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: eye lens opacity ...

Opacity: visible light scattering ...

Scattering centers of visible light?

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

Physiological changes

Causes: aging, drugs, diabetes, congenital, ...

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

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

Optical / Electron microscopies are limited for investigating:

- distributions of scattering center and density fluctuations
- on extended tissues (cm scale/entire lenses)

Nuclear fiber cell compaction...
Christopher D. Freel

Images of human cataractous lens...
W.L. Jongebloed.
**Why X-rays?**

**X-ray can see through entire lenses:**
- minimum of scattering (angular spreading $\approx \frac{\lambda}{D}$)
- refraction and diffusion scattering are **angular resolved** processes
- selective mapping of:
  - density fluctuations and
  - distributions of scattering centers

**Refraction**

\[
\Delta \alpha = \frac{\Delta n}{n} \tan \alpha \quad \text{(Snell's Law)}
\]

\[
n = 1 - \delta \quad \text{(index of refraction)}
\]

\[
\delta = \Gamma \rho_e \approx 10^{-5}, \ E \approx 20\text{keV}
\]

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

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

\[
\Delta \alpha \approx 1\mu\text{rad}
\]
Refraction

Half width $= 0.58 \frac{\lambda}{D}$
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) ($\Delta I/I \sim 30\%$ per $\mu$rad)

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

FWHM = $3.2\mu$rad @ 18keV / $1.5\mu$rad @ 40 keV

"High contrast radiography of normal and cataractous canine lenses"
A. Antunes, M.G. Hönnicke, C. Cusatis and S.L. Morelhão

"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

FWHM = 50μrad
DEI of entire lenses (@ 20 KeV)

Discovery of calcificated tissue in the eye lens (canine)

Calcificated tissues (Ca, Micro X-ray fluorescence)
Refraction: $\Delta \theta = -1.2 \, \mu\text{rad}$

Diffuse scattering:
$\Delta \theta = -4.5 \, \mu\text{rad}$

Mass absorption image
Localized calcification and the partial cataract cases

Refraction:
\[ \Delta \theta = -1.5 \text{ } \mu \text{rad} \]

Diffuse scattering:
\[ \Delta \theta = -4.5 \mu \text{rad} \]

Air bubble

\[ D < 5 \mu \text{m} \]
Fiber cell compaction and the total cataract cases

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

Refraction: $-2 \mu \text{rad} < \Delta \theta < -1 \mu \text{rad}$
White/dark contrast due to compaction of fiber cells

(a) X-rays ($\lambda \sim 0.1\,\text{nm}$) with $10\%$ of compaction and $\sim 1\mu\text{rad}$

(b) Fiber compaction with $\Delta\theta_A' < 0$

Left shoulder of the analyzer window
Size reduction of fiber-cells promotes compaction towards sites A and low-density fissures at sites B.
3D configuration of lesions
- localization of calcificated tissues, i.e. nuclear/cortical
- extension of fiber compaction

Correlations to be established
- ophthalmmic 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)
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