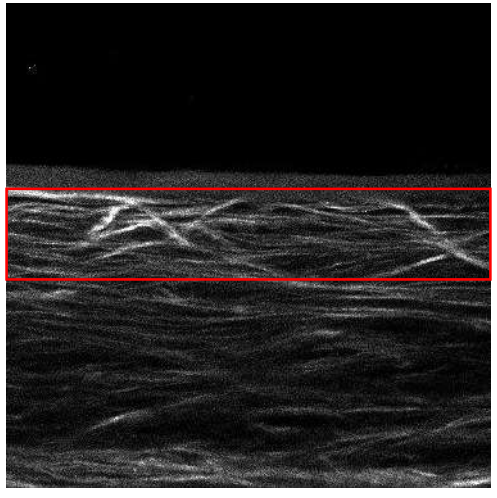
Conic shape




S. Akhtar et al. Mol Vis. 2013; 19: 1526–1537

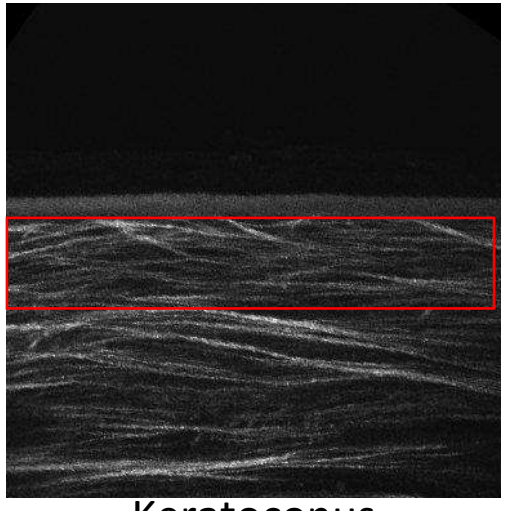
# SHG & Keratoconus

- SHG investigation Sagittal Optical Sectioning
  - Different morphology of the sutural lamellae, immediately below Bowman's membrane
  - In keratoconus, sutural lamellae are more oriented parallel to corneal surface than in healthy cornea
  - ROI of about 30 mm depth below Bowman's membrane within stroma



Healthy Cornea

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

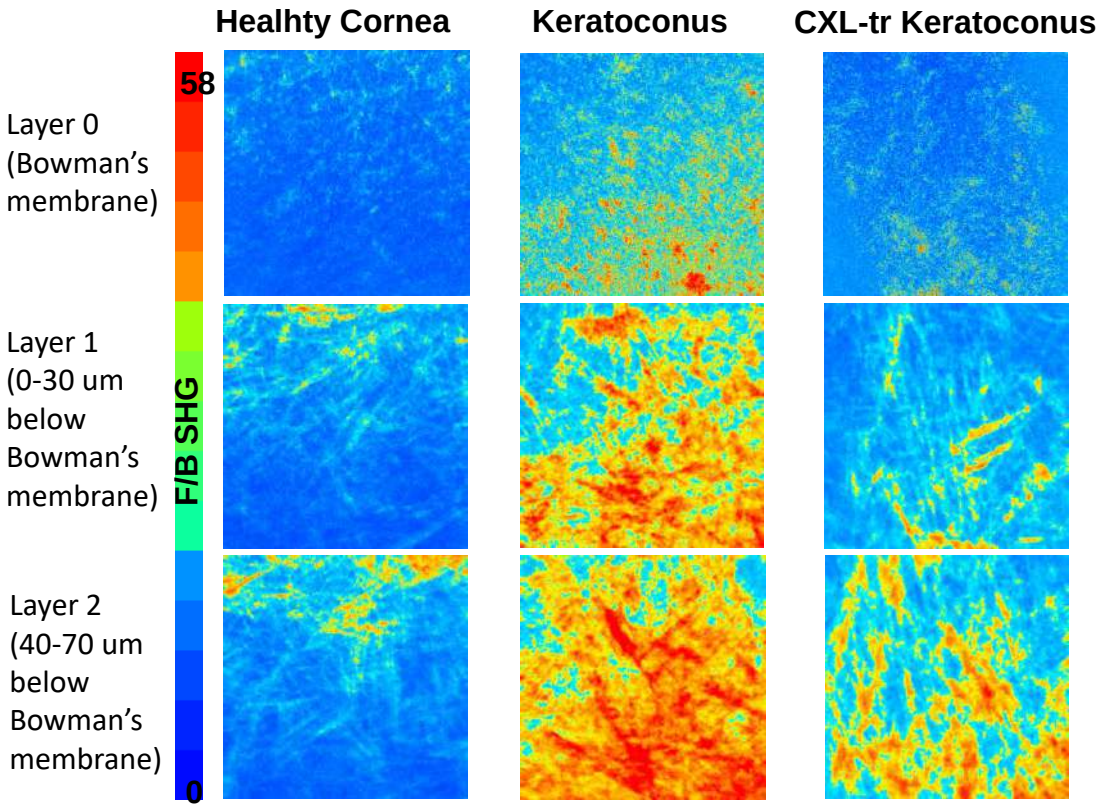


Keratoconus


*R. Mercatelli, F. Ratto, F. Rossi, F. Tatini, L. Menabuoni, A. Malandrini, R. Nicoletti, R. Pini, F. S. Pavone, R. Cicchi, J. Biophoton.* ***10****, 75-83(2017)*

# SHG & Keratoconus

- FW/BW SHG Ratio:
  - Different orientation of corneal lamellae monitored by simultaneus detection of F-SHG and B-SHG
  - F/B SHG ratio in three different layers: Bowman's membrane, 0-30 um depth, 40-70 um depth




Excitation wav.: 840 nm
Detection wav: 420 nm

Layer 0: 0 μm depth
Layer 1: 0-30 μm depth
Layer 2: 40-70 μm depth

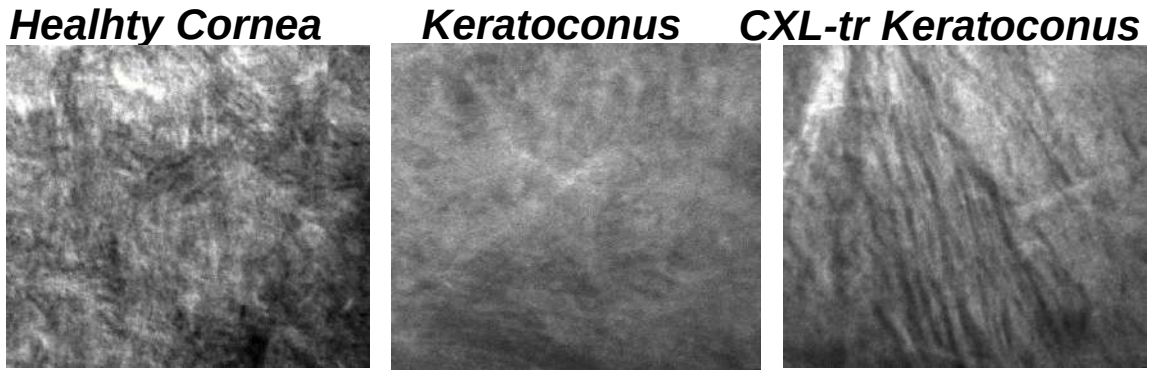
Detection type: B-SHG + F-SHG
Pixel dwell time: 20 μs
FOV: 400 μm
Sectioning: «En-face»

# SHG & Keratoconus

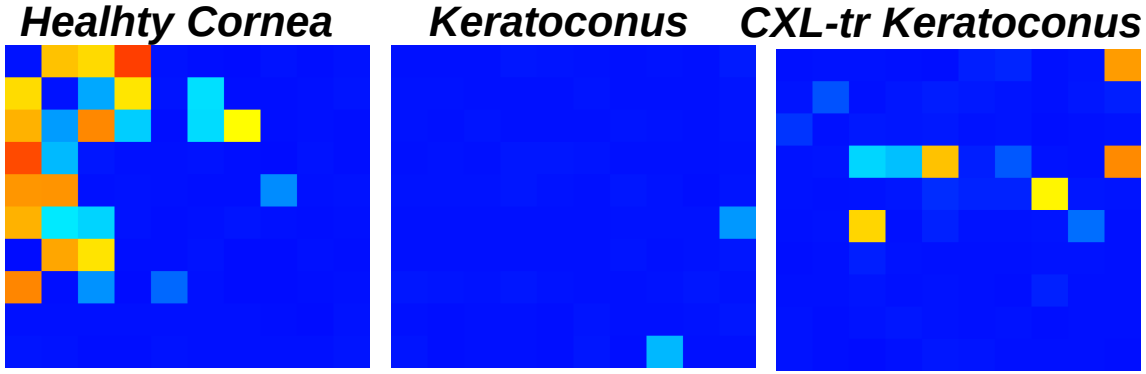
- Mapping the lamellar orientation:
  - Use of both F-SHG and B-SHG image stack
  - Evaluation of the correlation lengths – Average values within each domain - Ratio between axial and radial correlation length – Mean orientation angle wrt the Bowman's membrane

*• Sutural lamellae in Keratoconus are oriented at smaller angles wrt the Bowman's membrane than healthy cornea*

*• Orientation partially recovered after CXL-treatment*



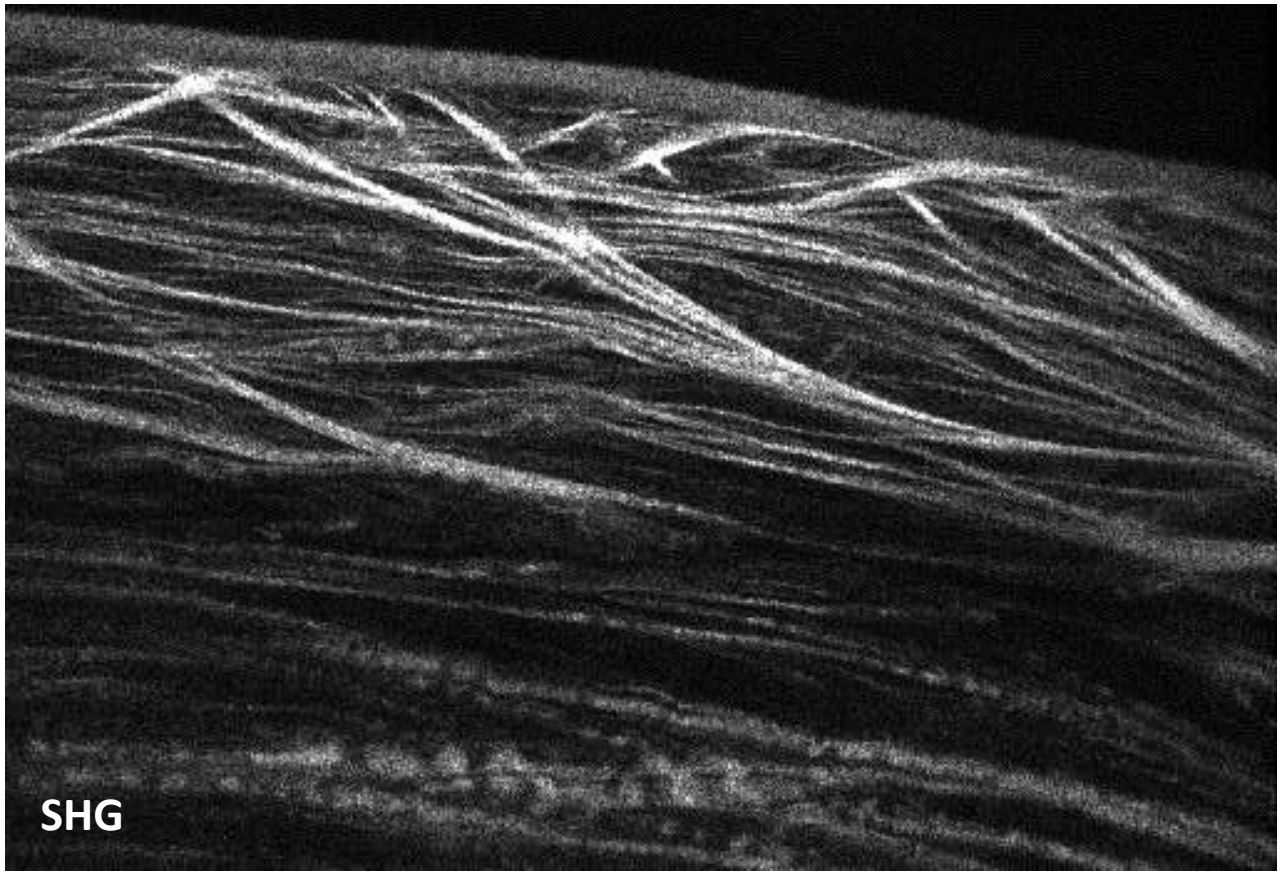

***Angular distribution of sutural lamellae***




*Excitation wav.: 840 nm*
*Detection wav: 420 nm*
*MIP: 30 μm*
*Detection type: B-SHG*
*Pixel dwell time: 20 μs*
*FOV: 150 μm*
*Sectioning: «En-face»*

# Morpho-Mechanics investigation

- Focus on *Sutural lamellae*

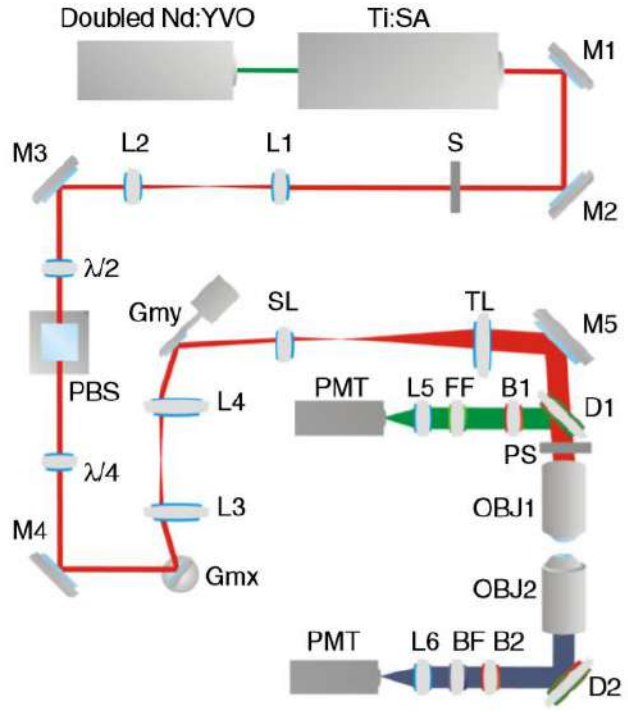


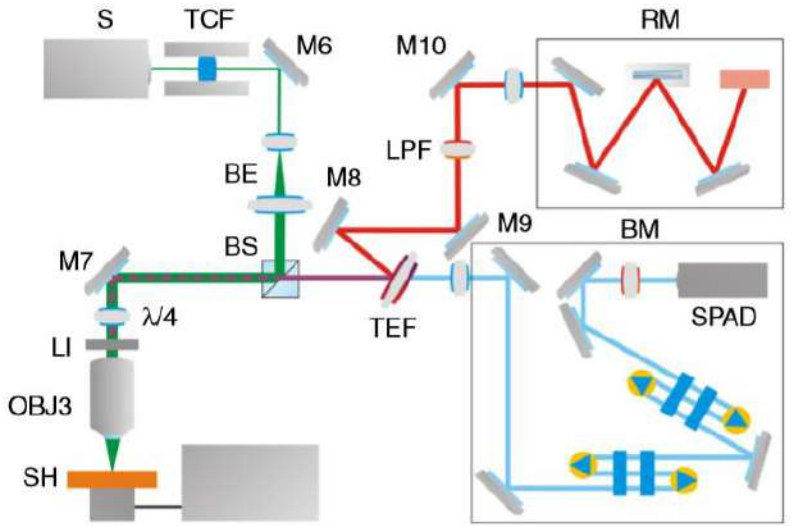
# Materials & Methods: Combined Microscopy

- SHG sketch and Brillouin/Raman setup

Excitation wav.: 840 nm
Detection wav: 420 nm
Pixel dwell time: 20 μs
Laser Power: 8 mW

Detection type: F-SHG
FOV: 200x200 $\mu m^2$
Sectioning: *En-face*
Spatial resolution: 300 nm radial
1000 nm axial




Excitation wav.: single mode 532 nm
Spatial Resolution 2X2X10 $\mu m^3$

Image Acquisition time: 65s

Laser power @sample: 10 mW

Mercatelli et al., Nature Comms Bio (2019) 2:117. https://doi.org/10.1038/s42003-019-0357-y

# Microscopia Brillouin: proprietà meccaniche

Scarcelli et al. IOVS, January 2012, Vol. 53, No. 1

*IOVS*, January 2012, Vol. 53, No. 1

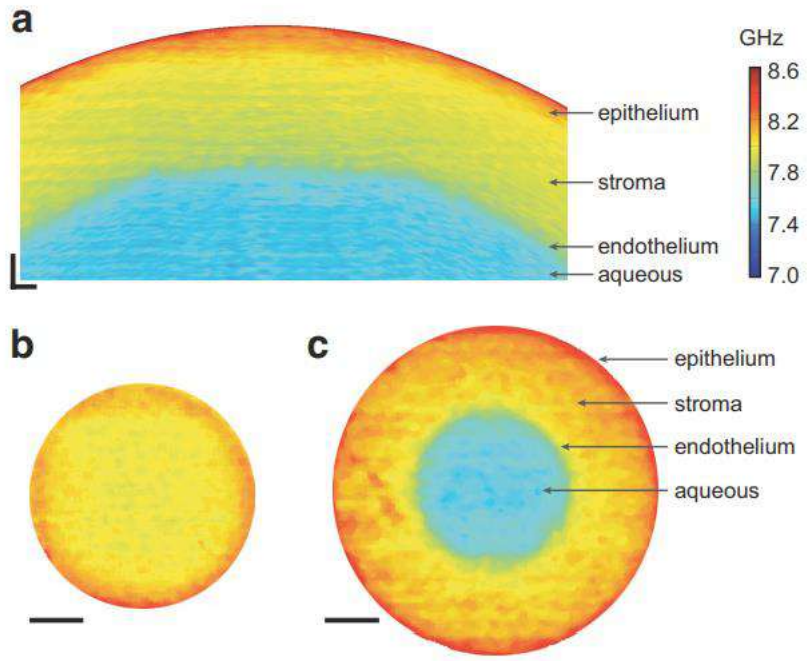



**FIGURE 2.** Brillouin imaging of the cornea. (**a**) A cross-sectional Brillouin image of bovine cornea, revealing the decreasing modulus with depth. The horizontal ($x$) and vertical ($z$) span is $5 \times 0.5$ mm. (**b**) En face Brillouin image of the cornea optically sectioned at a shallow depth. (**c**) A Brillouin image of a deeper section. Scale bars: (**a**) 200 $\mu$m; (**b**, **c**) 1 mm.

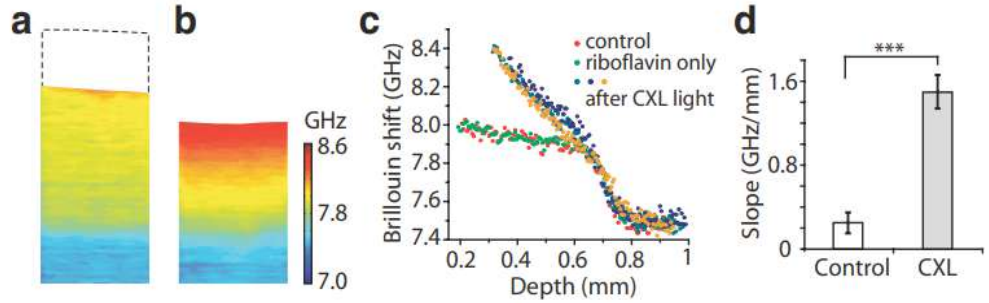
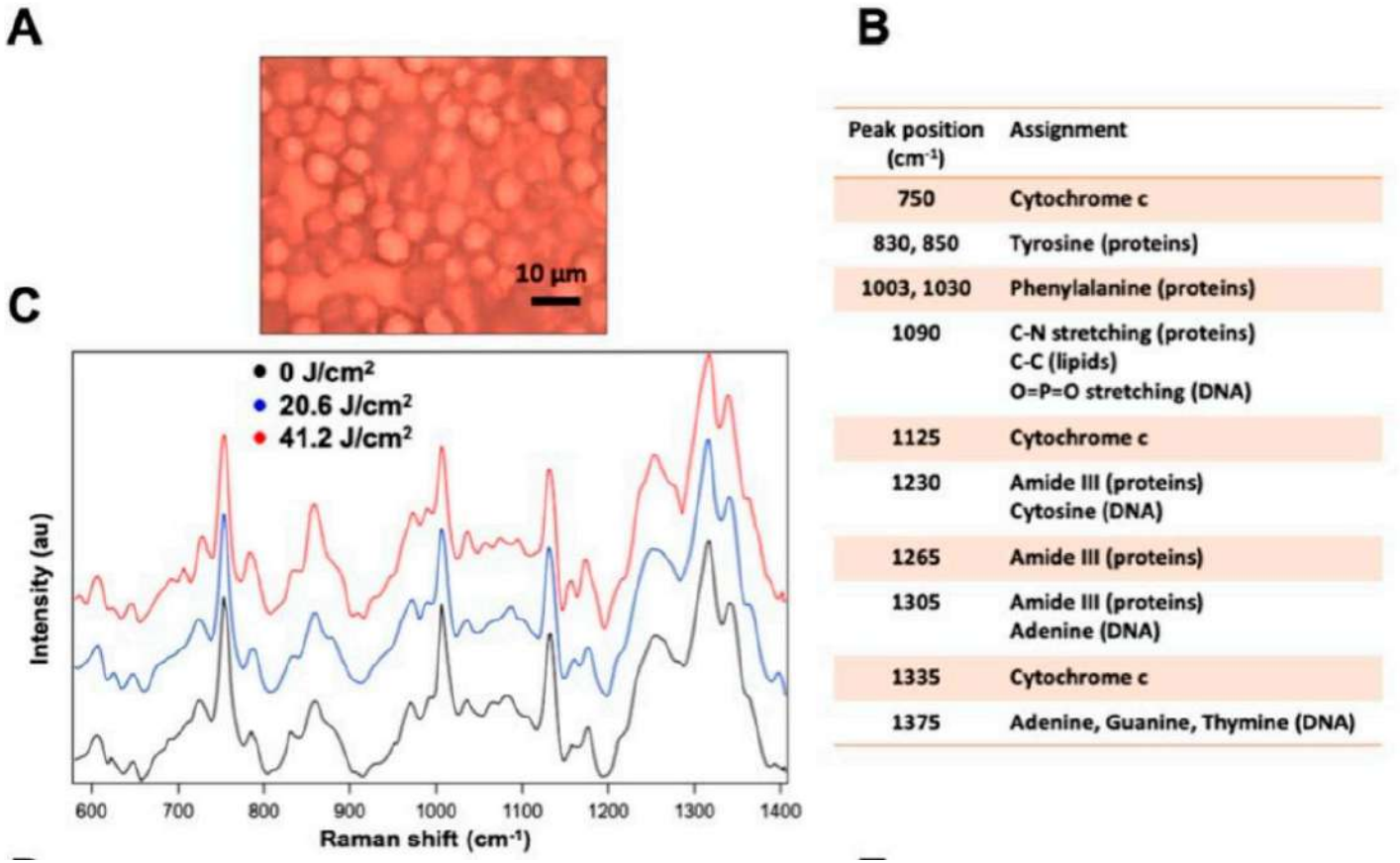



**FIGURE 5.** Brillouin measurement of the CXL procedure. (**a**) A cross-sectional ($x$-$z$) Brillouin image of untreated cornea without the epithelium. (**b**) A Brillouin image of the cornea after CXL treatment. (**c**) Brillouin depth profiles of the de-epithelialized cornea before treatment, after Riboflavin soaking, and after illumination of the treatment light (*orange*, *cyan*, and *blue circles*). (**d**) The slope of the Brillouin frequency in the stroma before and after the CXL treatment. Error bars, SD ($n = 4$). $^{***}P < 0.001$.

## Brillouin Light Scattering

Named after the French physicist Léon Brillouin, Brillouin light scattering (BLS) is a physical phenomenon that was first reported (in 1922) to occur when light interacts with material and undergoes scattering. All solid materials are made up of atoms and structures that are constantly vibrating but remain in fixed positions in relation to each other. Waves of vibration constantly run through solids, especially crystalline solids. These elastic vibrational waves can cause light to scatter in different ways when it

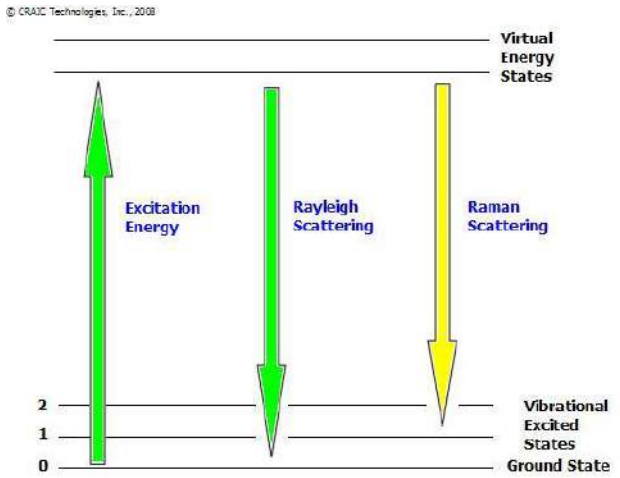
# Microscopia Raman: proprietà chimiche




B

| Peak position ($cm^{-1}$) | Assignment |
|---|---|
| 750 | Cytochrome c |
| 830, 850 | Tyrosine (proteins) |
| 1003, 1030 | Phenylalanine (proteins) |
| 1090 | C-N stretching (proteins)<br>C-C (lipids)<br>O=P=O stretching (DNA) |
| 1125 | Cytochrome c |
| 1230 | Amide III (proteins)<br>Cytosine (DNA) |
| 1265 | Amide III (proteins) |
| 1305 | Amide III (proteins)<br>Adenine (DNA) |
| 1335 | Cytochrome c |
| 1375 | Adenine, Guanine, Thymine (DNA) |

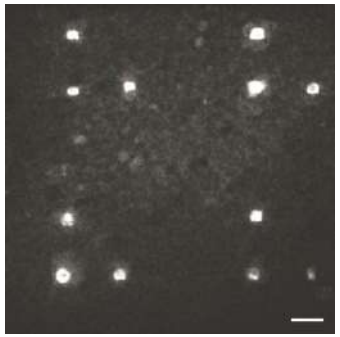
- La luce (laser) interagisce con le molecole del campione
- Restituisce l'*impronta digitale* del composto analizzato



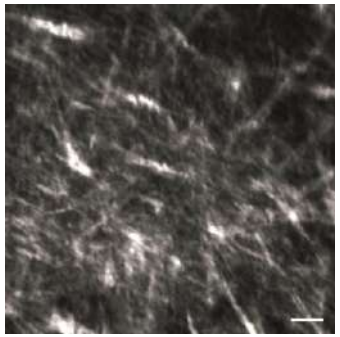

F. Rossi et al. *Photobiomodulation of Human Fibroblasts and Keratinocytes with Blue Light: Implications in Wound Healing*. Biomedicines. 2021; 9(1):41. https://doi.org/10.3390/biomedicines9010041

# Materials & Methods

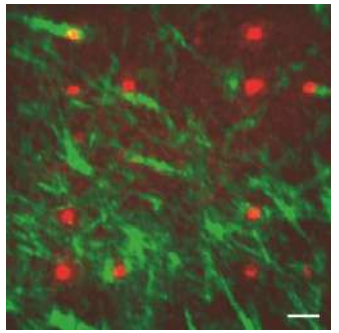
- 5 human healthy corneas (Veneto Eye Bank)
- Laser ablation spots (30 mW, 10x10 $\mu m^2$) as a fiducial marker



Corneal epithelium



Below epithelium

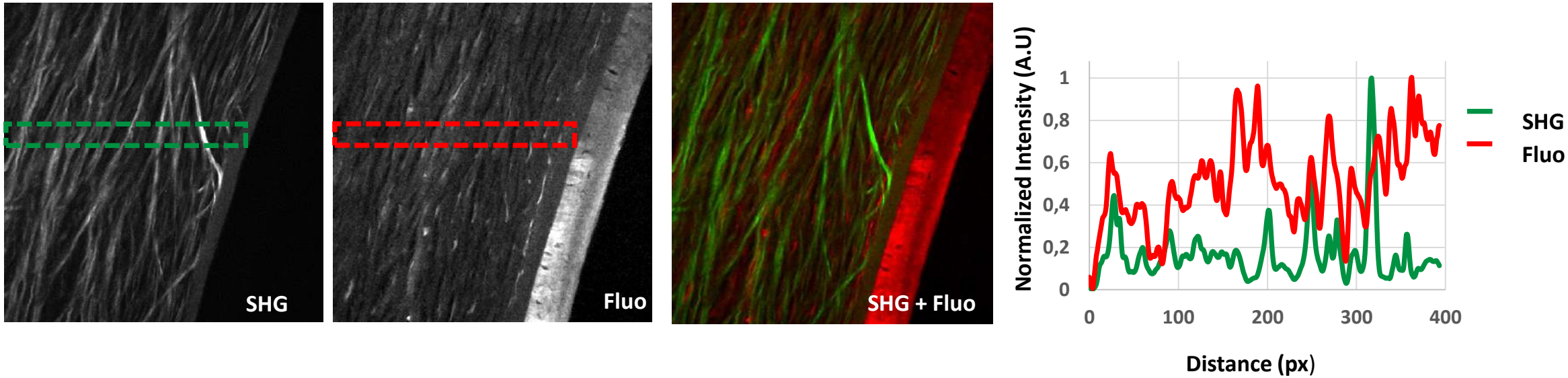


Combined

R. Mercatelli et al. *Morpho-mechanics of human collagen superstructures revealed by all-optical correlative micro-spectroscopies*. Nature Communications Biology (2019) 2:117, https://doi.org/10.1038/s42003-019-0357-y.

# Morpho-Mechanics investigation

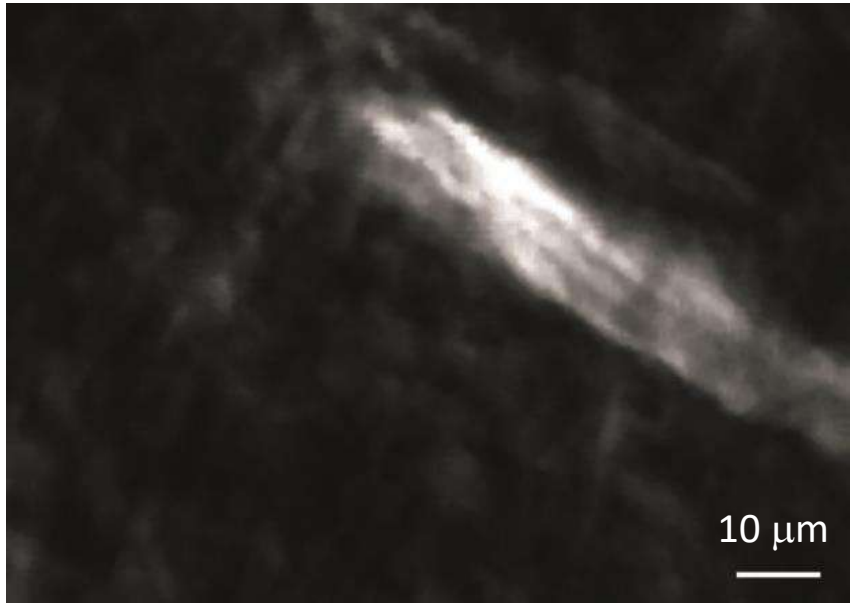
- Sutural lamellae: first evidences in healthy human corneas



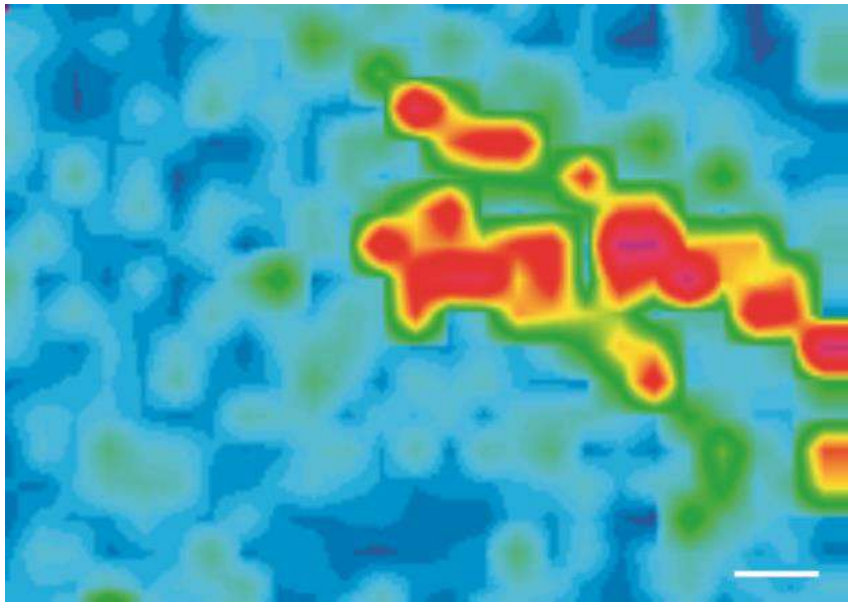
# Results- SHG

- En face optical sectioning
- Starting 10 μm below Bowman membrane

SHG image



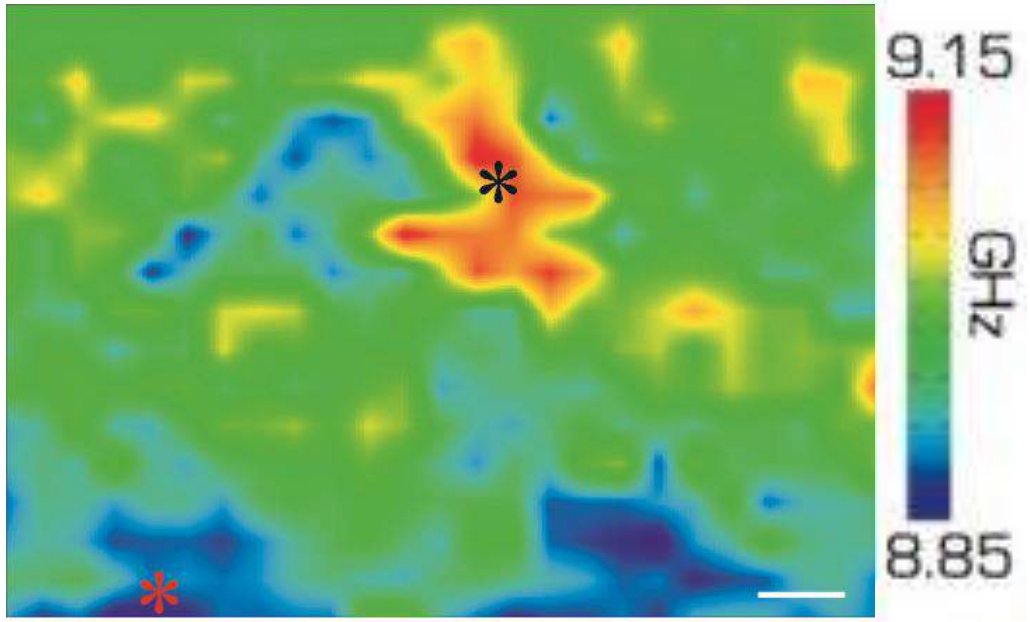

Inclination map

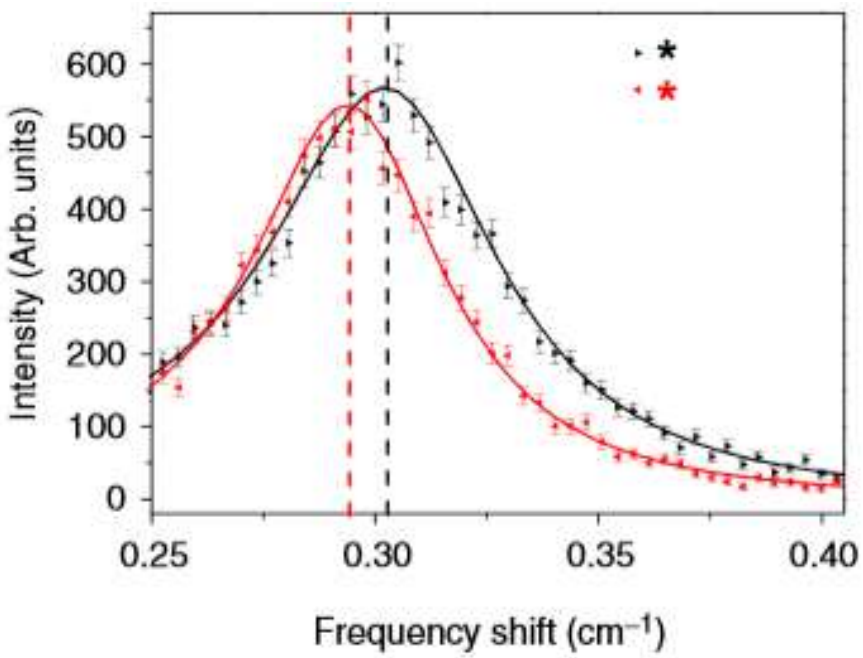


# Results- Brillouin

- Frequency and linewidth shift in Brillouin analysis

Brillouin frequency shift

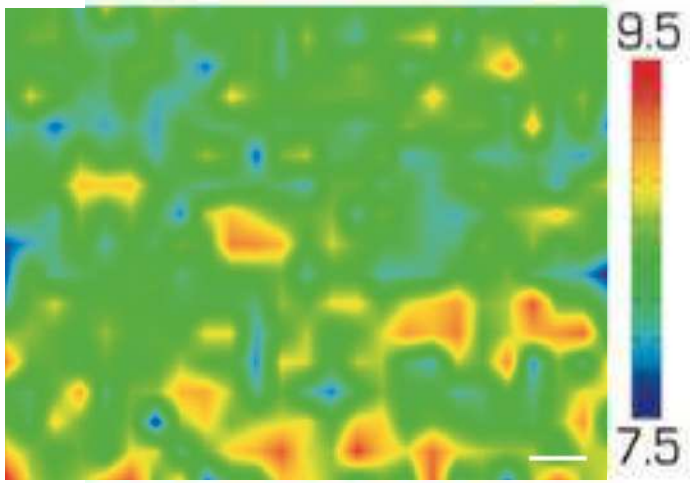


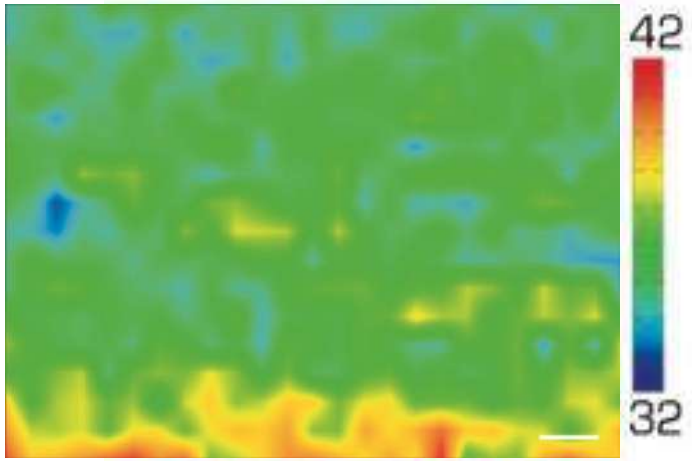



# Results- Raman

- Slight inhomogeneity
- No correlation with mechanical modulation

Intensity ratio between the CH stretching region (2800–3100cm−1) and the amide I region (1600–1750 cm−1). Mean error 6%

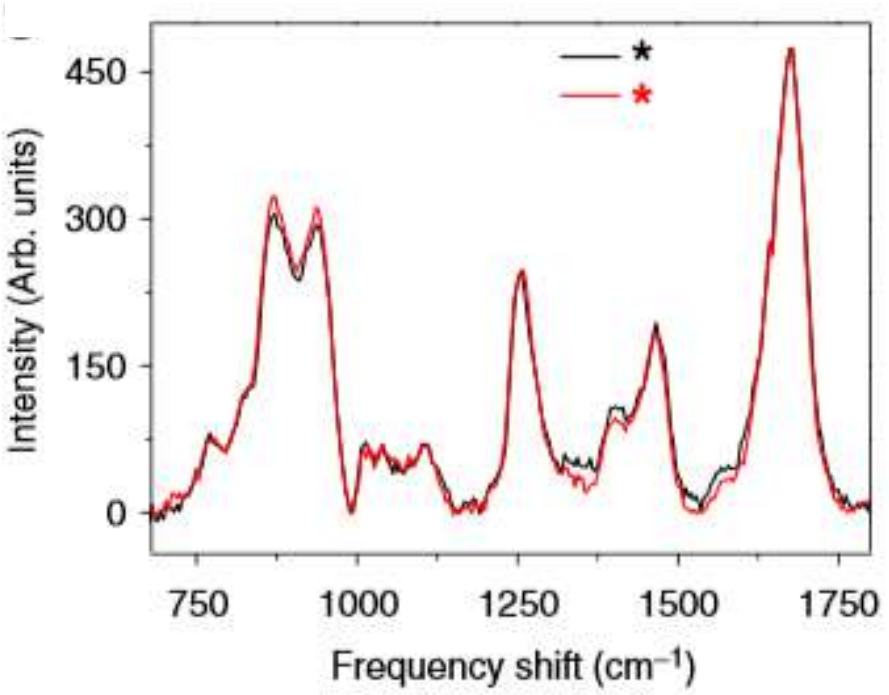


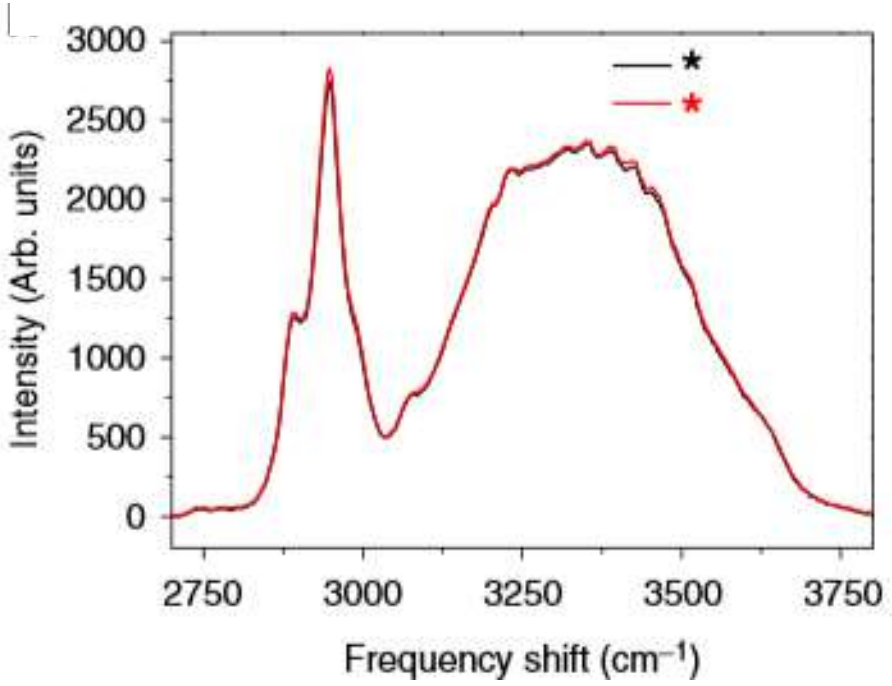




Intensity ratio between the OH stretching region (3100–3800cm−1) and the amide I region (1600–1750 cm−1). Mean error 6%

# Results- Raman

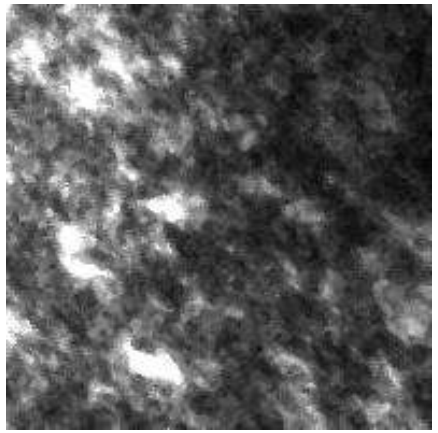
- No significant frequency shift



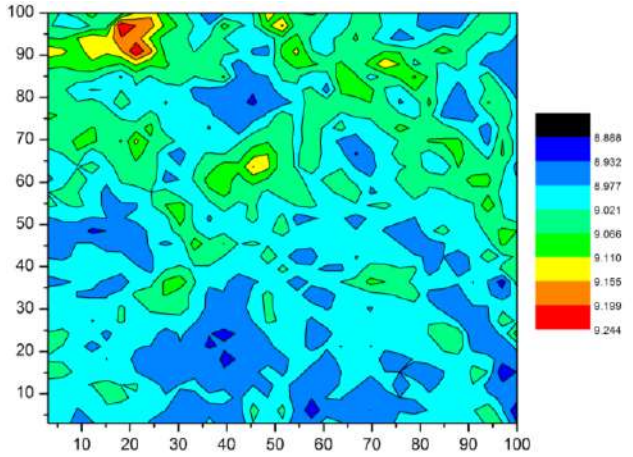



# Results- Brillouin

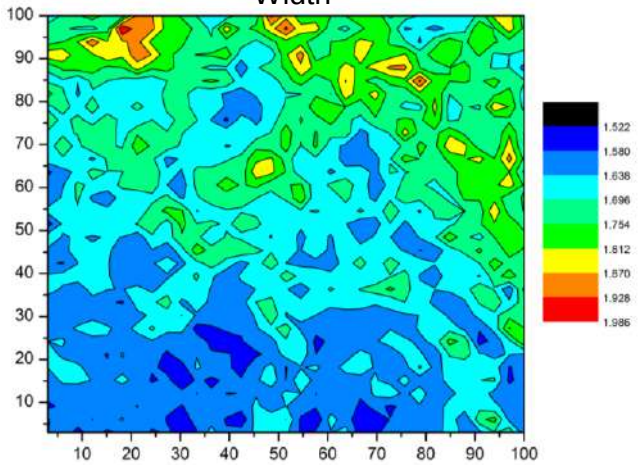
z 48 µm



Frequency

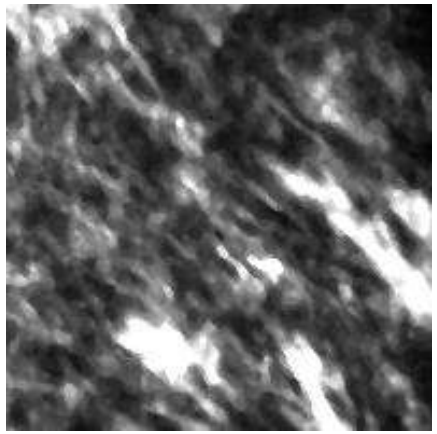



Width

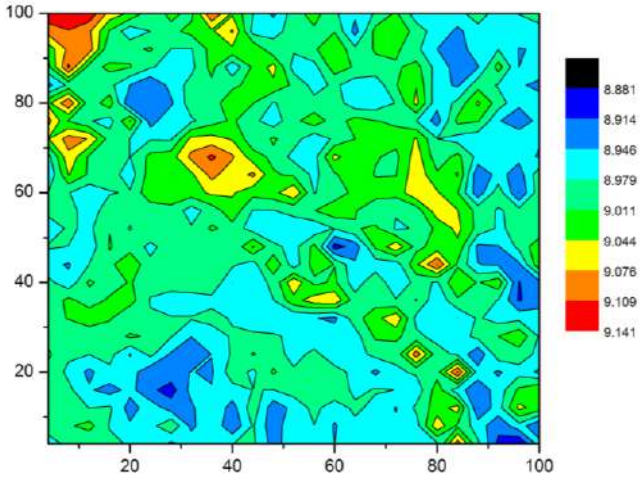



mapping: 33 x 33 (step 3 µm)

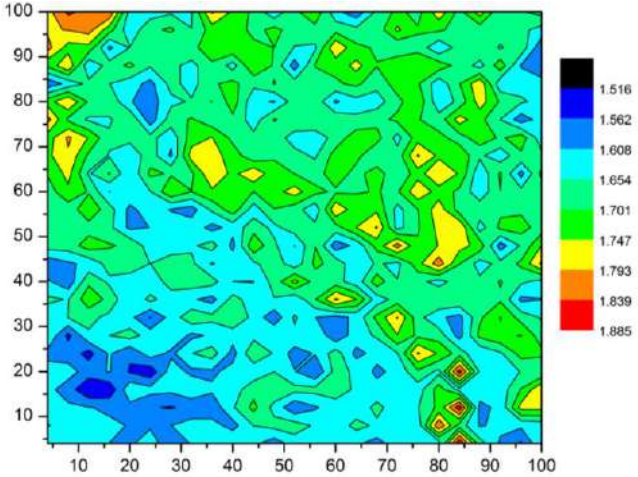
z 58 µm



Frequency




Width

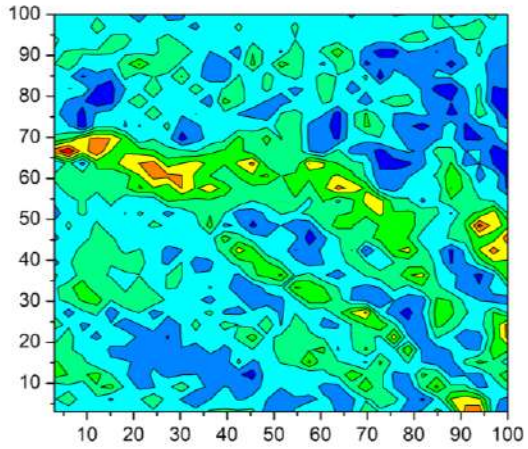



mapping: 25 x 25 (step 4 µm)

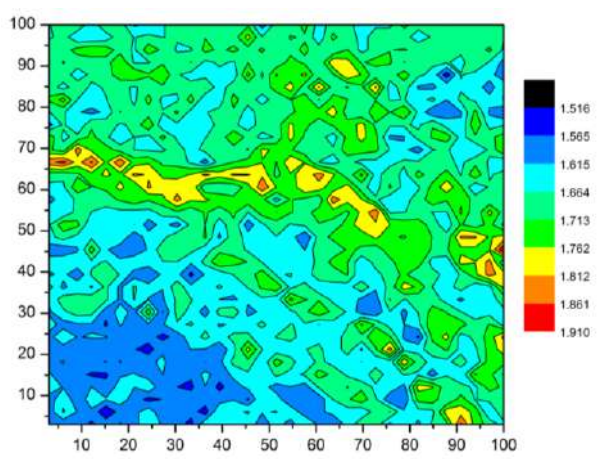
# Results- Brillouin

z 69 µm

Frequency

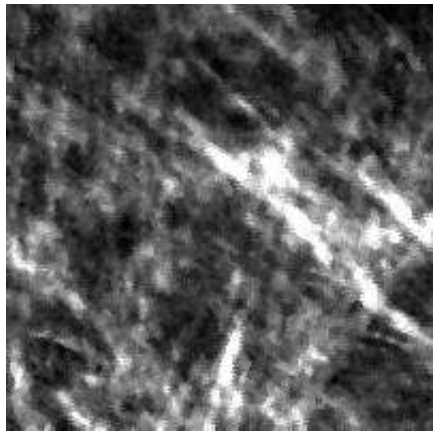



Width

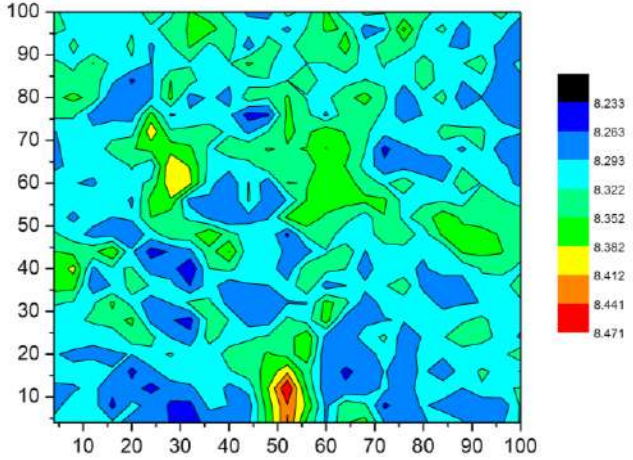



mapping: 33 x 33 (step 3 µm)

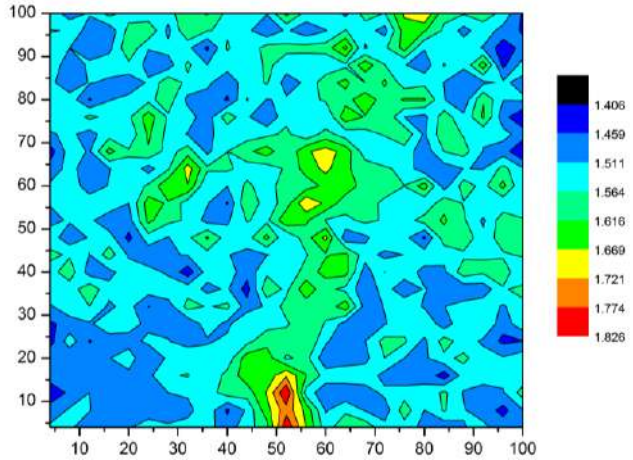
z 88 µm



Frequency




Width




mapping: 25 x 25 (step 4 µm)

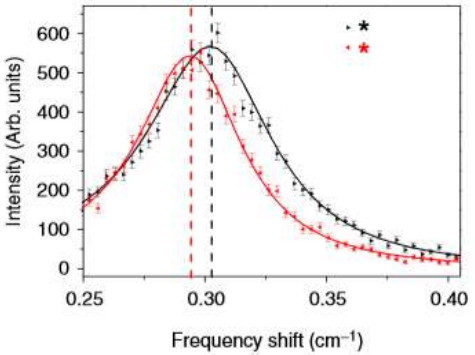
# Results- Brillouin

- Frequency and linewidth shift in Brillouin analysis:
  - The real part of the longitudinal modulus is

$$M' = \left(\frac{\lambda_i}{4\pi}\right)^2 \frac{\rho}{n^2} \omega_b^2$$

  - The imaginary part of the longitudinal modulus is

$$M'' = \omega_b \Gamma_b \frac{\rho}{q^2}$$




Modulation <0.3%
In human cornea

$\lambda_i$ = incident light wav.

$\Gamma_b$ = HWHM Brillouin curve

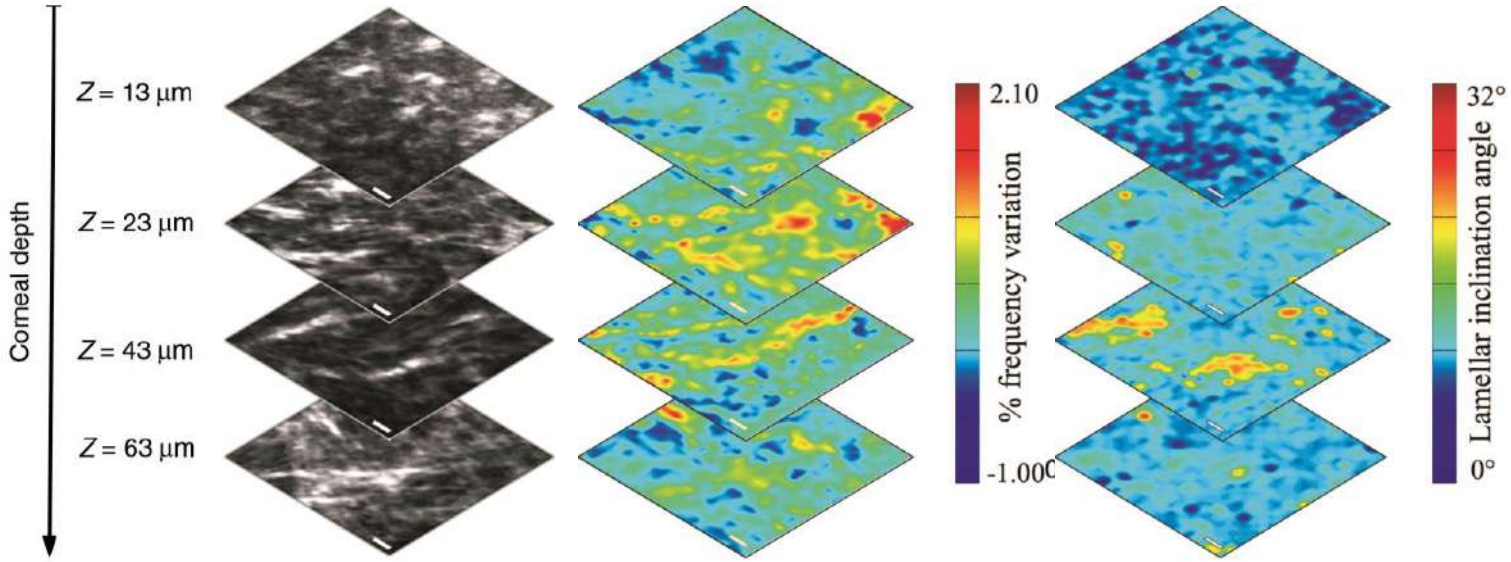
$q = 2nk_i$ = exchanged momentum

➢ Freq shift: increase in the longitudinal elastic modulus

➢ Linewidth shift: increase in viscosity

# In-depth analysis of lamellar collagen

- Poor correlation between elastic eterogeneity and lamellar inclination




R. Mercatelli, S. Mattana, L. Capozzoli, F. Ratto, F. Rossi, R. Pini, D. Fioretto, F. S. Pavone, S. Caponi and R. Cicchi, Communications Biology 2:117 (2019)

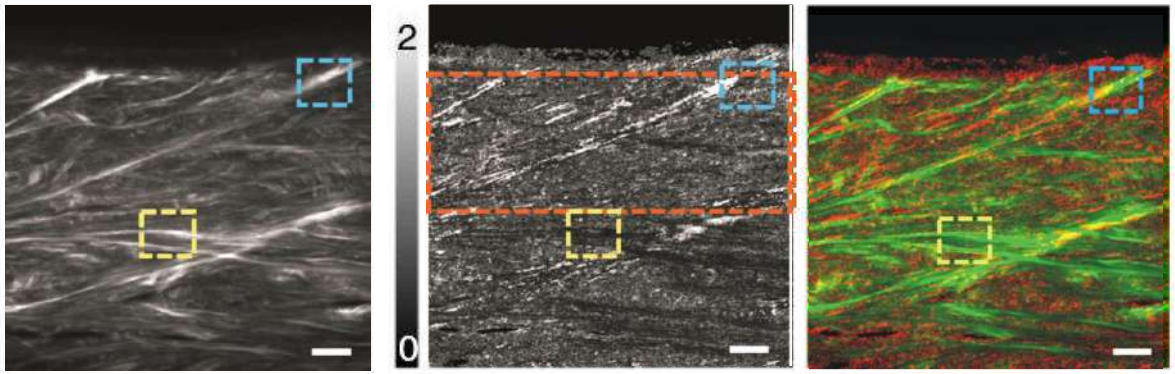
# In-depth analysis of lamellar collagen

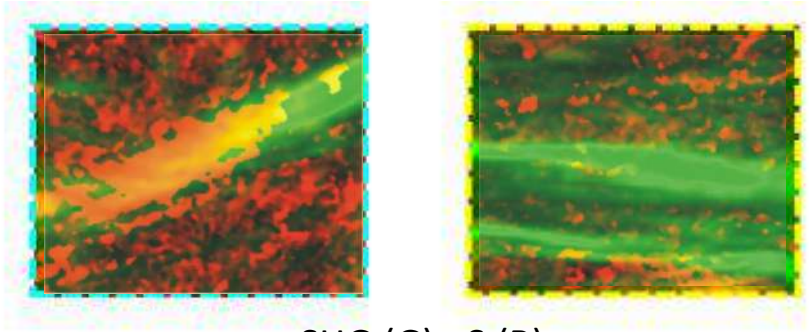
- P-SHG Symmetry analysis (trigonal simmetry)

SHG

Symmetry parameter S

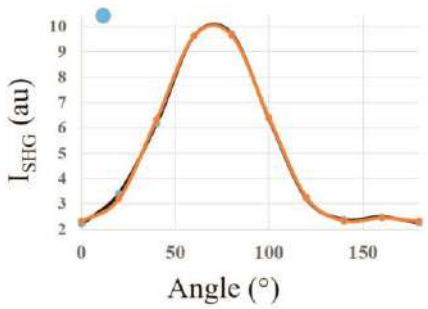
SHG (G)+ S (R)



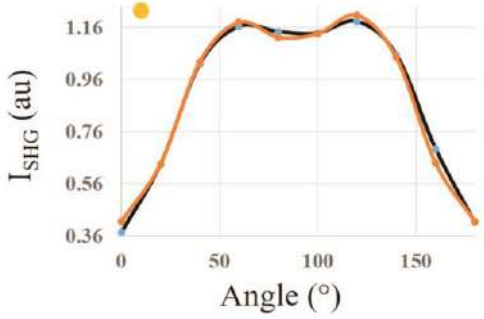



SHG (G)+ S (R)

Trigonal Symmetry



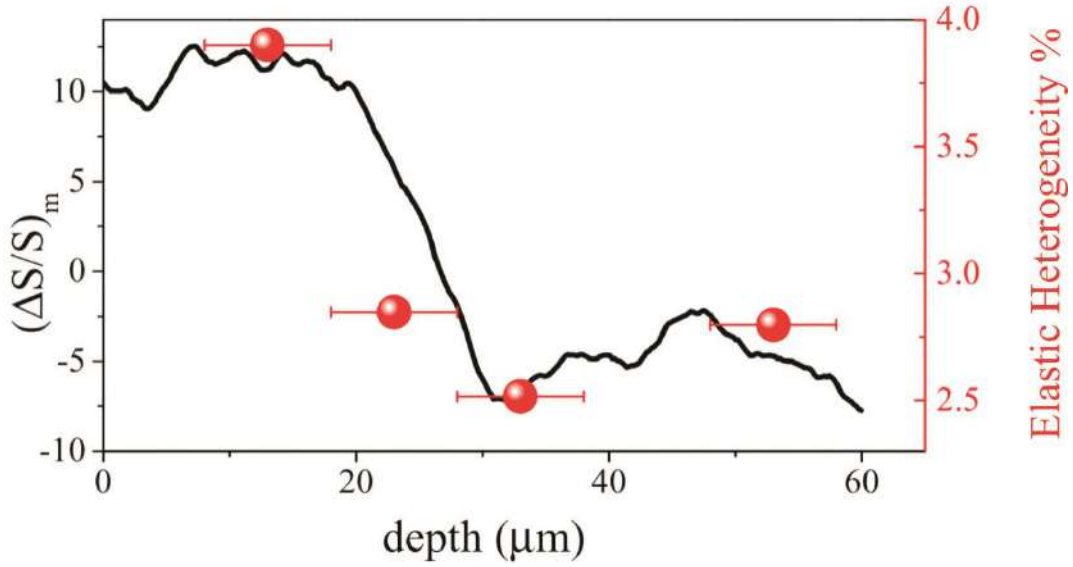

Cylindrical Symmetry




R. Mercatelli, S. Mattana, L. Capozzoli, F. Ratto, F. Rossi, R. Pini, D. Fioretto, F. S. Pavone, S. Caponi and R. Cicchi, Communications Biology 2:117 (2019)

# In-depth analysis of lamellar collagen

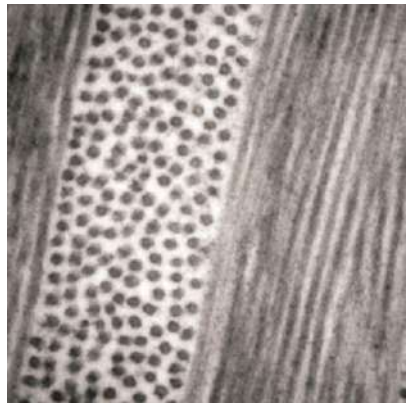
- P-SHG Symmetry analysis (trigonal simmetry):
  - Good match with elastic heterogeneity and TEM analysis




Sutural lamellae

Helicoidal distribution of collagen fibrils
Trigonal symmetry

Deep stroma



Adjacent collagen fibrils oriented in the same direction and staggered side-by-side
Cylindrical symmetry

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# Le conclusioni di questo studio:

- The combined use of SHG-Brillouin-Raman microscopy is feasible in corneal tissue
- It provides info on tissue morphology, mechanics and chemistry with an all-optical non-contact approach
- Sutural lamellae are characterized by:
  - Different supramolecular symmetry (SHG)
  - Different stiffness (Brillouin)
  - Same biochemistry (Raman)
- The collagen in sutural lamellae has a different supramolecular organization

# Conclusioni

- Le «nuove» microscopie consentono analisi della cornea in vivo su paziente
- La microscopia non-lineare e la combinazione di più approcci di nuove microscopie permetteranno diagnostica «puntuale» in vivo
- Possibile applicazione: diagnosi precoce di alcune patologie (e.g. keratocono)

# Approfondimenti

- Istologie: https://histologyguide.com/slideview/MHS-227a-eye/20-slide-1.html?x=10499&y=35551&z=3.1&page=1
- I Microscopi: Microscopy_for_Dummies (wiley-vch.de)
- Microscopia Non-lineare: https://youtu.be/4mESMygp5EU
- Microscopia Brillouin: Brillouin Microscopy (photometrics.com)
- Microscopia Raman: Confocal Raman Microscopy (The Basics) | JASCO (jascoinc.com)
- Corneal Imaging: An Introduction (uiowa.edu)

In generale i siti dei produttori (Olympus, Nikon, Leica ecc. hanno la sezione «education»)

# Microscopia *classica* - Istologia