



POLARIMETRIA IN OFTALMOLOGIA

Eugenio LIPARI

XVI Congresso Società Oftalmologica Calabrese

CATANZARO – 07 Ottobre 2016



Fibrils Geometry and Intrastromal Arrangement

X-rays analysis

[Craig Boote, Christina S. Kamma-Longer, et al.](#), Quantification of Collagen Organization in the Peripheral Human Cornea at Micron-Scale Resolution- [Biophysical Journal](#), Vol 101, July 2011, 33-34

[McCally RL, Farrell RA](#) - Small-angle light scattering and birefringence properties of chick cornea - [J Refract Surg](#). 1999 Nov-Dec;15(6):706-10, [Meek KM, Boote C](#). –The use of X-ray scattering techniques to quantify the orientation and distribution of collagen in the corneal stroma - [Prog Retin Eye Res](#), 2009 Sep; 28(5) :369-92

[Abahussin M., Hayes S, Knox Cartwright NE, Kamma-longer CS, Khan Y, Marshall J, Meek KM](#) – 3D collagen orientation study of the human cornea using X-ray diffraction and femtosecond laser technology – [Invest Ophthalmol Vis Sci](#), 2009 Nov; 50(11): 5159-64

[Boote C, Dennis S, Newton RH, Puri H, Meek KM](#) - Collagen fibrils appear more closely packed in the prepupillary cornea: optical and biomechanical implications - [Invest Ophthalmol Vis Sci](#). 2003 Jul;44(7):2941-8

[Boote C, Christina S. Kamma-Longer, Sally Hayes, Jonathan Harris, Manfred Burghammer, Jennifer Hiller, Nicholas J. Terril and Keith M. Meek](#) – Quantification of Collagen Organization in the Peripheral Human Cornea at Micron-Scale Resolution – [Biophysical Journal](#), Vol.11 July 2011 (33-42)

[Hossein Aghamohammadzadeh, Richard H. Newton, and Keith M. Meek*](#) - X-Ray Scattering Used to Map the Preferred Collagen Orientation in the Human Cornea and Limbus - [Structure](#) 2004 Feb;12(2):249-56

...

SHG (Second Harmonic Generation)

[Naoyuki Morishige, Yuki Takagi, Tai-ichiro Chikama, Atsushi Takahara and Teruo Nishida](#), Three-Dimensional Analysis of Collagen Lamellae in the Anterior Stroma of Human Cornea Visualized by Second Harmonic Generation Imaging Microscopy- [IOVS](#), February 2011, vol 52, No 2

[Nicolas Olivier, Florent Aptel, Karsten Plamann, Marie-Claire Schanne-Klein and Emmanuel Beaupaire](#), Harmonic microscopy of isotropic and anisotropic microstructure of the human cornea- [Optic Express](#) 5028 – March 2010/vol.18, No.5

...

Combined Techniques (SHG, P-SHG, THG, X-rays)

[Latour G, Gusachenko I, Kowalczyk L, Lamarre I, Schanne-Klein MC](#), In vivo structural imaging of the cornea by polarization-resolved second harmonic microscopy - [Biomed Opt Express](#) 2012 Jan 1;3(1): 1-15

[Petsche SJ, Pinsky PM](#) - The role of 3-D collagen organization in stromal elasticity: a model based on X-ray diffraction data and second harmonic-generated images - [Biomech Model Mechanobiol](#). 2013 Jan 4

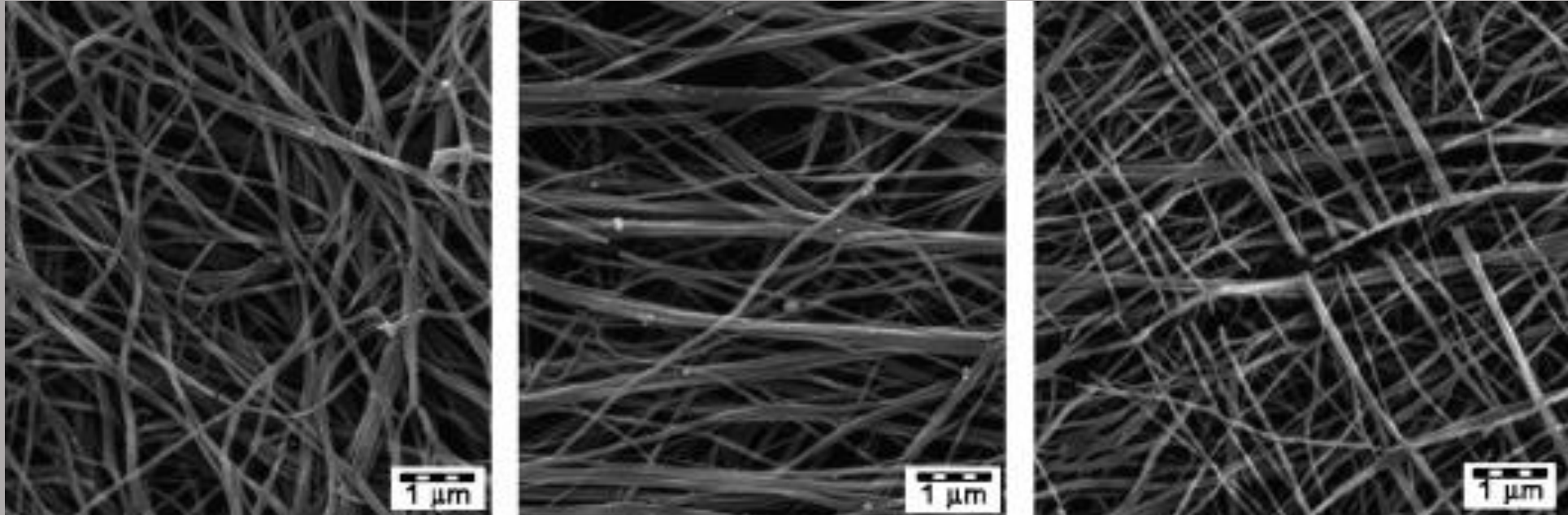
...



Stroma Structure

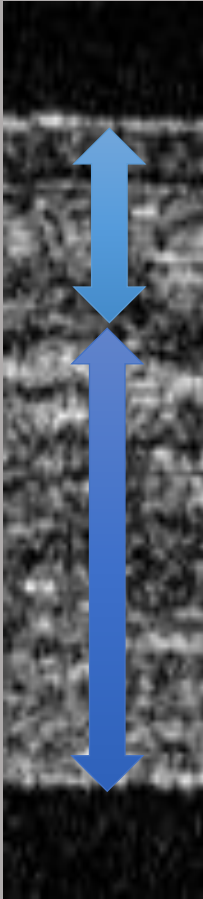
Collagen Fibrils:

- 20-30 nm avg. diameter
- organized in lamellae, average thickness of 10 μm
- $\sim$ 50 layers through the full cornea thickness





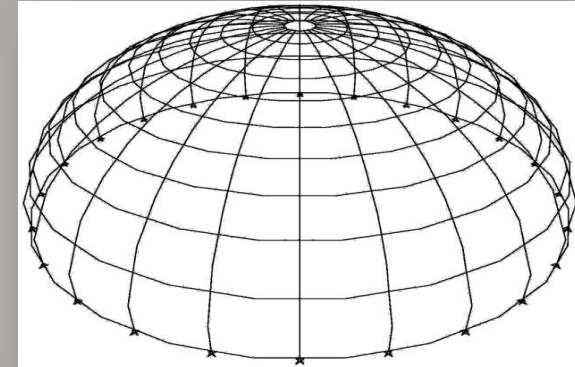
Fibrils Geometry and Intrastromal Arrangement



First third of cornea (anterior stroma):

“ISOTROPIC” Meridian Distribution

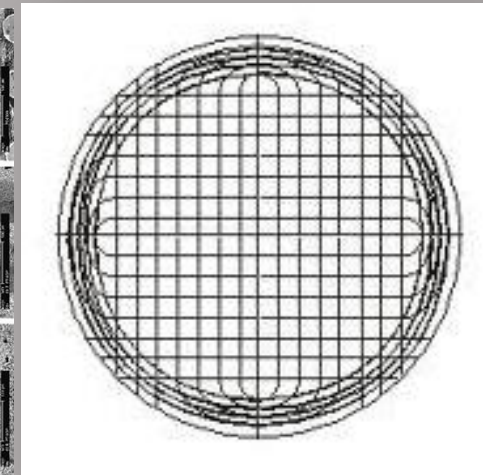
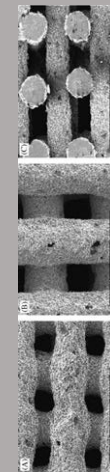
- Antero-posterior lamellar interconnections
- Responsible for cornea robustness



Two-thirds of cornea (posterior stroma):

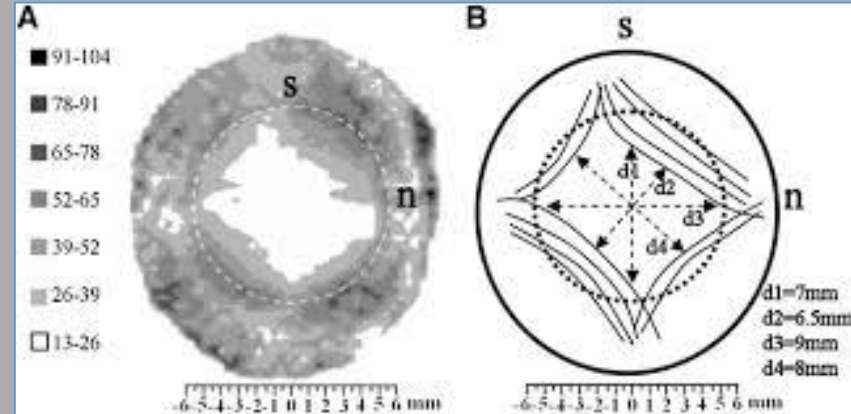
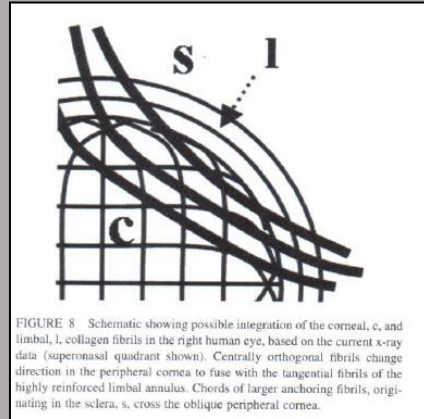
“ANISOTROPIC” (Orthogonal) Distribution

- Superior-inferior and nasal-temporal lamellar interconnections

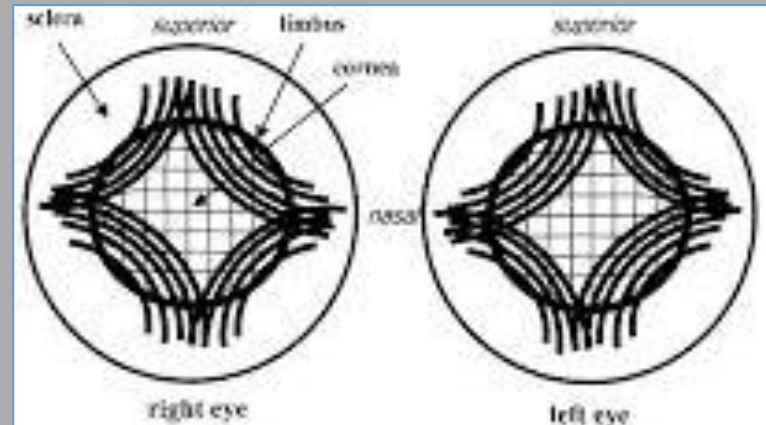




Peripheral Collagen Fibrils Arrangement



[Craig Boote, Christina S. Kamma-Longer, et al.](#), Quantification of Collagen Organization in the Peripheral Human Cornea at Micron-Scale Resolution- [Biophysical Journal](#), Vol 101, July 2011, 33-34



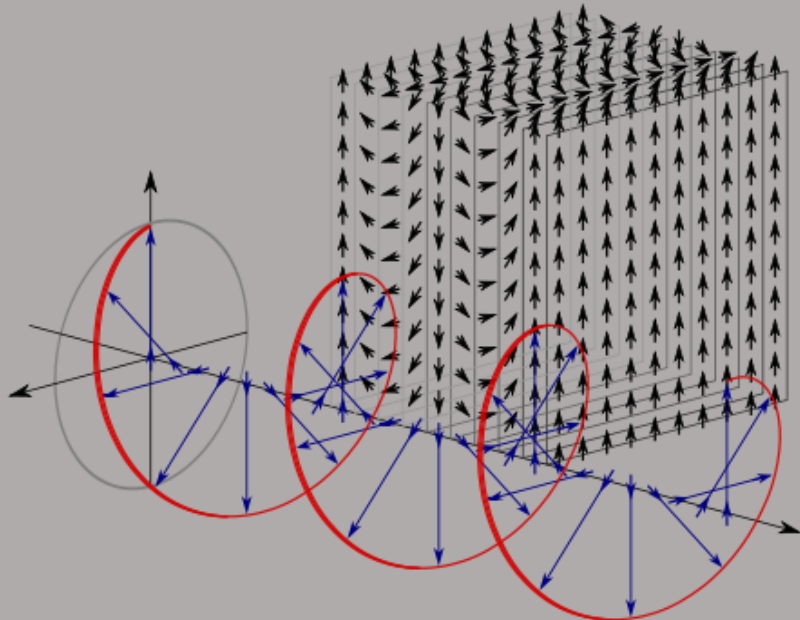
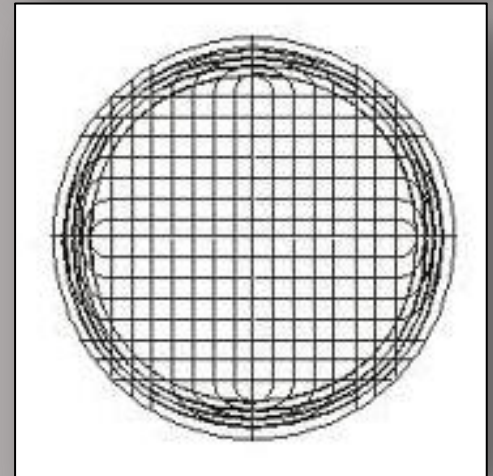
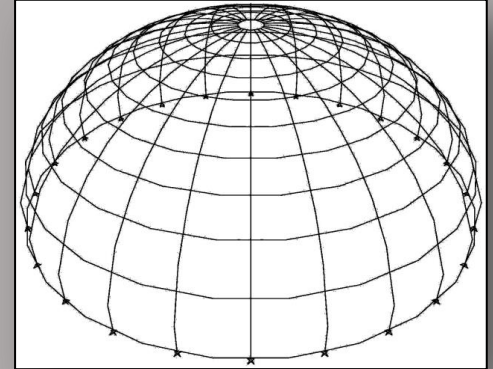
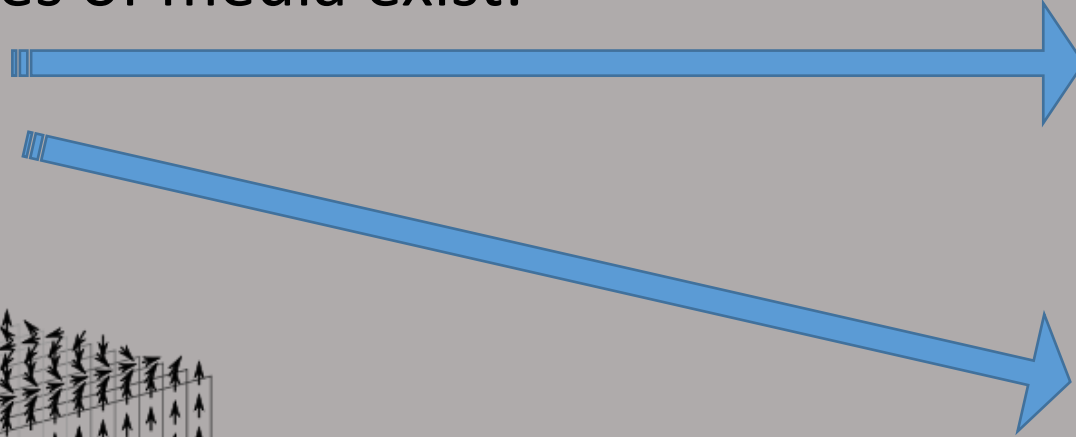
[Meek K.M.](#), The Cornea and Sclera – Collagen, Structure and Mechanics, Springer, Cap 13, 2008, 359-396



Light transmission in transparent medium

Two main categories of media exist:

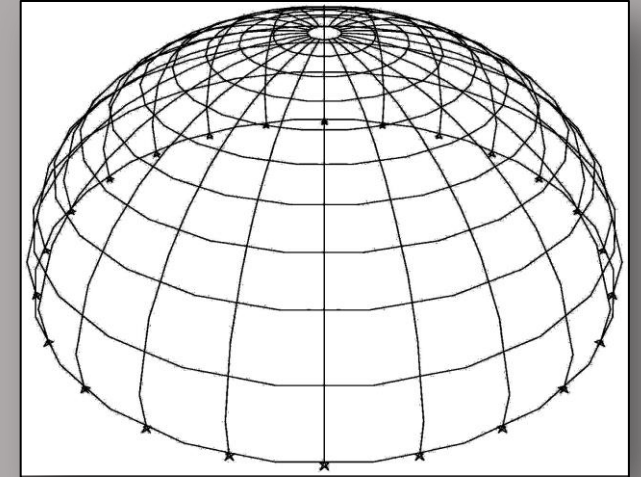
- **ISOTROPIC**
- **ANISOTROPIC**



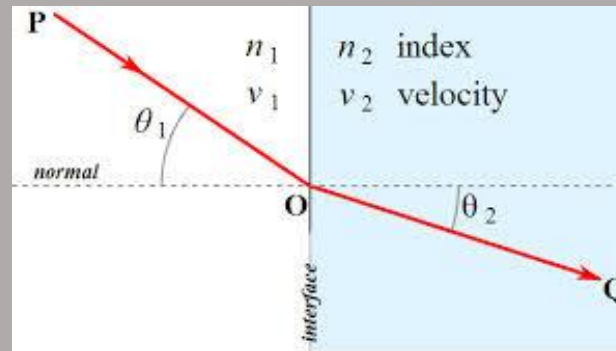


Isotropic Materials (gases, liquids, glasses ...):

- Light travels with equal velocity in all directions
- Constant direction-independent refractive index



Isotropic materials transmit light in any polarized state without alteration !!!

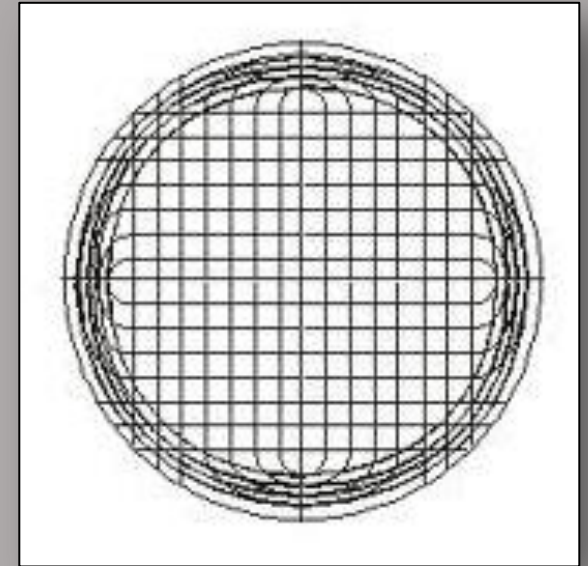
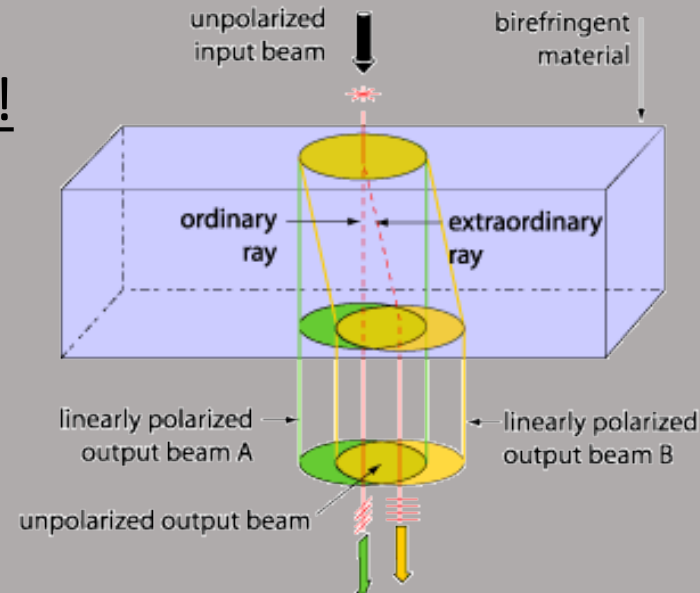
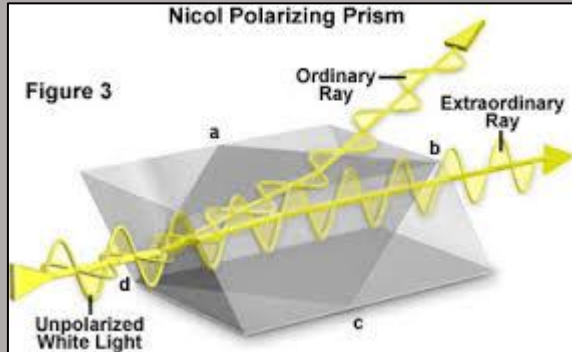




Anisotropic Materials

- Light velocity is direction-dependent according with the anisotropy of the molecular binding forces or structural features

BIREFRINGENCE OCCURS !

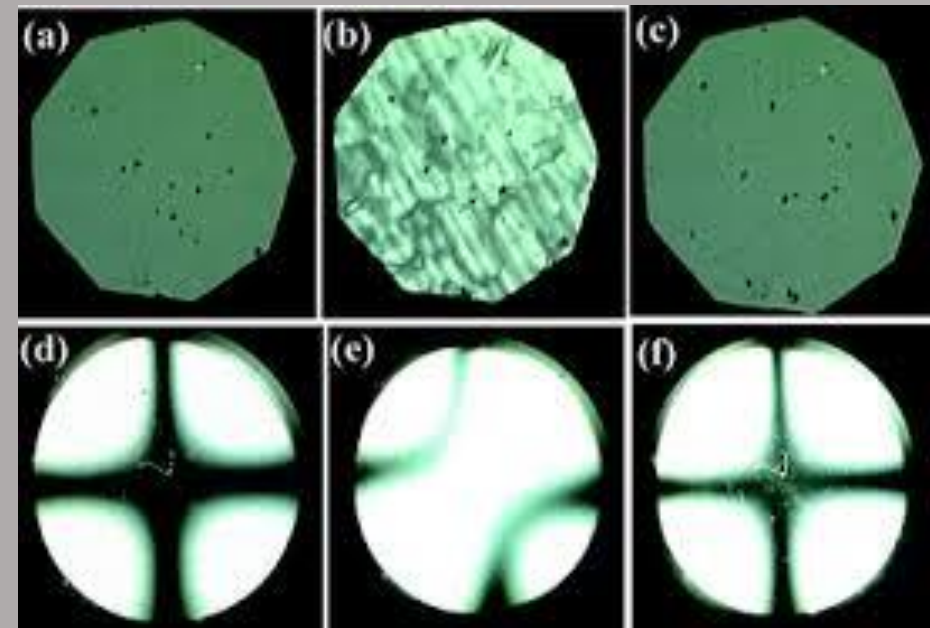
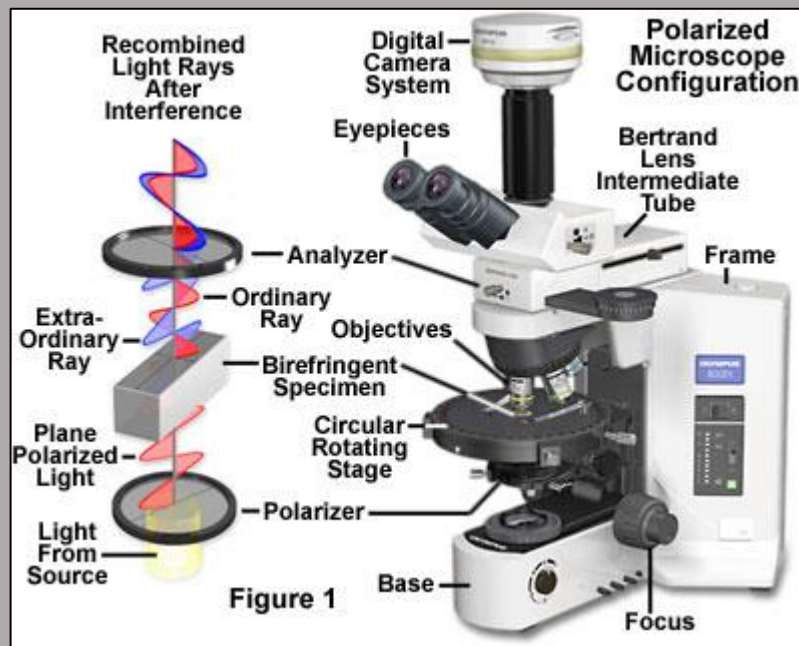


Birefringence phenomena is the “splitting of incident light into two orthogonally polarized light rays with different velocities and hence different refractive indices”.



Birefringence and Conoscopy

Optical property of birefringent medium can be analyzed with a polariscope and isogyres generation





Birefringence and Stroma properties

Measurements of the corneal birefringence with a liquid-crystal imaging polariscope

Juan M. Bueno and Fernando Vargas-Martín

116 APPLIED OPTICS / Vol. 41, No. 1 / 1 January 2002

IS THERE A LINK BETWEEN CORNEAL STRUCTURE AND THE 'CORNEAL CROSS'?

BARBARA K. PIERSCIONEK¹ and ROBERT A. WEALE^{2,3}

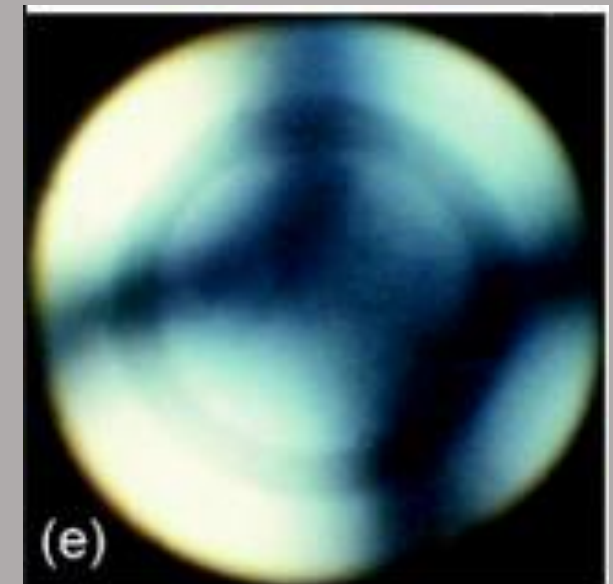
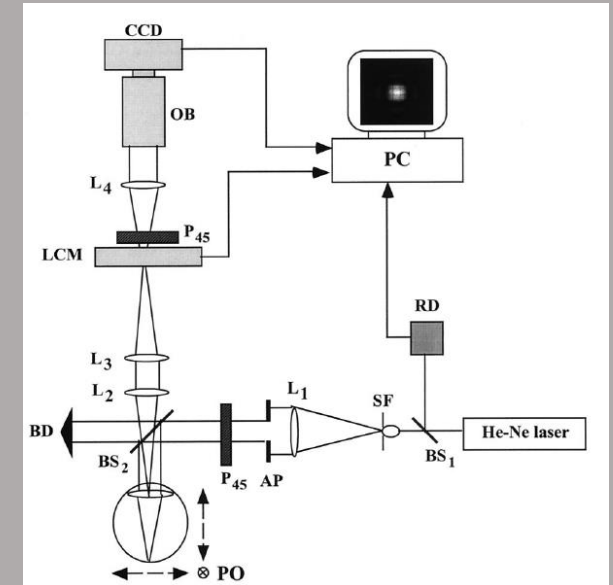
Eye (1997) 11, 361-364 © 1997 Royal College of Ophthalmologists

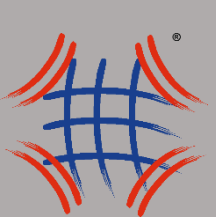
Optical anisotropy of the human cornea determined with a polarizing microscope

Richard A. Bone and Grenville Draper [»View Author Affiliations](#)

Applied Optics, Vol. 46, Issue 34, pp. 8351-8357 (2007)

<http://dx.doi.org/10.1364/AO.46.008351>





Lumaxis[®]: Polarimetric Interferometry

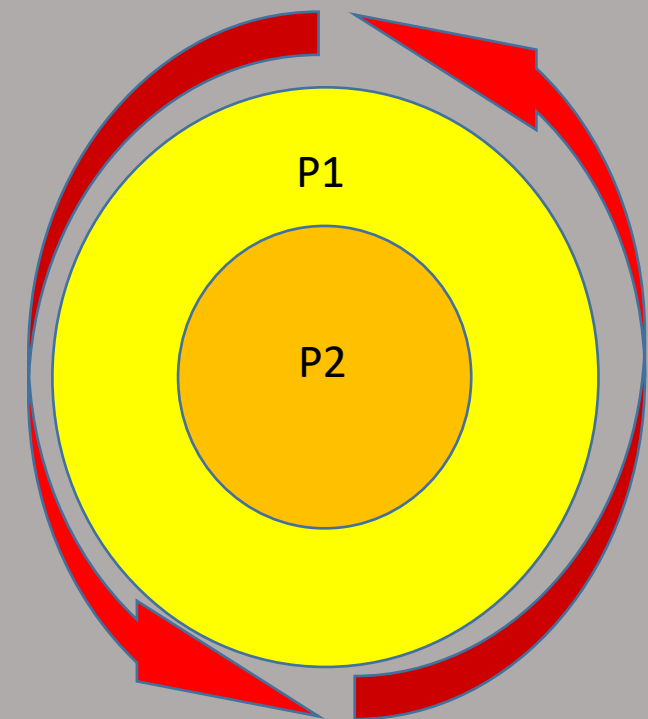


LUMAXISTM
CORNEAL LAMELLA PHASE ALIGNMENT



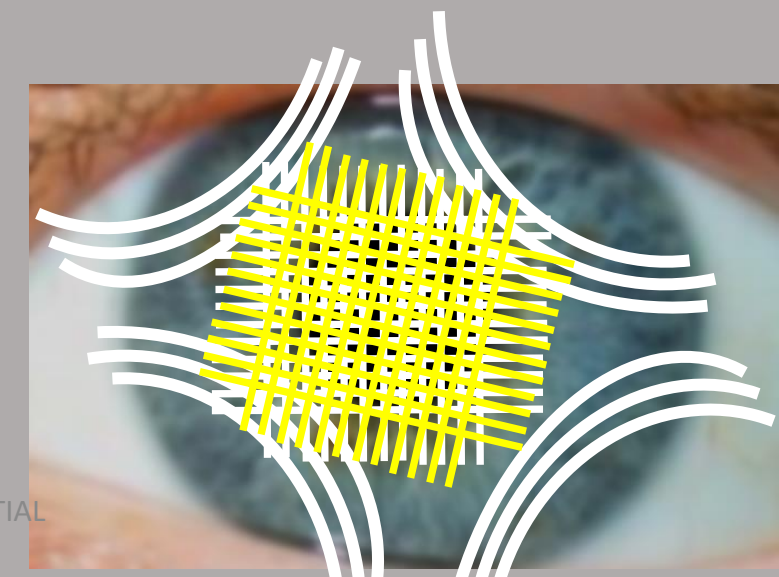
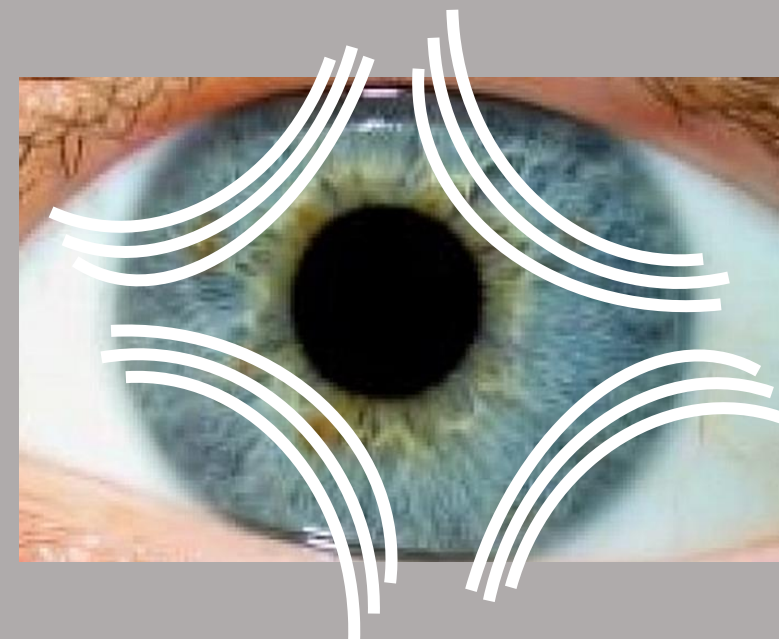
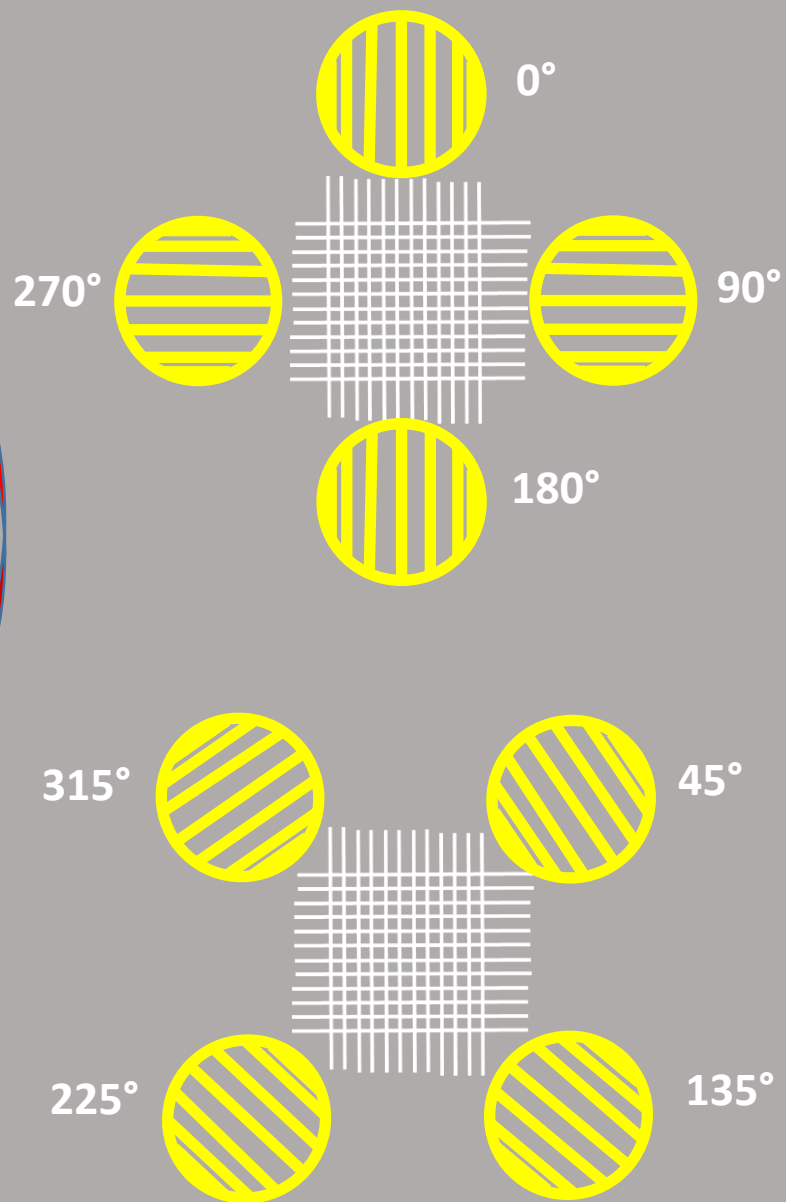


Lumaxis: Detection of collagen fibrils orientation



Patented Technology:

- PCT/IB2013/058263
- PCT/IB2014/063482



CONFIDENTIAL



Real-Time Lumaxis Imaging

No Visible Effect from External View

CROSS image

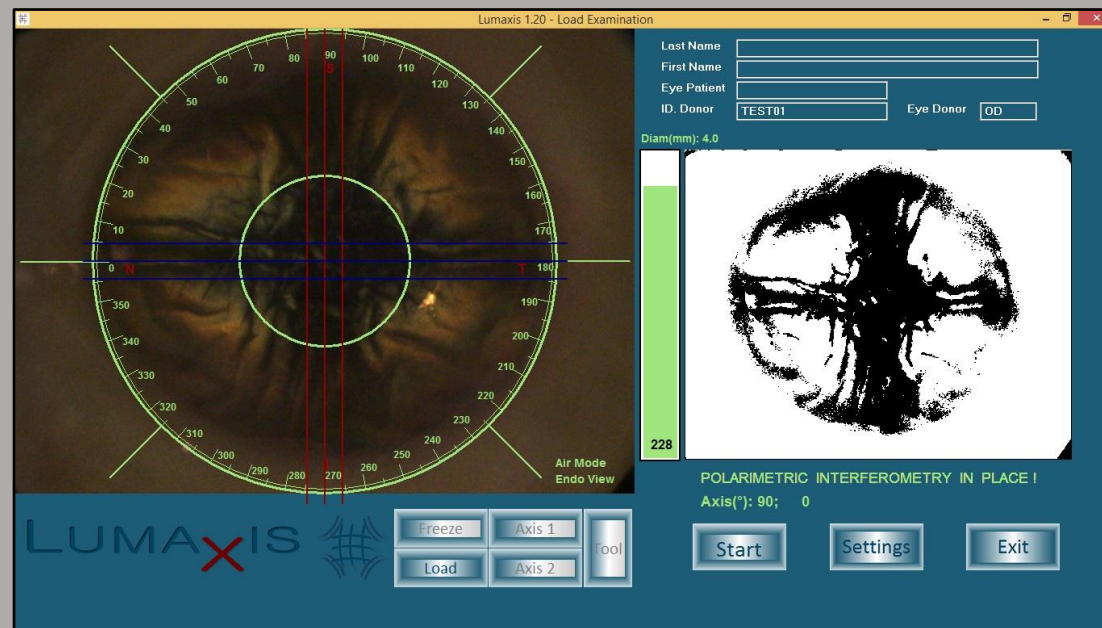
Stromal Scattering Visible if not Coupled

Software interface showing patient information (Last Name: EUGENIO, First Name: LIPARI, C.I.D., ID. Curves) and a 'CROSS' image. The interface also displays a circular image of an eye with a green circular overlay and a green crosshair. The text 'LUMAXIS' is visible at the bottom of the interface.



Real-Time Lumaxis Imaging for Donor Cornea

«'CORNEAL CROSS' IS CREATED ONCE POLARIZERS ARE ALIGNED WITH DEEP STROMA COLLAGEN FIBRILS DIRECTIONS»



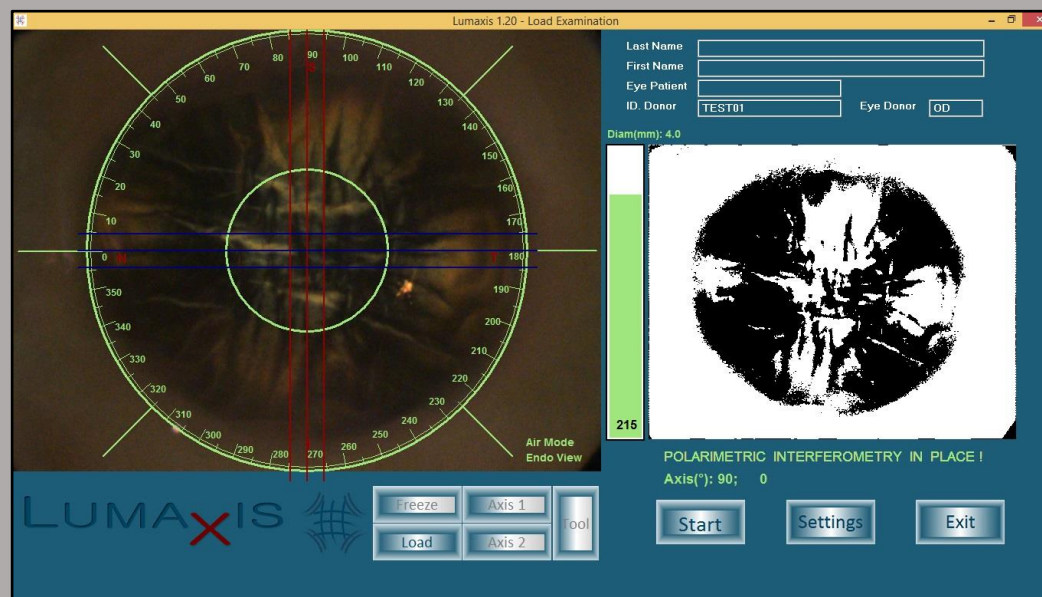
The Dark Bands (Isogyres)
correspond to points of the
cornea stroma where the state
of polarization of the incident
Light is unchanged

HUMAN CORNEA IS ANALYZED «ON AIR»



Real-Time Lumaxis Imaging for Donor Cornea

**«'CORNEAL CROSS' DISAPPEARS TO GENERATE TWO HYPERBOLAE SHAPED PATTERN
ONCE POLARIZERS ARE 45° OFF TO THE DEEP STROMA COLLAGEN FIBRILS
DIRECTIONS»**

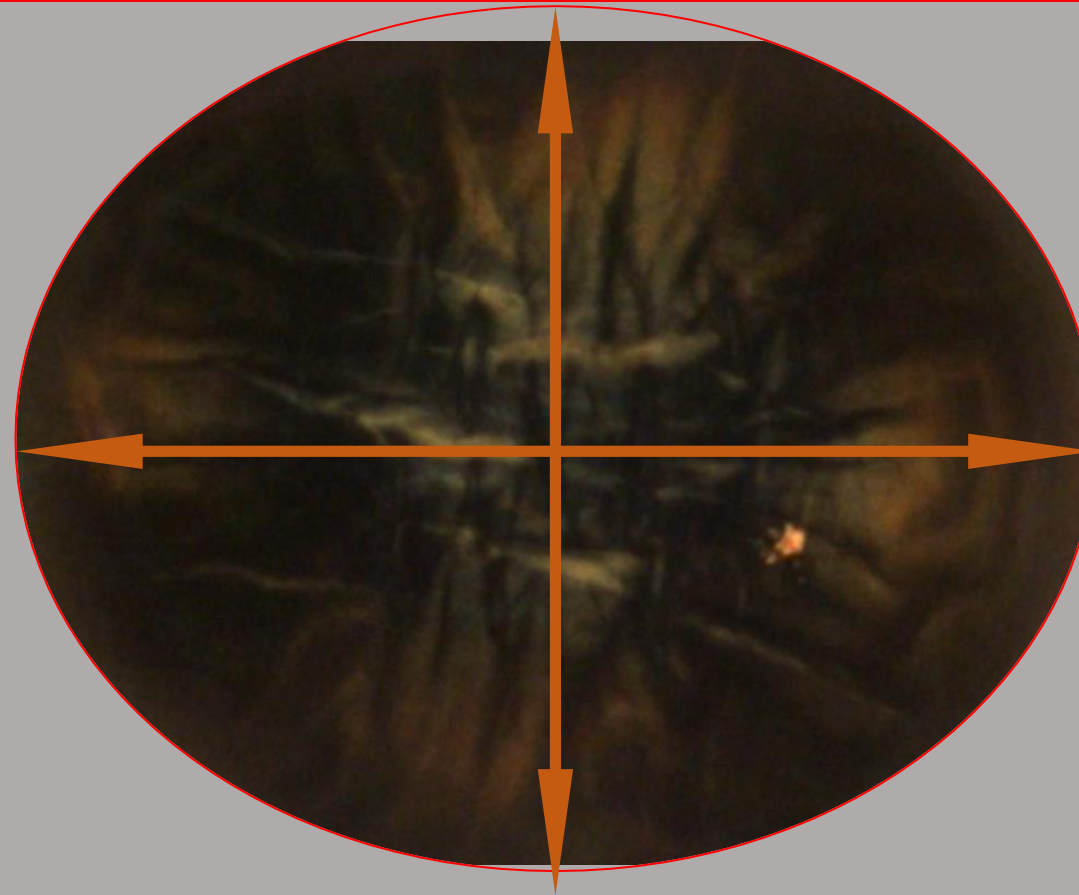


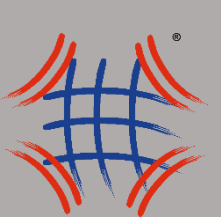
HUMAN CORNEA IS ANALYZED «ON AIR»



Real-Time Lumaxis Imaging for Donor Cornea

LIVE SCAN !!

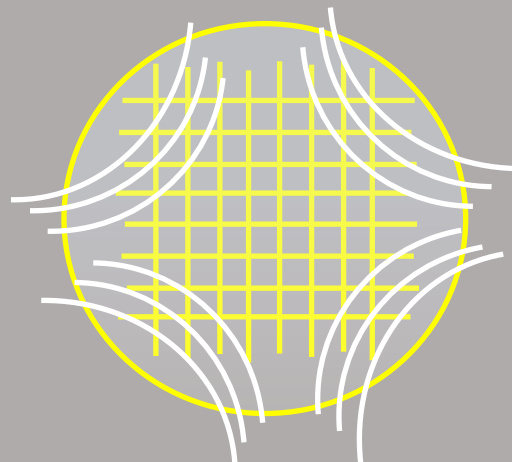
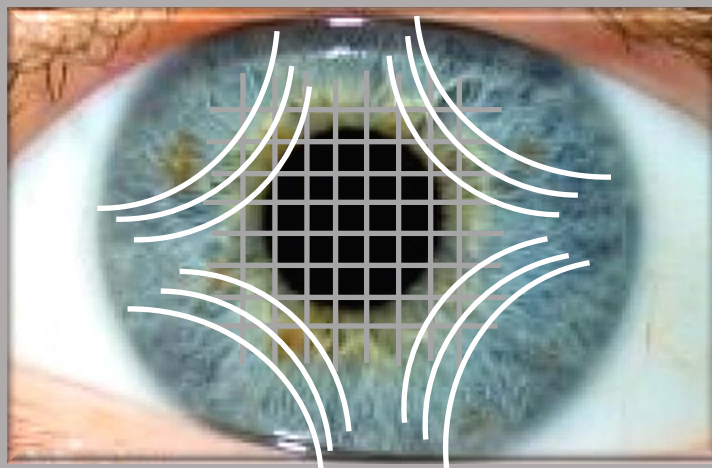




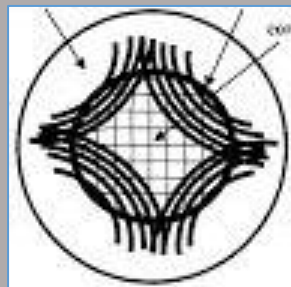
WHAT IS THE CLINICAL IMPORTANCE OF LUMAXIS?

Corneal Keratoplasty:

- **Lamellar Keratoplasty (Anterior and Posterior)**: Lumaxis scans both donor and patient cornea and allows the surgeon to align each other to optimize the postop visual outcome;

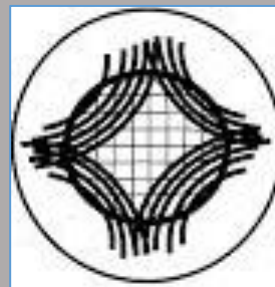


- **Penetrating Keratoplasty**: Are Right and Left Eye Corneas Interchangeable???



OD

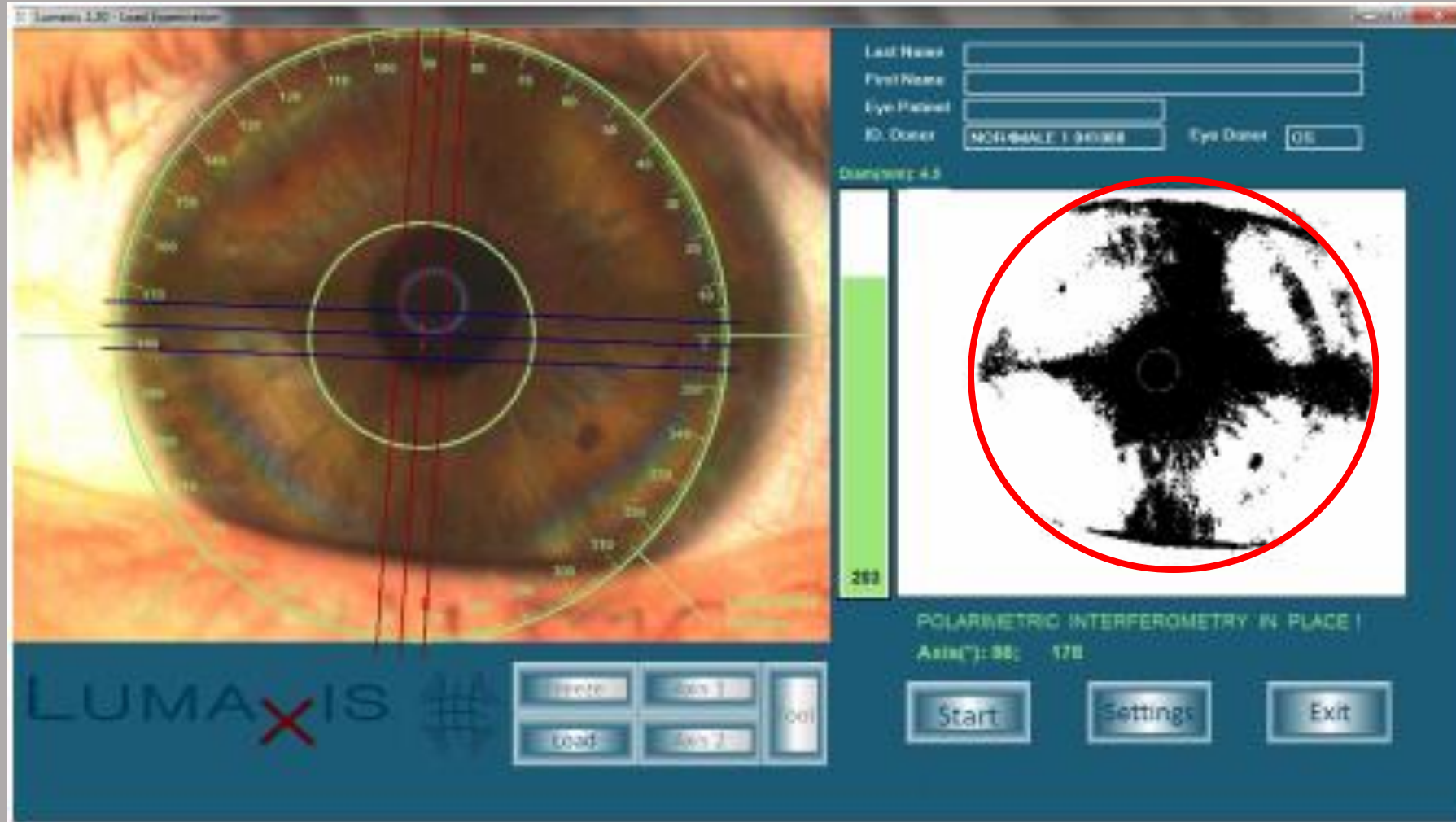
Vs.

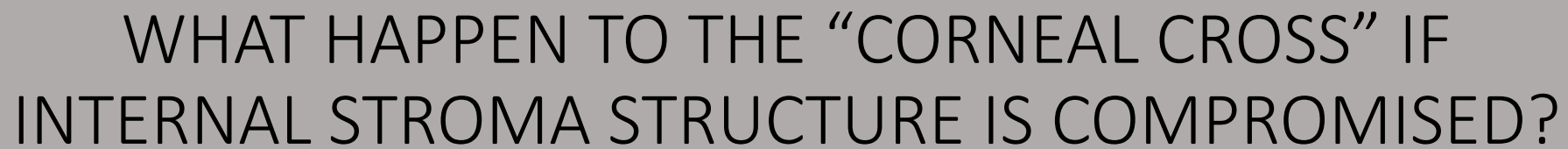


OS



“CORNEAL CROSS” PATTERN IS REGULAR AND SIMMETRIC
AS CONSEQUENCE OF THE FIBRILS ANISOTROPY



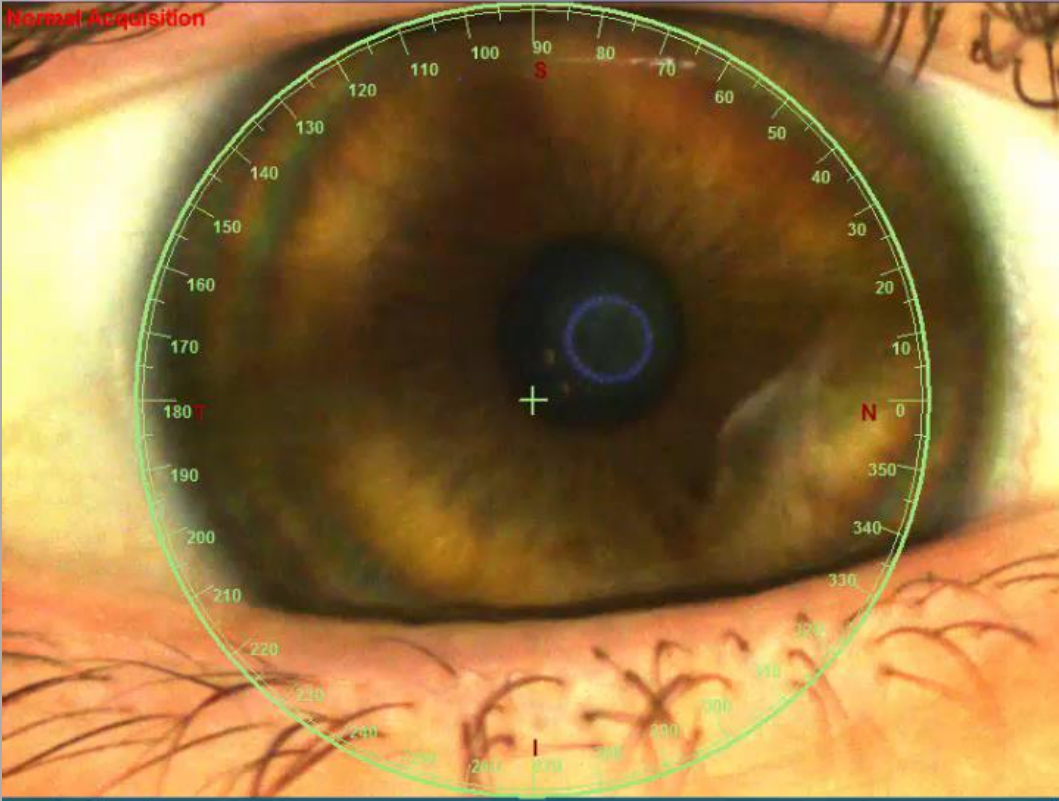




Acquisition on regular Eye

Lumaxis 1.20 - Load PATIENT Examination

Normal Acquisition



Last Name: ANDREAS
First Name: OCULUS
Eye Patient: EYE OD ACQUISITION
ID. Donor: Eye Donor:



68

CLICK TO START VIDEO

LUMAXIS

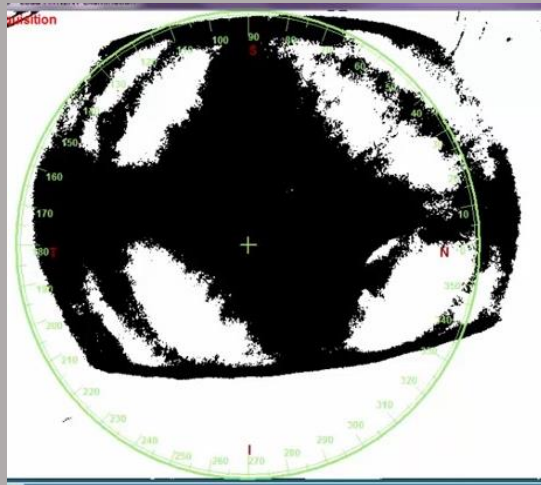
Freeze Axis 1
Load Axis 2 Tool

Stop Settings Exit

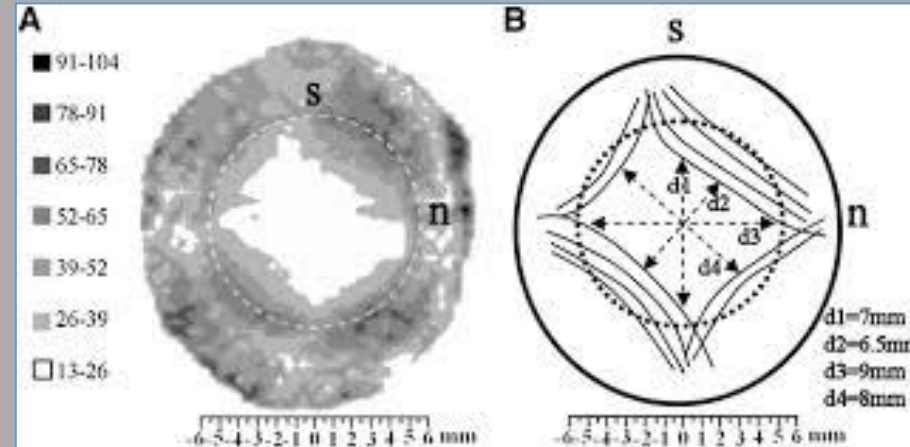
THRESHOLD 68



Acquisition on regular Eye: E.L. OD



VS.



[Craig Boote, Christina S. Kamma-Longer, et al.](#), Quantification of Collagen Organization in the Peripheral Human Cornea at Micron-Scale Resolution- [Biophysical Journal](#), Vol 101, July 2011, 33-34

Lumaxis[®]
(EUGENIO)

X-Rays Analysis
(Only Ex-Vivo)

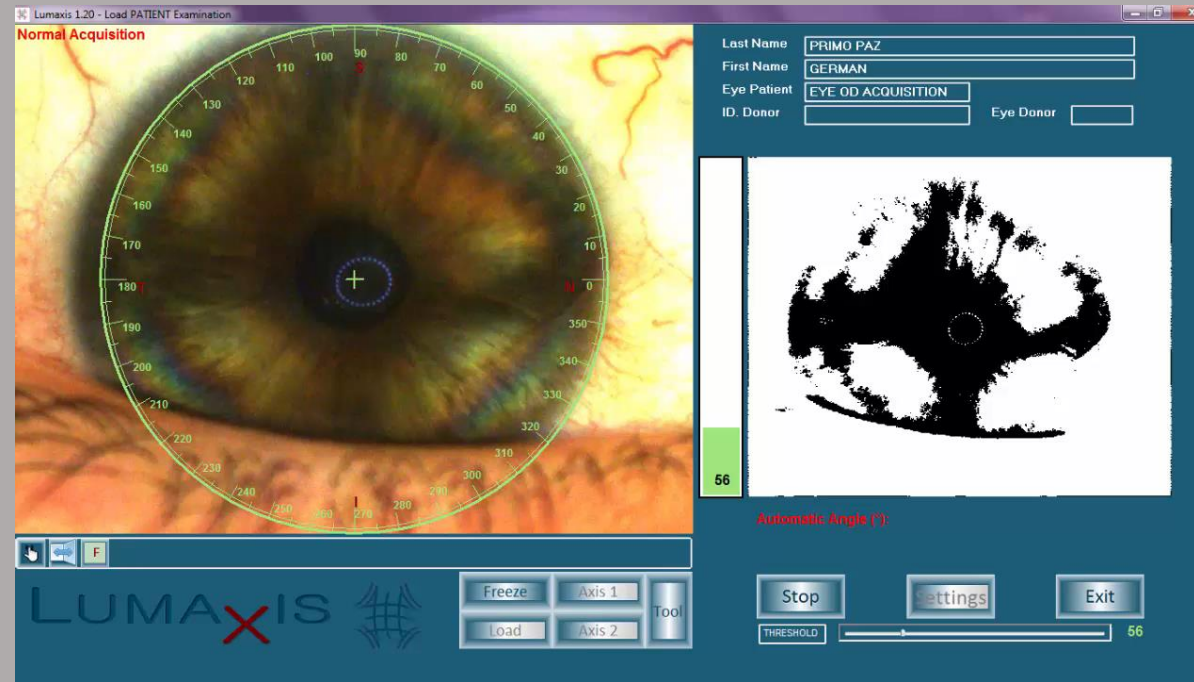
Lumaxis Analysis is real time and not invasive technique which shows same pattern obtained with the X-Rays Analysis. The cross-like pattern is a consequence of the birefringences properties of the stroma.



Acquisition on «LOW KC» : Patient #1

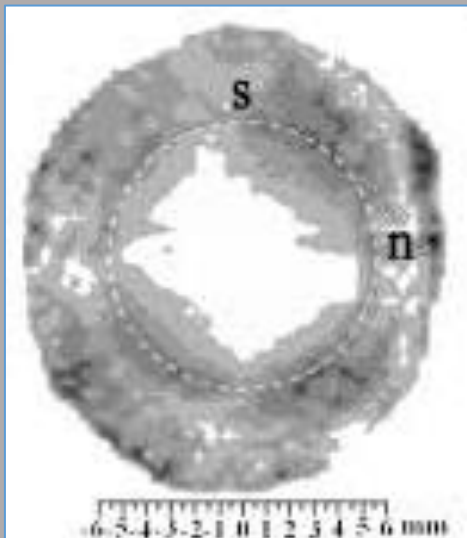
«OD»

VIDEO

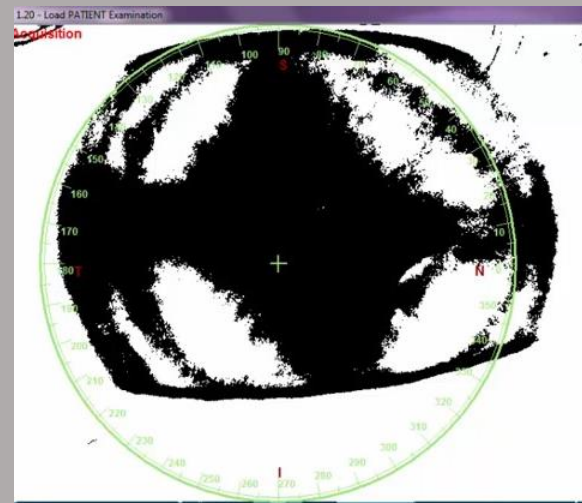


Once the stroma pathology occurs,
Consequently the optical quality is
Compromised and the cross pattern
Move away from the theoretical shape.
The arms of the cross start to modify
And the center of the cross (optical axis)
Moves away from the center of the
Cornea.

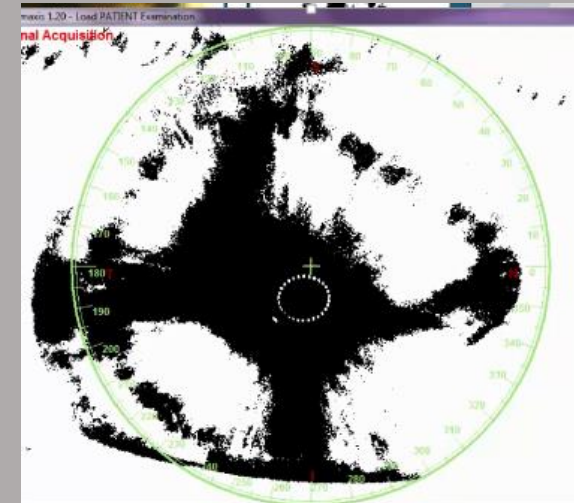
Theoretical
(X.Rays)



Regular
(E.L.)



Irregular
(Low KC)



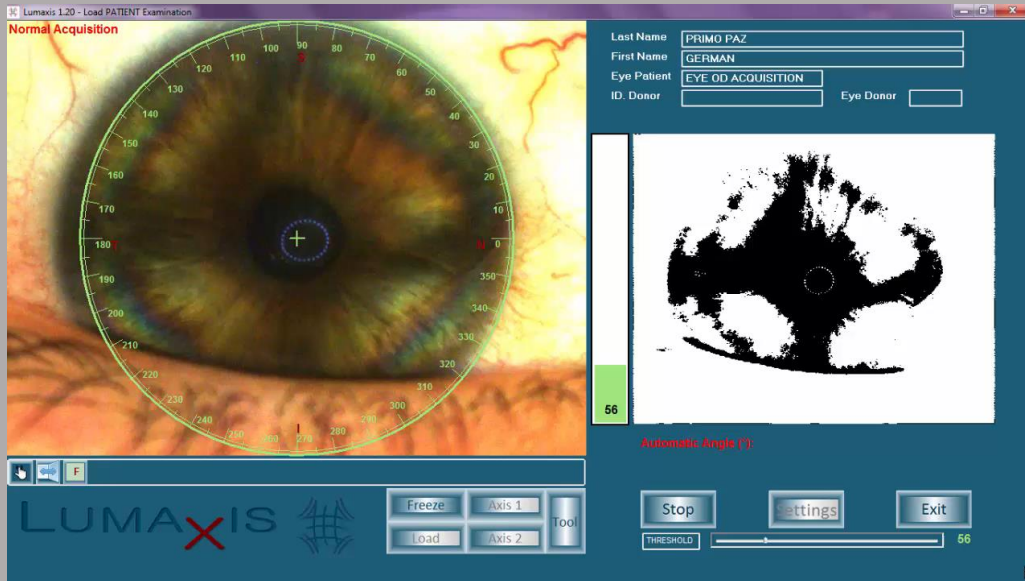


Acquisition on «LOW KC» : Patient #1

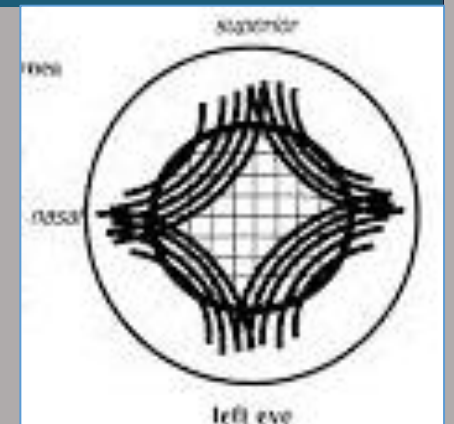
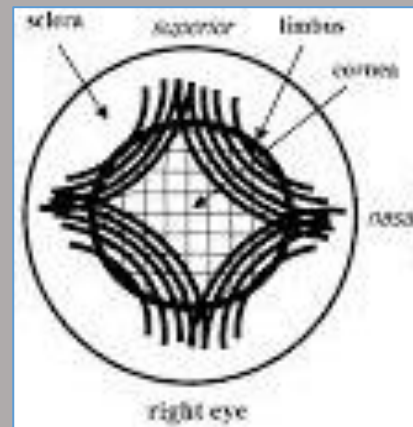
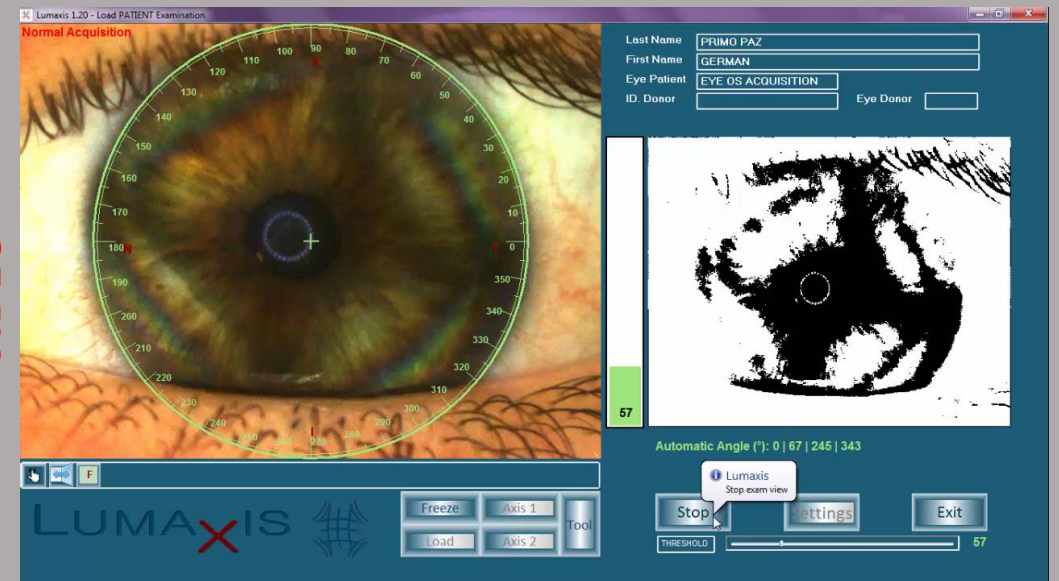
«OD»

«OS»

VIDEO



VIDEO



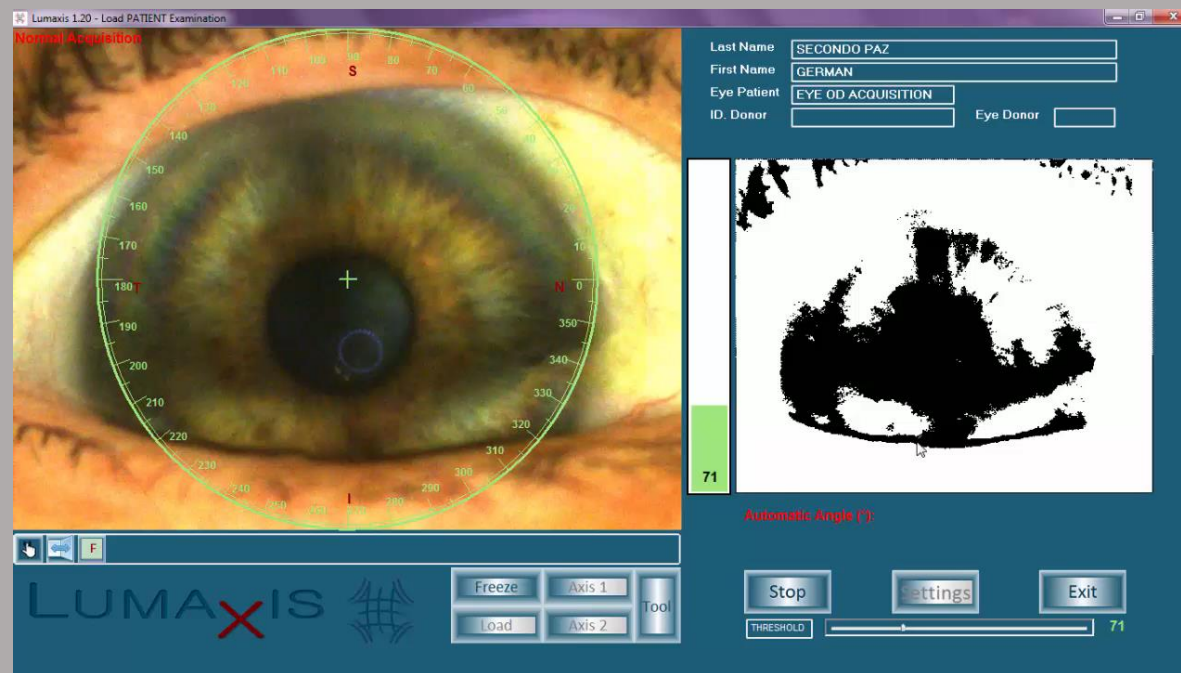
Cross-shaped collagen fibrils distribution is A-Symmetric ODvsOS as described by the X-Rays studies



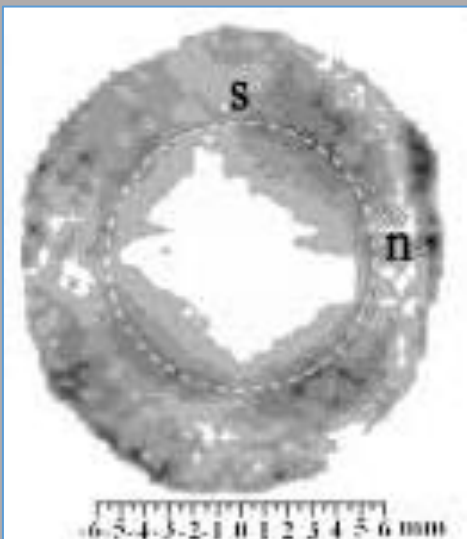
Acquisition on «MODERATE KC» : Patient #2

«OD»

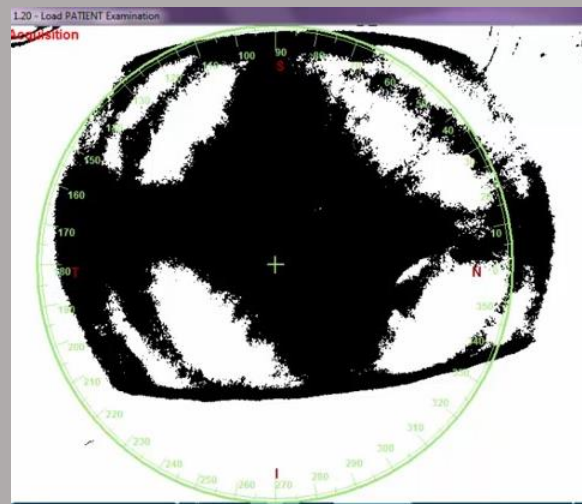
VIDEO



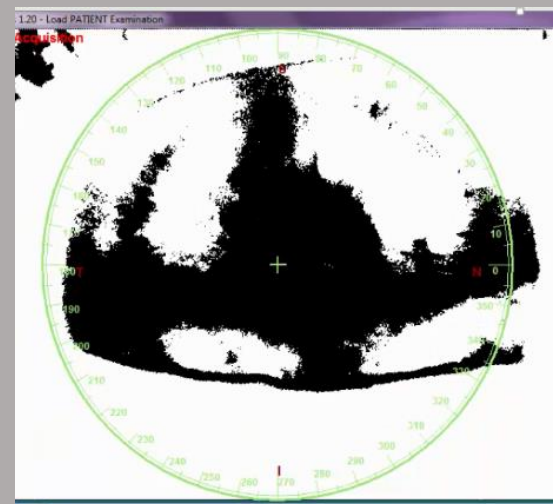
Theoretical
(X.Rays)



Regular
(E.L.)



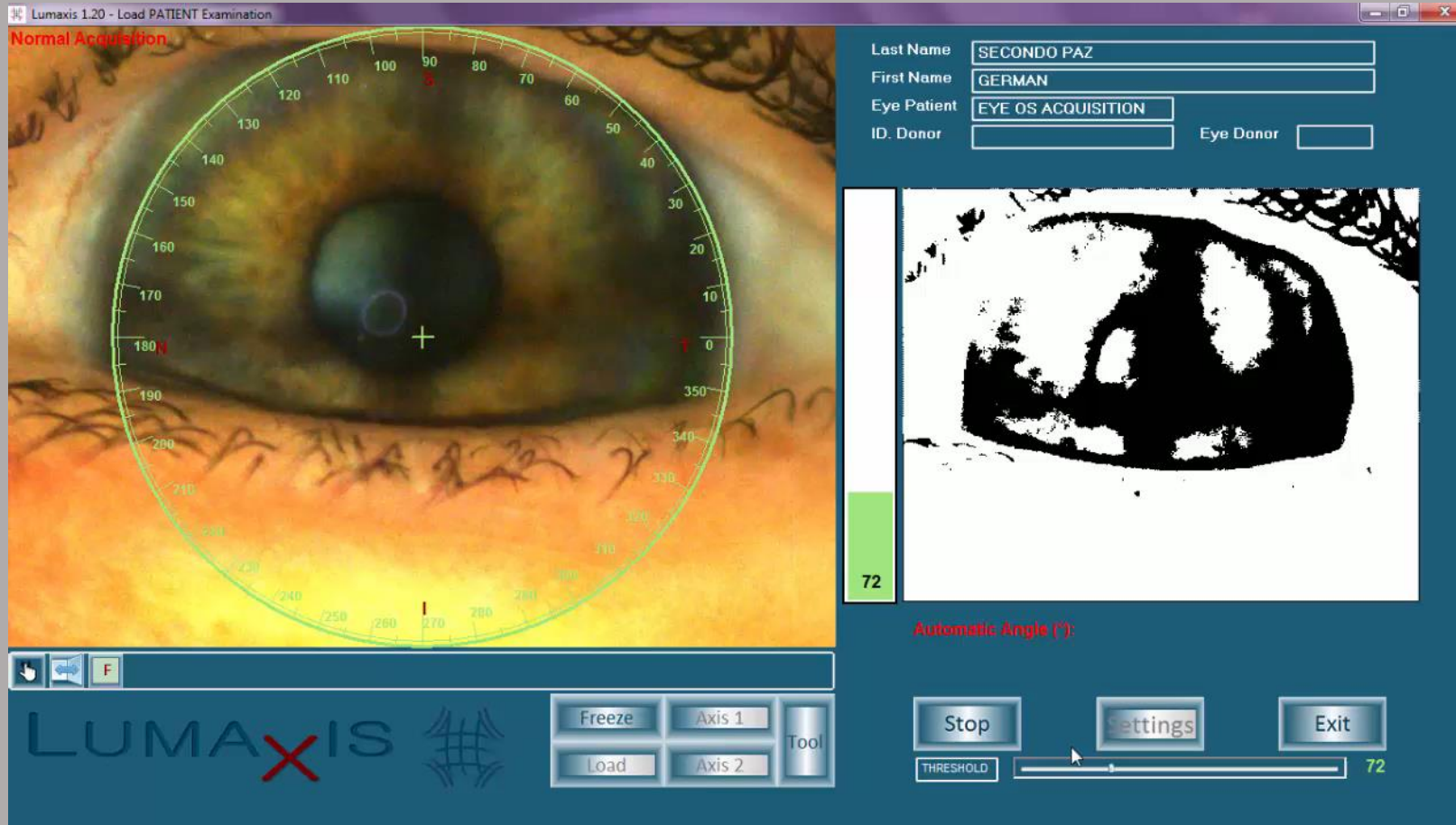
Irregular
(Moderate KC)





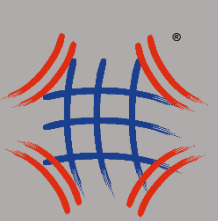
Acquisition on «MODERATE KC» : Patient #2

«OS»

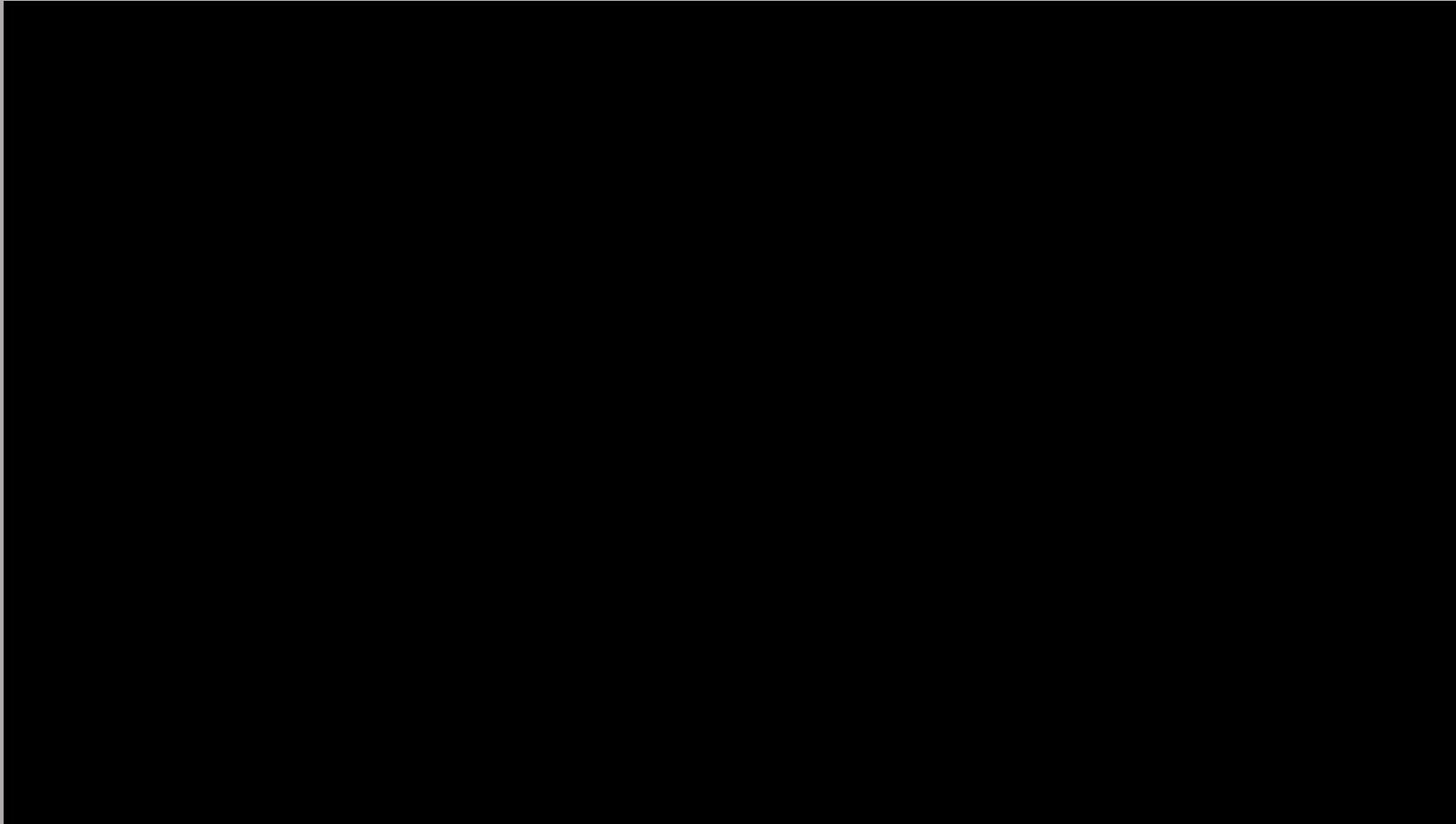


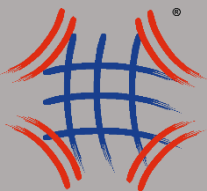
This video clearly shows how the center of
The cross is moving out from the center of the
Corneal. During the scan is evident how the
Arms are not any more simmetric therefore
The optical property of the stroma are going
To be more and more compromised.
Once the cross shape pattern will not be visible
Any more, than the stroma tissue will not
Optically work any more and the only indication
For the patient will be the PK (penetrating
Keratoplasty)

VIDEO



DSAEK & POLARIMETRIC INTERFEROMETRY





Format: Abstract

[Cornea](#). 2016 Apr;35(4):519-22. doi: 10.1097/ICO.0000000000000776.

Polarimetric Interferometry for Assessment of Corneal Stromal Lamellae Orientation.

[Iovieno A](#)¹, [Yeung SN](#), [Nahum Y](#), [Teichman J](#), [Lipari E](#), [Busin M](#), [Fontana L](#).

Author information

Abstract

PURPOSE: To analyze corneal stromal lamellae orientation in normal subjects and patients with various corneal conditions using a polarimetric interferometer.

METHODS: Thirty-two healthy control subjects (59 eyes) and 16 patients (22 eyes) with corneal conditions or postkeratoplasty were included in the study. All patients were imaged using the interferometer and slit lamp photography. The interferometer produces an orthogonal cross-like image of stromal lamellae by illuminating the cornea in phase-light polarization angle. Corneal haze was graded on a scale from 0 to 4. Interferometer cross-like images were graded on a scale from 0 to 5 by a masked observer. Keratometry, corneal central pachymetry, and optical densitometry were obtained with Scheimpflug corneal tomography.

RESULTS: The cross-like image was observed in 31 of 32 healthy control subjects (58 of 59 eyes) and in 13 of 16 patients (19 of 22 eyes). The image was not detectable in 3 patients, 1 with total corneal neovascularization and scarring, 1 with central leukoma, and 1 with failed deep lamellar keratoplasty. Corneal haze was the main factor obscuring the cross-like image ($P < 0.05$). Clarity of the images was influenced by older age, steeper keratometry, higher pachymetry, and optical density ($P < 0.05$). There was no correlation between the orientation of the keratometric axes and the orientation of the 2 arms of the cross-like image ($P > 0.05$).

CONCLUSIONS: Corneal stromal lamellae orientation can be efficiently assessed and displayed as a cross-like image by the polarimetric interferometer. Stromal lamellae orientation imaging may have potential implications in corneal diagnostics and surgery.

Format: Abstract

[Cell Tissue Bank](#). 2016 Jun;17(2):233-9. doi: 10.1007/s10561-016-9546-9. Epub 2016 Feb 27.

Polarization of human donor corneas.

[Parekh M](#)¹, [Ruzza A](#)², [Ferrari S](#)², [Salvalaio G](#)², [Elbadawy H](#)², [Ponzin D](#)², [Lipari E](#)³.

Author information

Abstract

To investigate the de-orientation effect of DSAEK grafts by observing the cross patterns and polarization power of human donor corneas using a polarizing device (Lumaxis®). Forty human donor corneas were placed in small petri-plates with epithelial side facing up. Polarizing power (arbitrary unit) and crosses were monitored and recorded by the software. The tissue was marked at 'Superior' position to ensure that the base and the polarizer are in alignment with each other after the cut. The anterior lamellar cut was performed using microkeratome. The lenticule was placed back in the same position as marked to mimic the alignment. The tissue was further rotated by 45° ensuring that the base of the cornea and the polarizer were in alignment. The polarization power and 'crosses' were identified at each step. The average of forty corneas from pre-cut to post-45° angular change showed statistically significant difference ($p < 0.05$) in terms of polarizing power. The cross-shaped pattern deformed and lost the sharpness towards 45° angle. However, multiple variances in terms of 'cross-patterns' were observed throughout the study. Lumaxis® was able to determine the worst quality tissue in terms of polarization (no black zone and crosses). Despite the quality of cross pattern which can be used as an additional objective parameter to evaluate the optical properties of the corneal tissue, this preliminary study needs to be further justified in terms of clinical relevance whether polarization changes with oriented or de-oriented grafts have any effects and consequences on the visual acuity.

KEYWORDS: Cornea; Eye bank; Isogyres; Lumaxis®; Polarizer



GRAZIE