XTOP 2006

X-ray Imaging in Advanced Studies of Ophthalmic Diseases

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Ophthalmic Diseases and X-rays

Disease:

- Alterations in optical/physiological properties of ocular tissues

Ocular Tissues:

- Those responsible for processing the visible light inside the eye, such as retina, cornea and crystalline (eye lens)

Cataract:

- Transparency loss of the eye lens. One of the most common disease, leading cause of eye surgery (8-10 million/year)

Drugs, R&D:

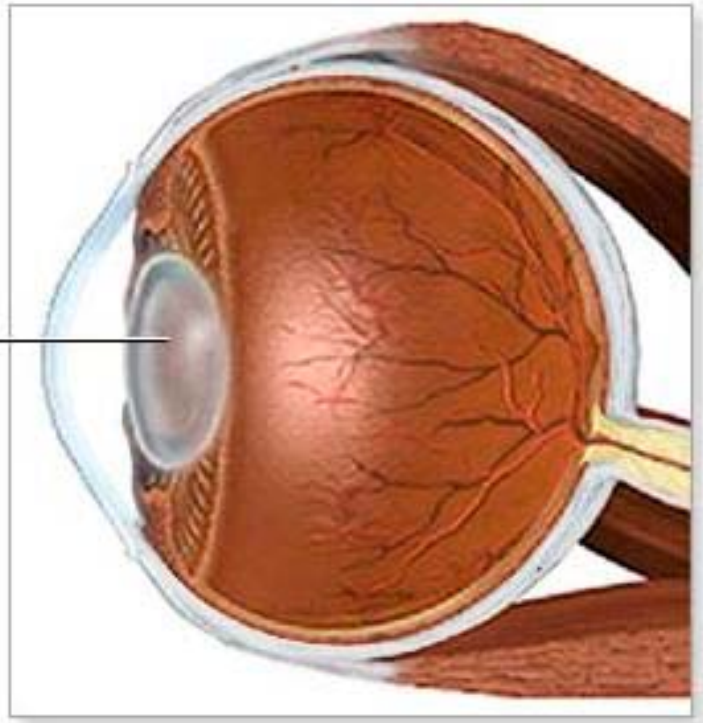
- For corrective/preventive treatments new tools are required with sensitivity to extended changes in the tissue & feasible on large ensembles

X-ray imaging: what are the benefits?

- Short wavelength, low scattering, good resolution/penetration ratio, fast data collection (50 samples/day)

Cataract

Cataract: eye lens opacity …



Opacity: visible light scattering …

http://www.tedmontgomery.com/the_eye/eyephotos/index.html

Physiological changes

Scattering centers of visible light ?

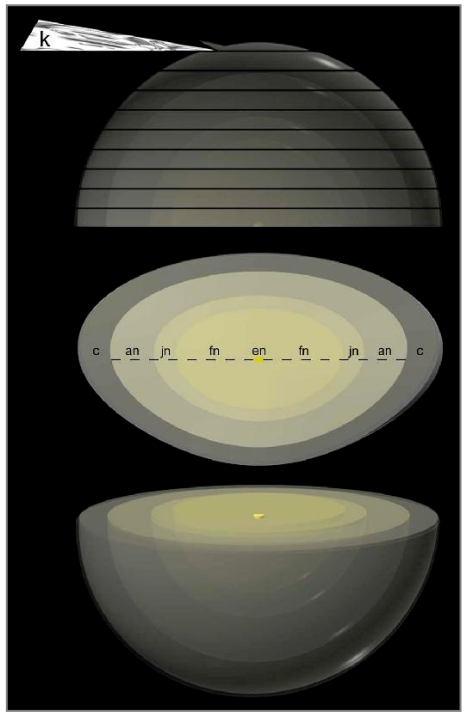
Any tissue damage, even external ones (a.f.i. UV exposure)

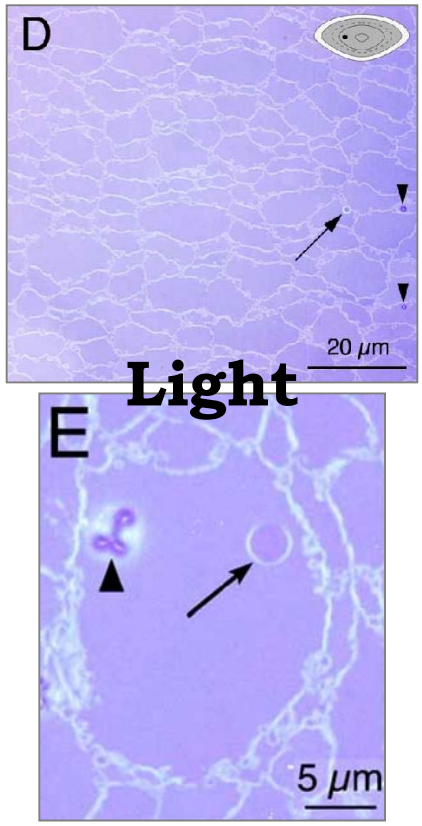
Causes:
aging, drugs, diabetes, congenital, …

Optical/Electron Microscopy

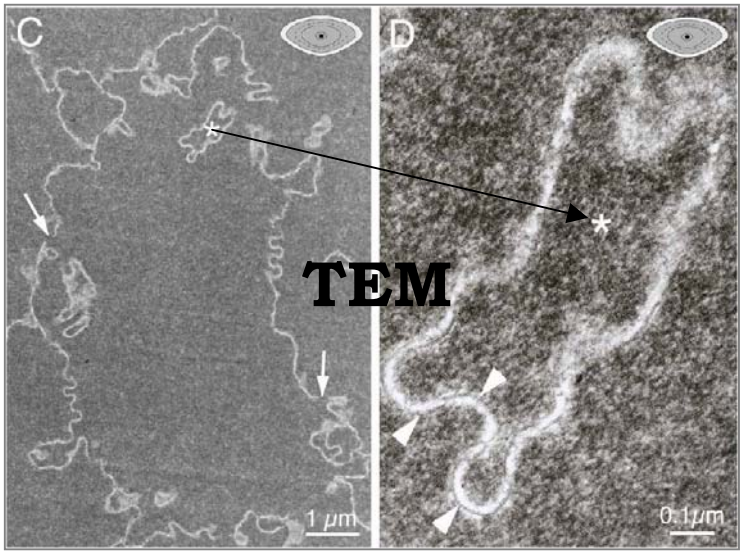
Visible light:
- too much diffuse scattering for structural analysis of the scattering centers by naked eye
- histological procedures required
- macro scale information is lost

Faculty of Veterinary Medicine, University of Sao Paulo - USP
Canine lens with cataract.

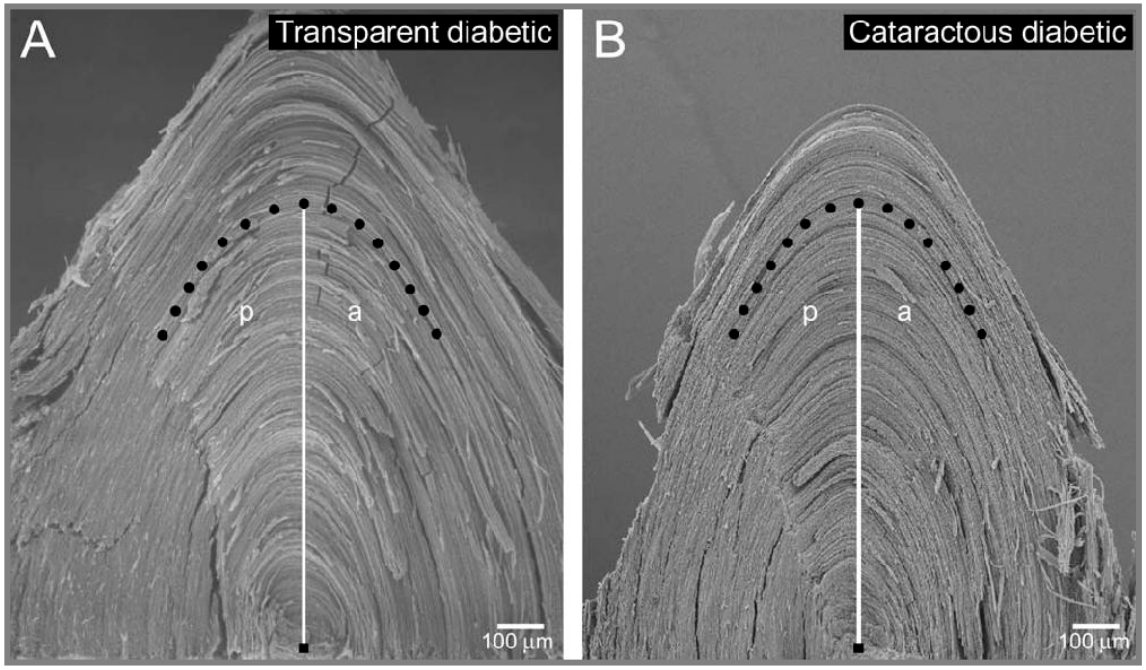




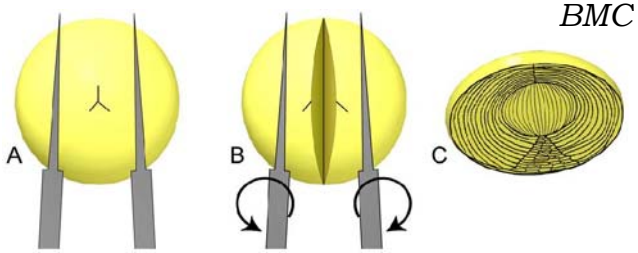
Multilamellar bodies as potential scattering particles...
K.O. Gilliland *et al.*
Molecular Vision 2000; **7**, 120 (2001)

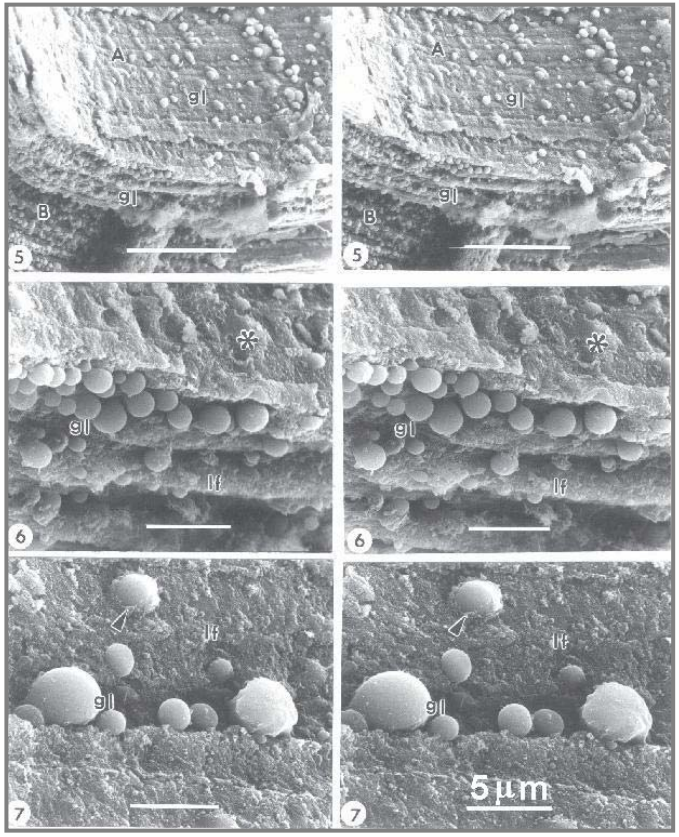


Scanning Electron Microscopy



Nuclear fiber cell compaction…
Christopher D. Freel
BMC Ophthalmology **3**, 1 (2003)





Images of human cataractous lens…
W.L. Jongebloed.
Scanning Microscopy **12**, 653 (1998)

Optical / Electron microscopies are limited for investigating:
distributions of scattering center and density fluctuations
on extended tissues (cm scale/entire lenses)

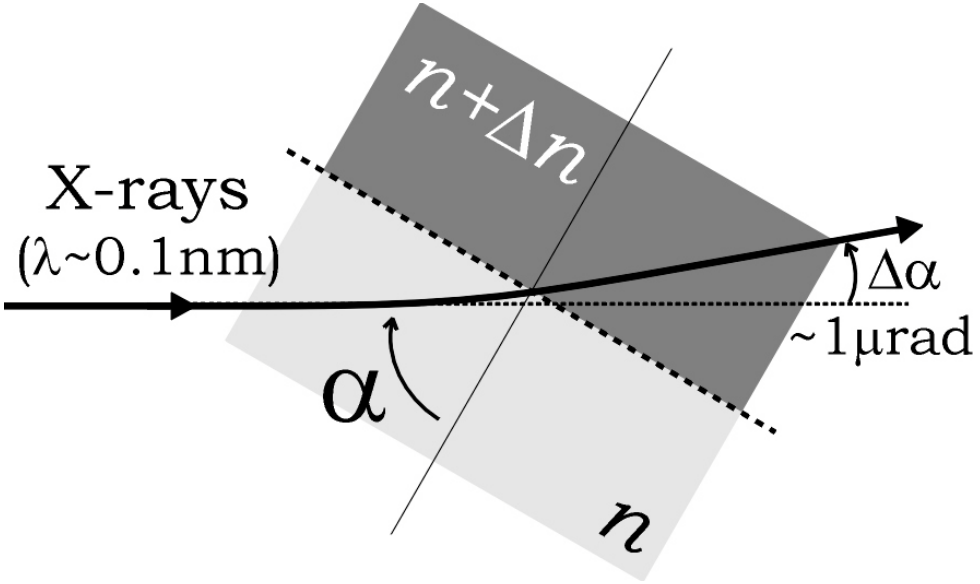
Why X-rays?

X-ray can see through entire lenses:

- minimum of scattering (angular spreading $\approx \lambda / D$)
- refraction and diffusion scattering are angular resolved processes
- selective mapping of:
  - density fluctuations and
  - distributions of scattering centers

Small angle scattering

Refraction



$$\Delta\alpha = \frac{\Delta n}{n}\tan\alpha \qquad \text{(Snell's Law)}$$

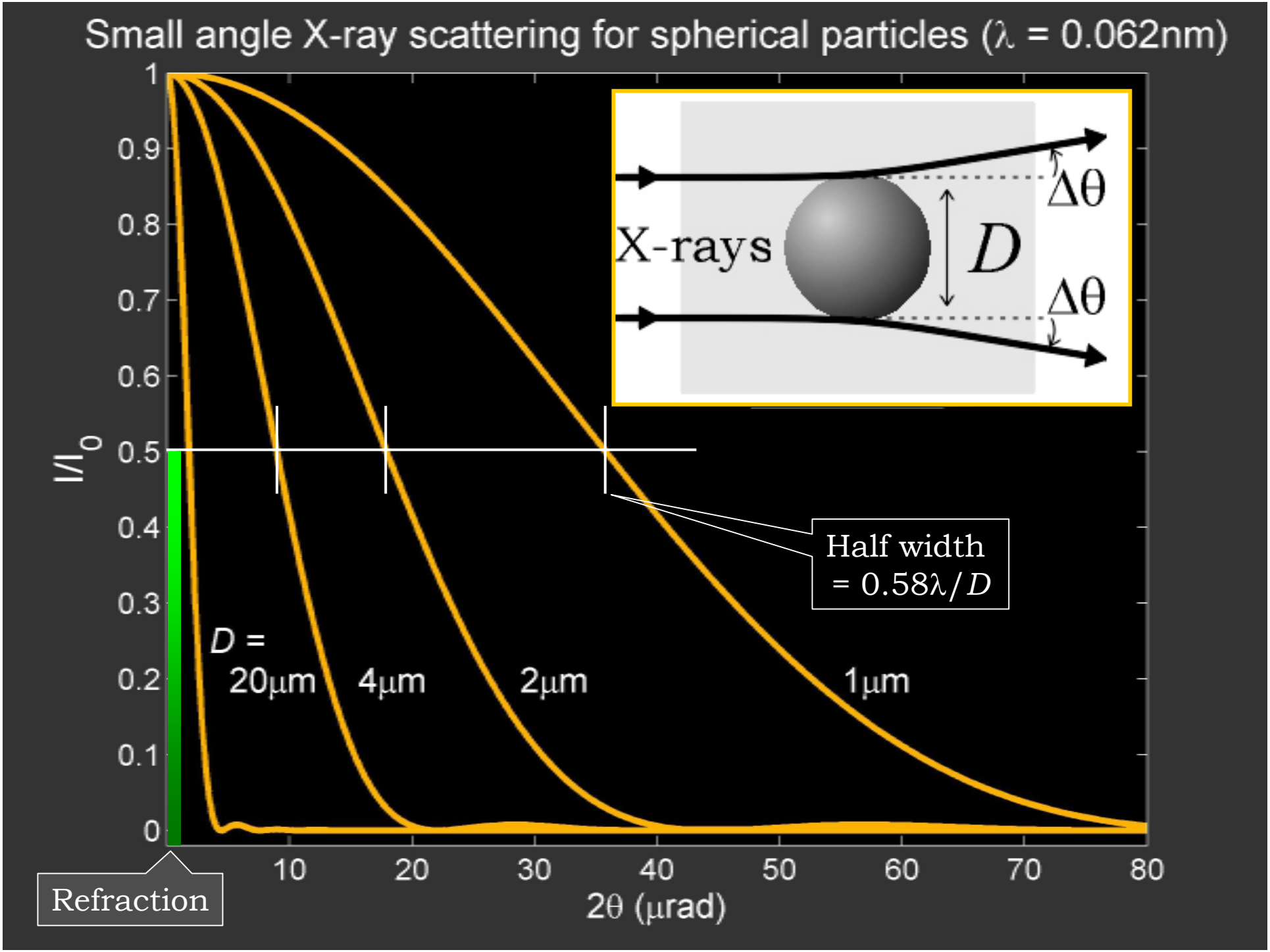
$$n = 1 - \delta \qquad \text{(index of refraction)}$$

$$\delta = \Gamma\rho_e \cong 10^{-5}, \quad E \approx 20\text{keV}$$

$$\left|\frac{\Delta n}{n}\right| = \Delta\delta = \delta\frac{\Delta\rho_e}{\rho_e}$$

$$\frac{\Delta\rho_e}{\rho_e} = 0.1 \Rightarrow \Delta\delta = 10^{-6}$$

$$\Delta\alpha \approx 1\mu\text{rad}$$


Small angle X-ray scattering for spherical particles (λ = 0.062nm)
I/I0
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
70
80
2θ (μrad)
X-rays
D
Δθ
Δθ
Half width
= 0.58λ/D
D =
20μm
4μm
2μm
1μm
Refraction

Diffraction enhanced X-ray imaging (DEI)

a) Experimental setup at X15A (NSLS).

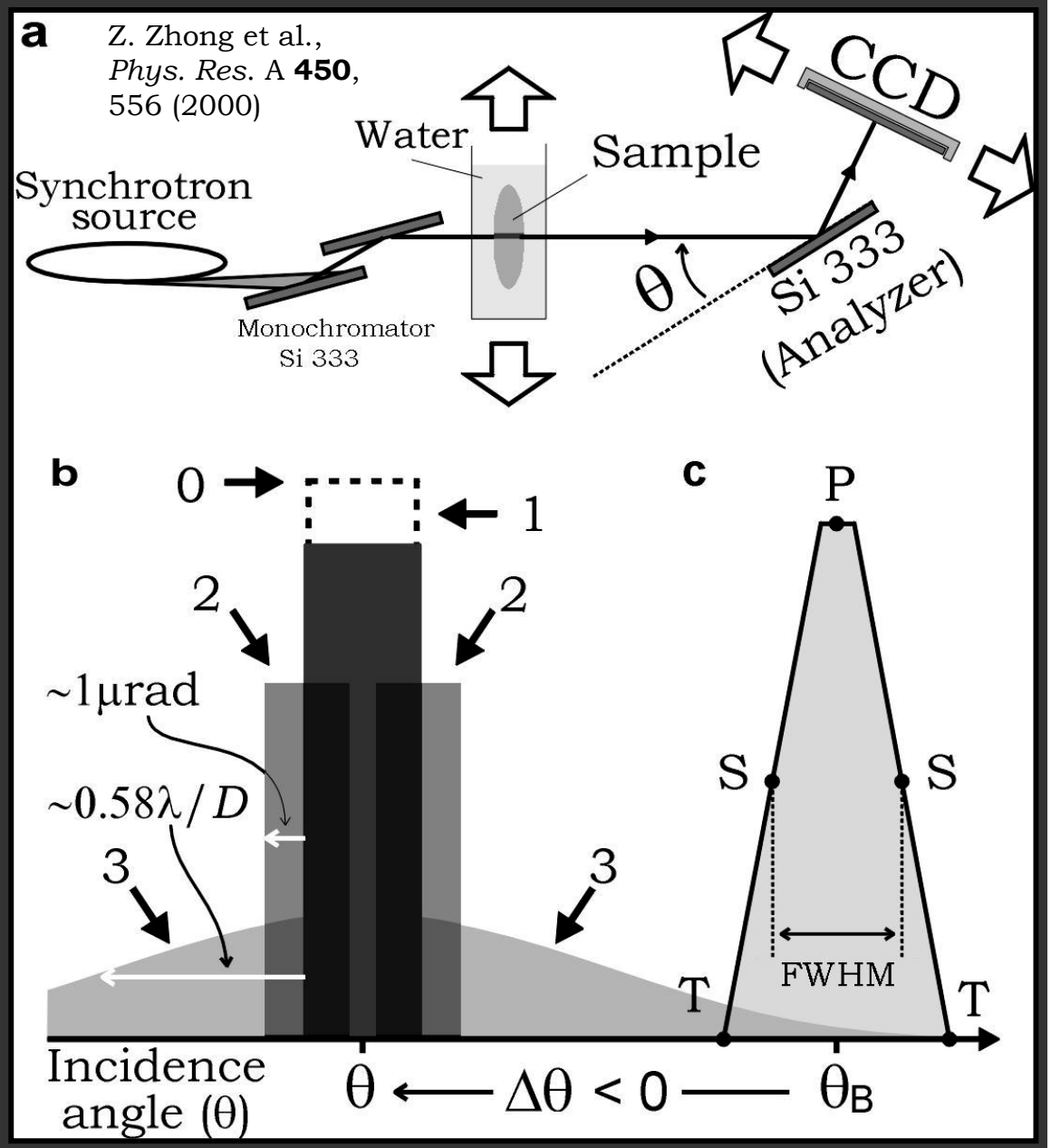
b) Incident beam (0), absorption (1), refraction (2), and diffuse scattering (3).

c) Analyzer window (acceptance angle for Bragg diffraction in the analyzer crystal)

P: center of reflection curve (absorption)

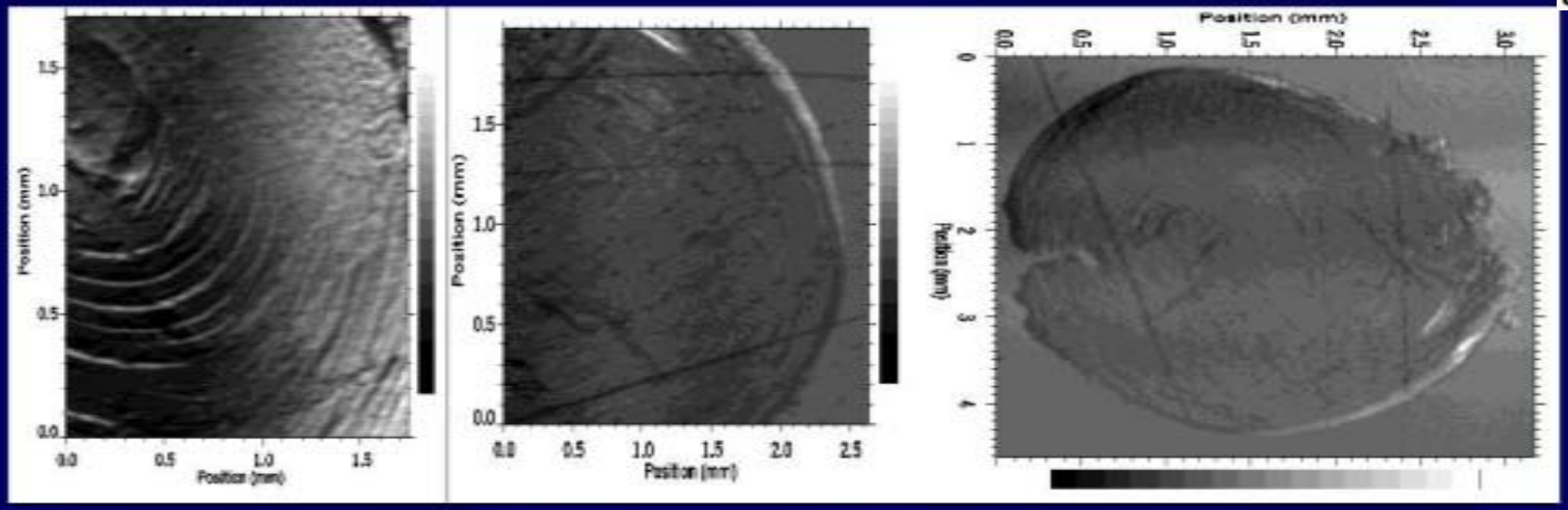
S: shoulder (refraction) ($\Delta I/I$ ~ 30% per μrad)

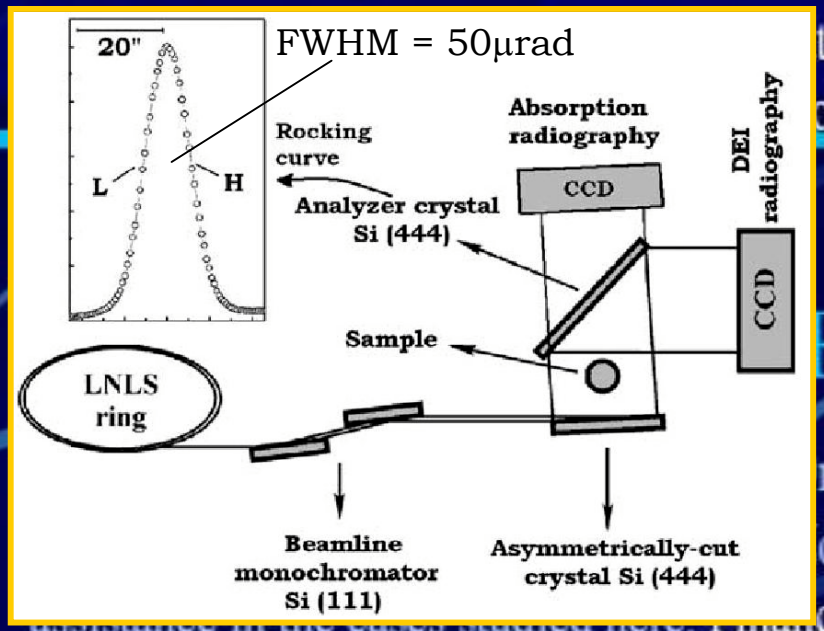
T: tail (diffuse scat.) $|\Delta\theta| > 5\mu rad$, $I/I_0 < 2\%$



FWHM = 3.2μrad @ 18keV / 1.5 μrad @ 40 keV

DEI @ LNLS (10.7KeV)



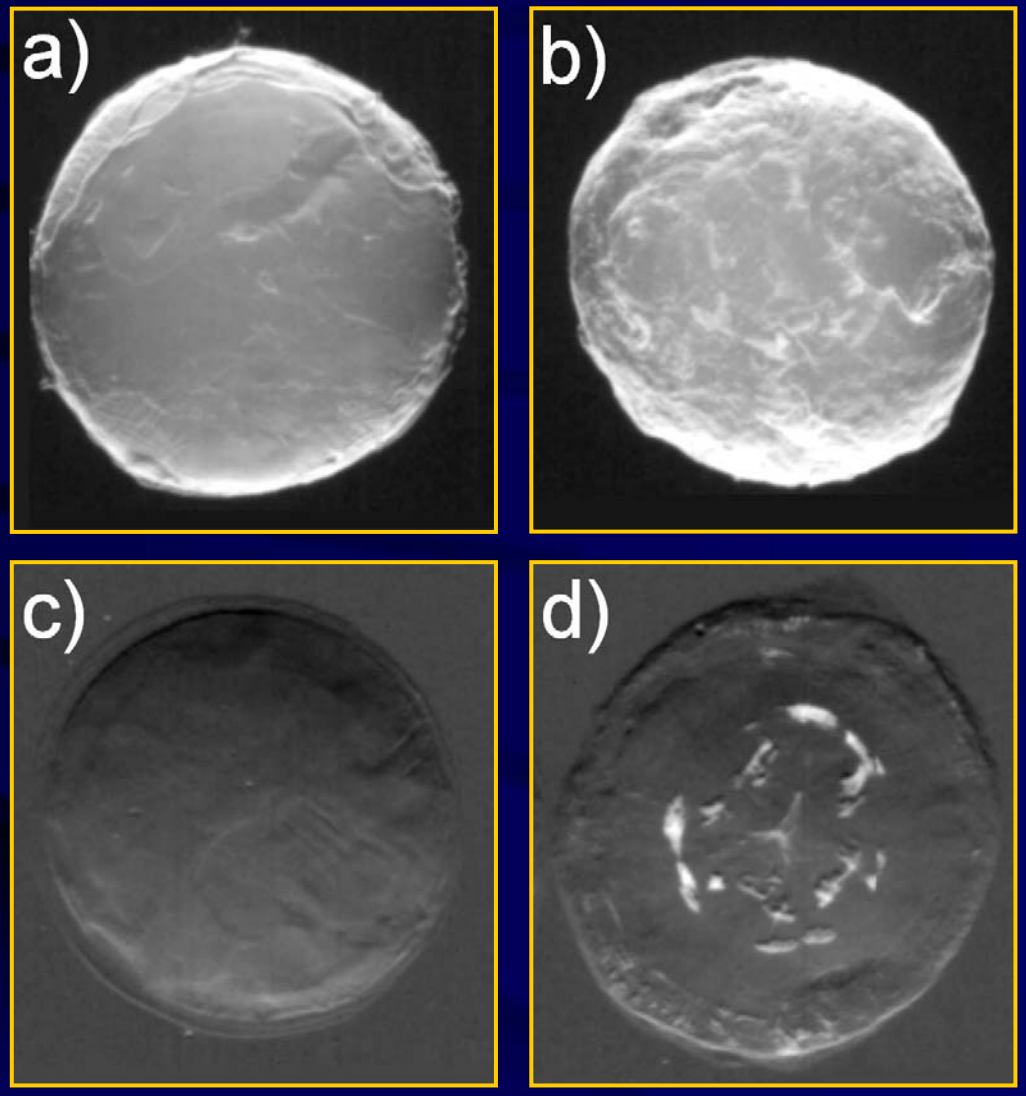


"High contrast radiography of normal and cataractous canine lenses"
A. Antunes, M.G. Hönnicke,
C. Cusatis and S.L. Morelhão
J. Phys. D: Appl. Phys. **38** A85-A88 (2005)

"Diffraction Enhanced X-Ray Imaging of Mammals Crystalline Lens"
A. Antunes, M. G. Hönnicke,
A. M. V. Safatle, C. Cusatis,
P. S. Moraes Barros and S. L. Morelhão
Nucl. Instr. Meth. B **238**, 28-31 (2005)

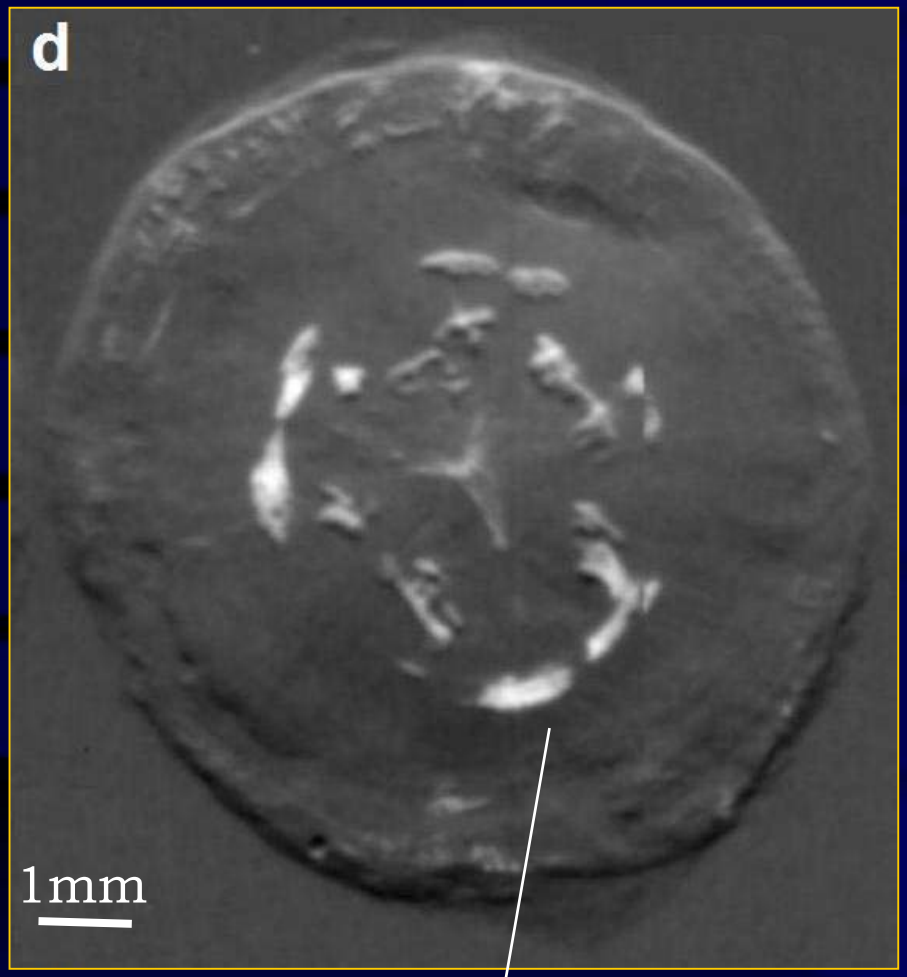
cial support: CNPq, Fapesp and PRP-USP.

DEI of entire lenses (@ 20 KeV)

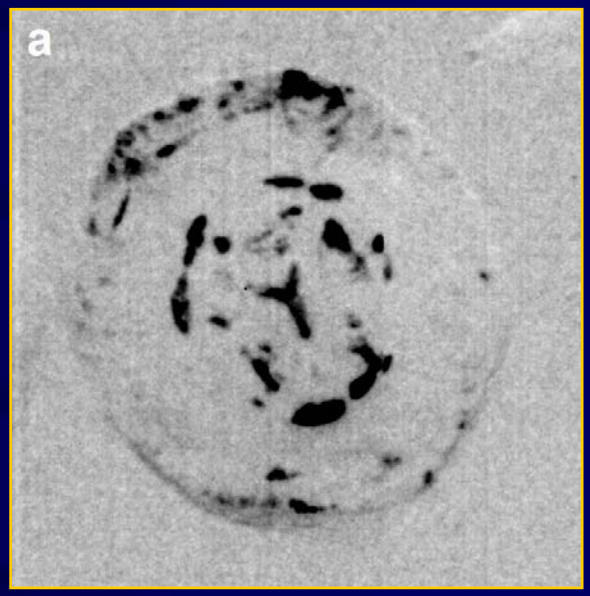


Med. Phys. **33**, 2338 (2006)

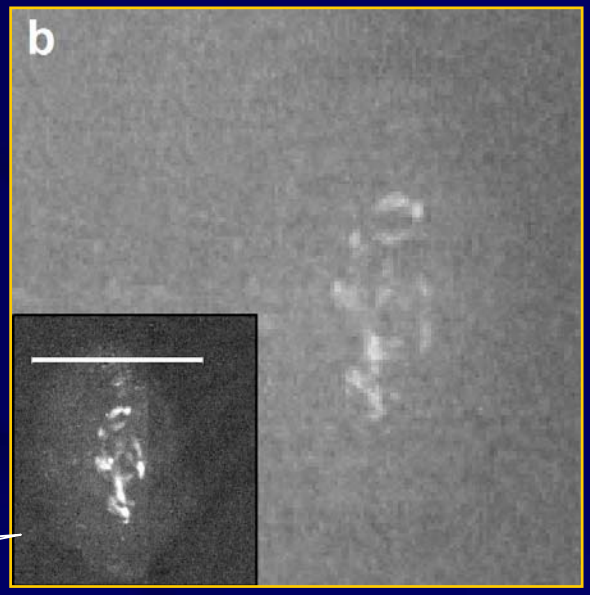
Discovery of calcificated tissue in the eye lens (canine)



Calcificated tissues
(Ca, Micro X-ray fluorescence)
Refraction: $\Delta\theta$ = -1.2 μrad



Diffuse scattering:
$\Delta\theta$ = -4.5μrad

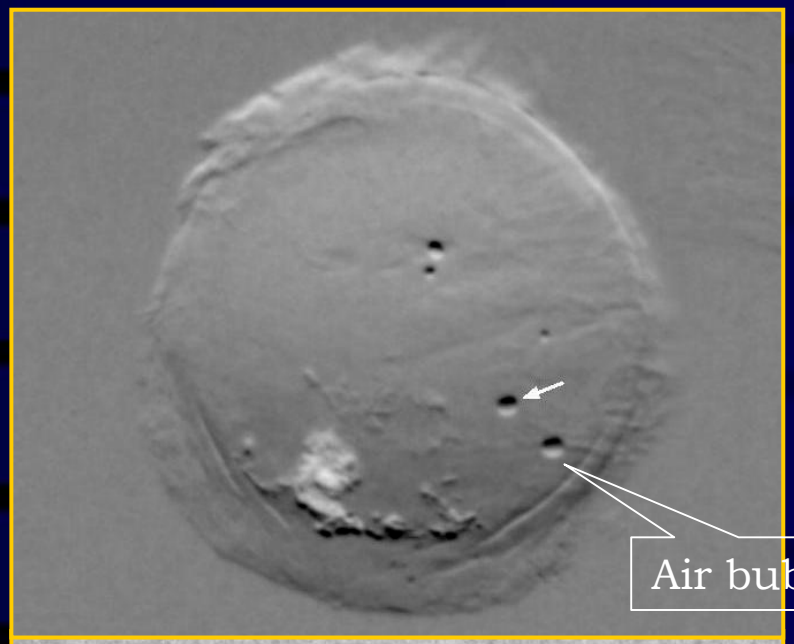


Absorption (before analyzer)

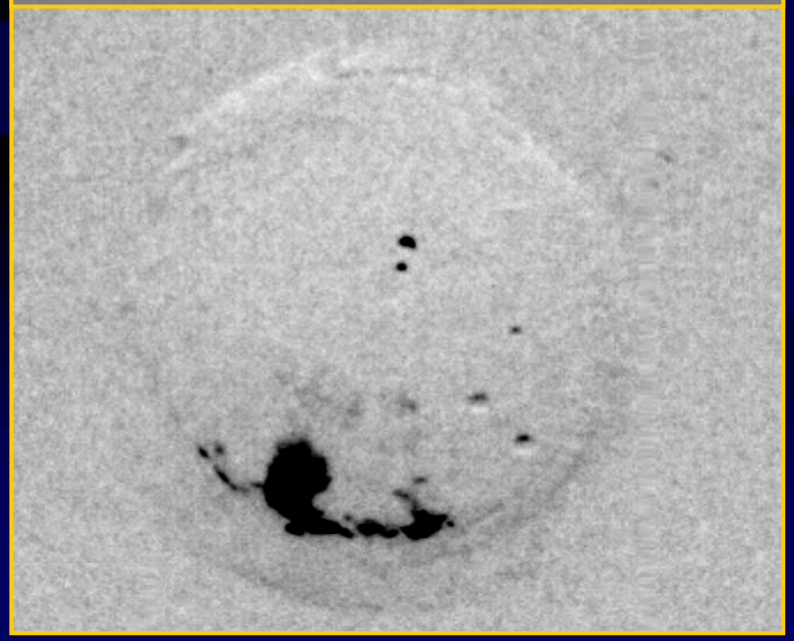
Side view

Mass absorption image

Localized calcification and the partial cataract cases



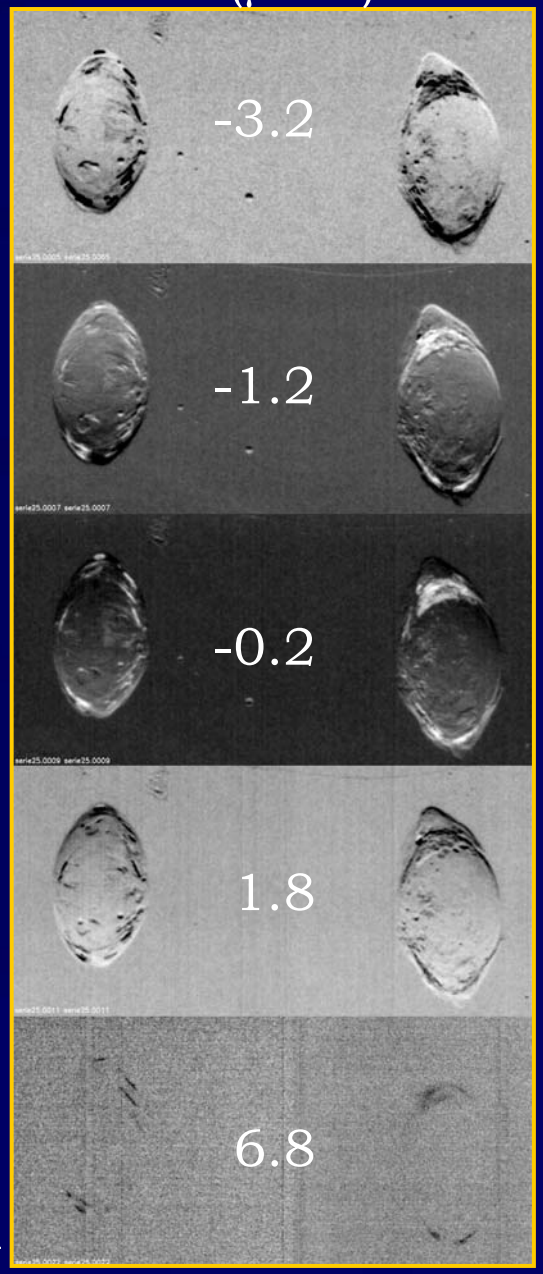
Refraction:
$\Delta\theta$ = -1.5 μrad



Diffuse scattering:
$\Delta\theta$ = -4.5μrad

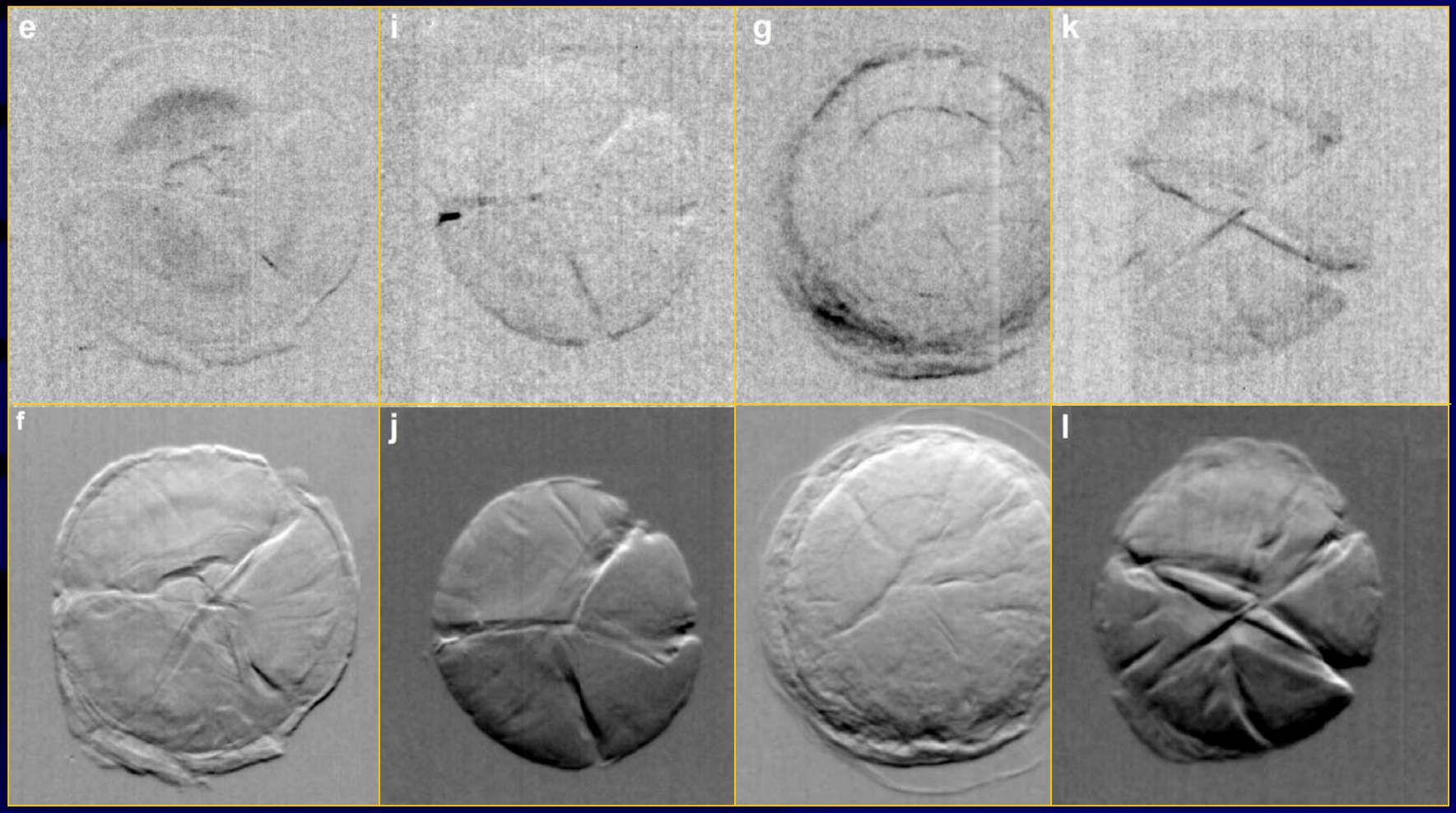
$D < 5\mu m$

$\Delta\theta$ (μrad)



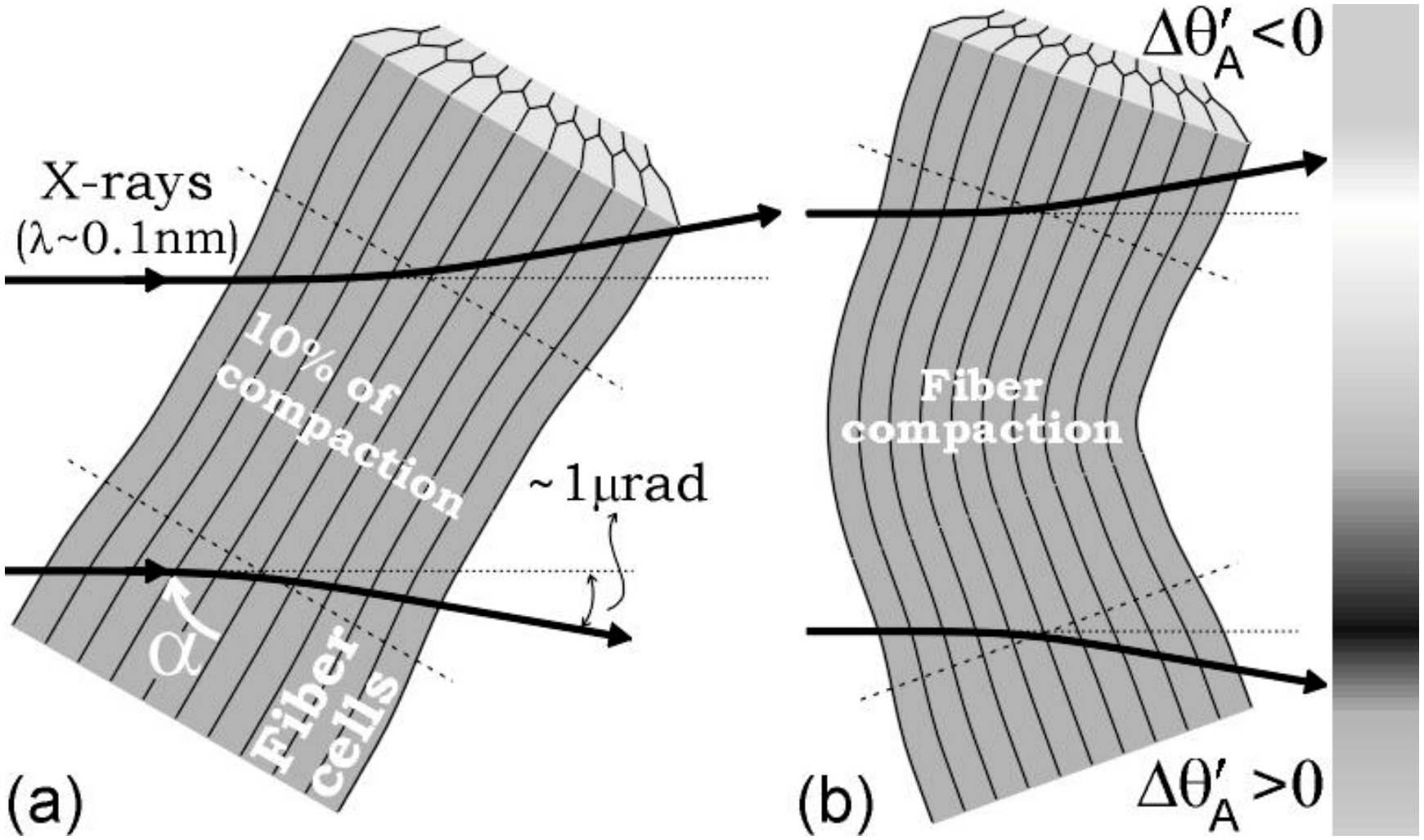
Fiber cell compaction and the total cataract cases

Diffusion scattering: $-5\mu rad < \Delta\theta < -4\ \mu rad$



Refraction: $-2\mu rad < \Delta\theta < -1\mu rad$

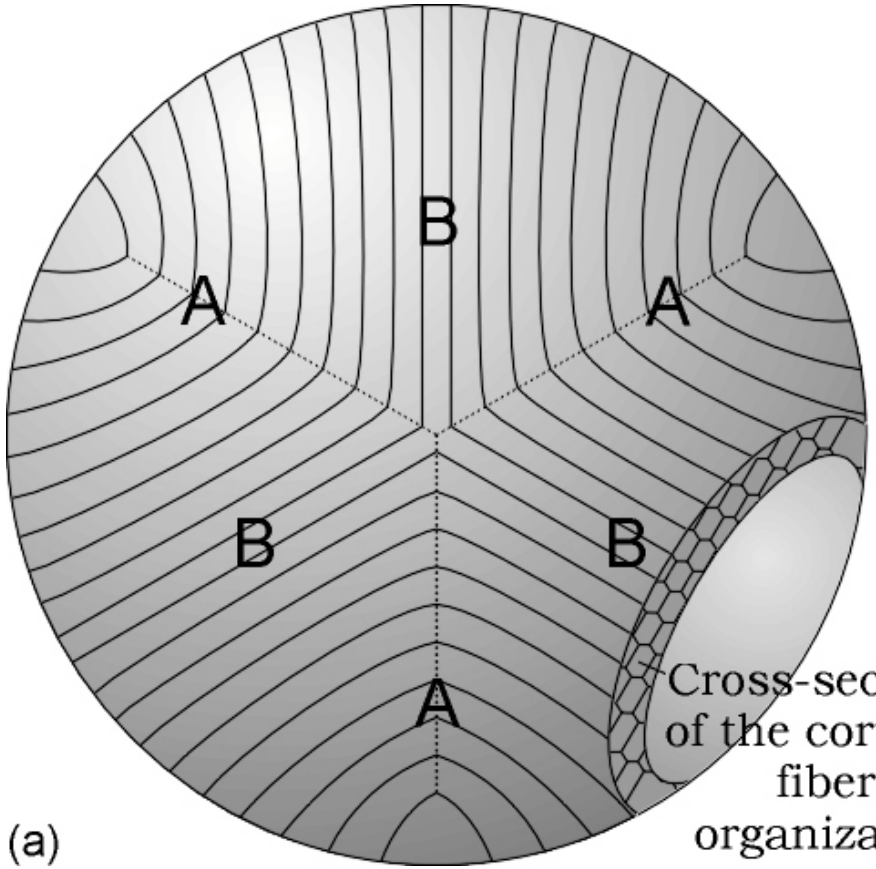
White/dark contrast due to compaction of fiber cells



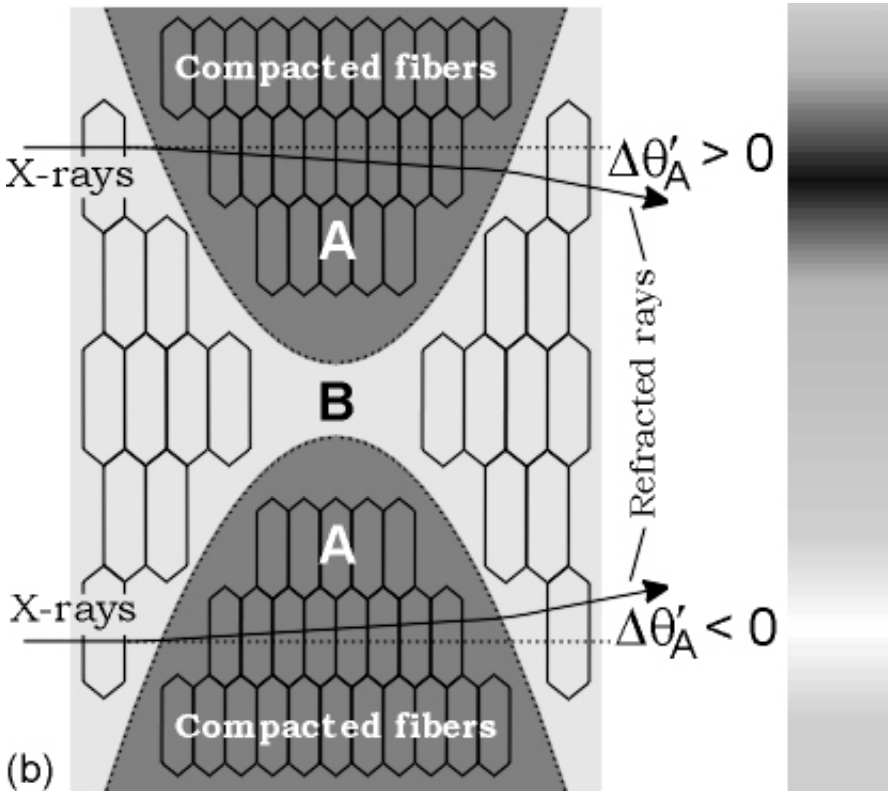
Left shoulder of the analyzer window

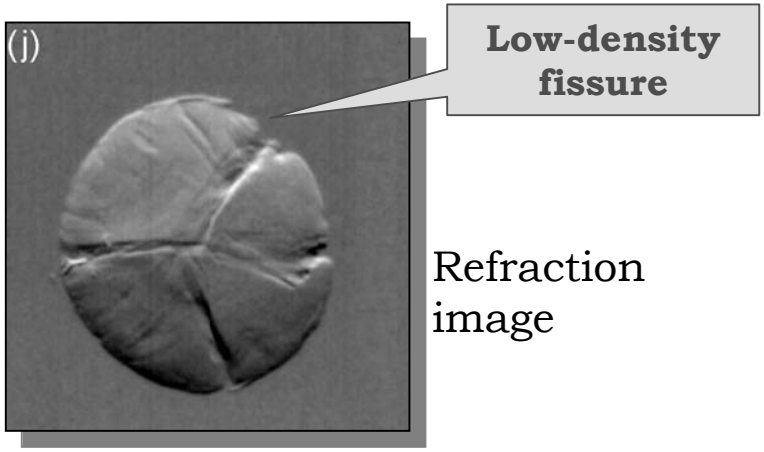
Cortical fiber-cell organization and low-density fissures

Size reduction of fiber-cells promotes compaction towards sites A and low-density fissures at sites B.



Cross-section of the cortical fiber-cell organization





Refraction image

Classification scheme of cataract by DEI

3D configuration of lesions

- localization of calcificated tissues, i.e. nuclear/cortical
- extension of fiber compaction

Correlations to be established

- ophthalmic exams (partial/total opacity) and type of lesions
- calcification:
  inductive agents and susceptible mammal species
- potential causes of cataract and type of lesions
- information from other techniques

Multi-disciplinary approach

- to fully understand the disease
- histological procedures; microscopic analysis
- UV and Raman spectroscopy; protein/molecular content
- X-ray fluorescence; elemental analysis

Researchers and Institutes

Dr. Andrea Antunes

- 4 years of Young Researcher fellowship, FAPESP
- to explore the multi-disciplinary aspect of the ophthalmic diseases
- Institute of Physics, Dept. Applied Physics, USP

Prof. Paulo S.M. Barros and Dr. Angélica Safatle

- Faculty of Veterinary Medicine, USP
- cataract surgery in mammals
- patient clinical records
- ophthalmic classification, i.e. extension of opacity

Dr. Maria Ines Borella

- Institute of Biomedical Sciences, USP
- histological analysis
- lab. for handling tissues (microtome)

Researchers and Institutes

Dr. Marcia Temperini

- Institute of Chemistry, USP
- Raman spectroscopy
- *Laser Phys. Lett.* **2**, 356 (2005)

Prof. Maria Luisa de Carvalho

- University of Lisbon, Portugal
- Institute of Atomic Physics
- X-ray fluorescence

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  U.S. Department of Energy
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