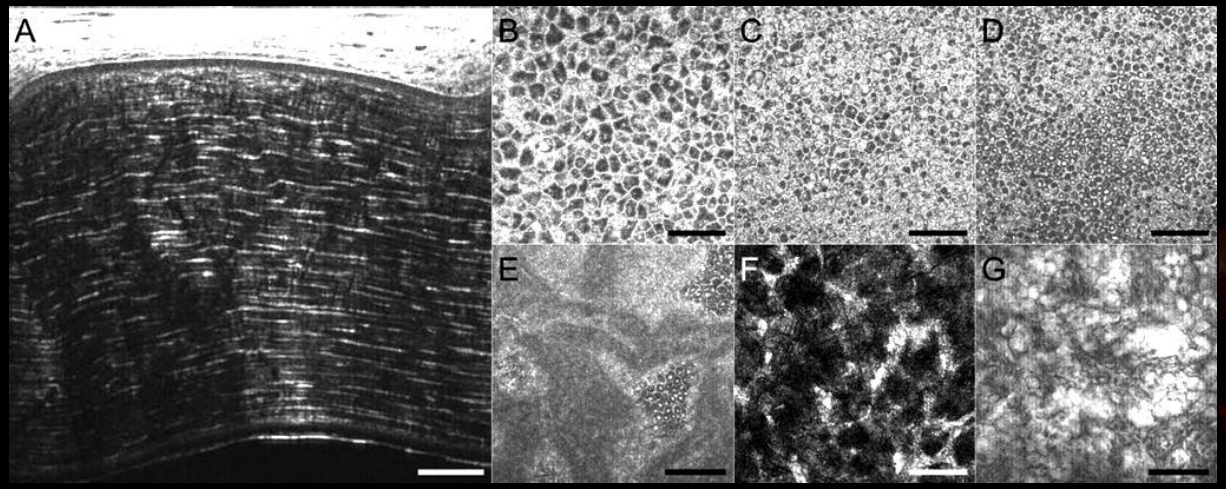
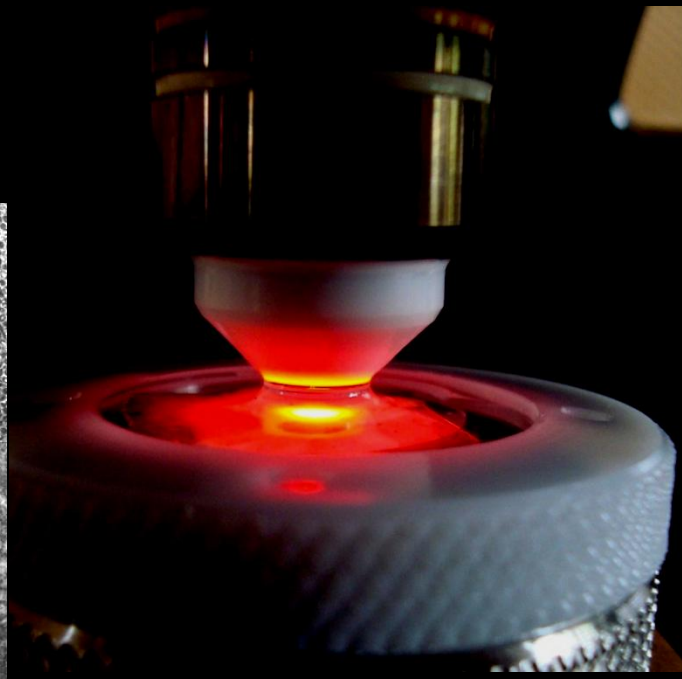
MicroEye
3D microscopy of ocular tissues using full-field OCT

Kate Grieve (Institut de la Vision), Laurent Vinet (CHNO des XV-XX)

Partners:
Michel Pâques (CHNO des XV-XX)
Odile Guerin (EFS Cornea Bank)
Vincent Borderie (Institut de la Vision)



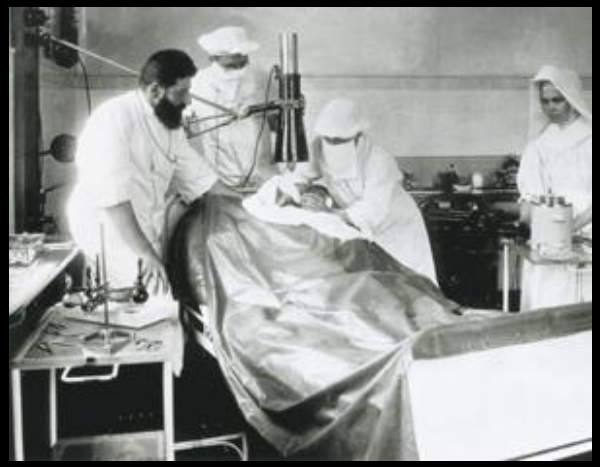


Imaging graft, cultured and surgical tissues

1. Preoperative evaluation of graft tissues
2. Evaluation of bioengineered artificial corneas
3. Check tissues removed during retinal and cataract surgery

Background - Corneal grafts

- First corneal graft: 1905 (Dr Zim)



- Regular improvement of the technique with:
  - Technological advancements (imaging, surgical tools)
  - Diversification of surgical techniques (TK, PLK, DSEK, DMEK)
  - Eye banks

Eye banks – current process (cornea)

Handling and processing

1. Graft collection and transportation to the Eye bank
2. Graft preservation and qualification of the donation
3. Corneal deturgescence & transportation to the operating room

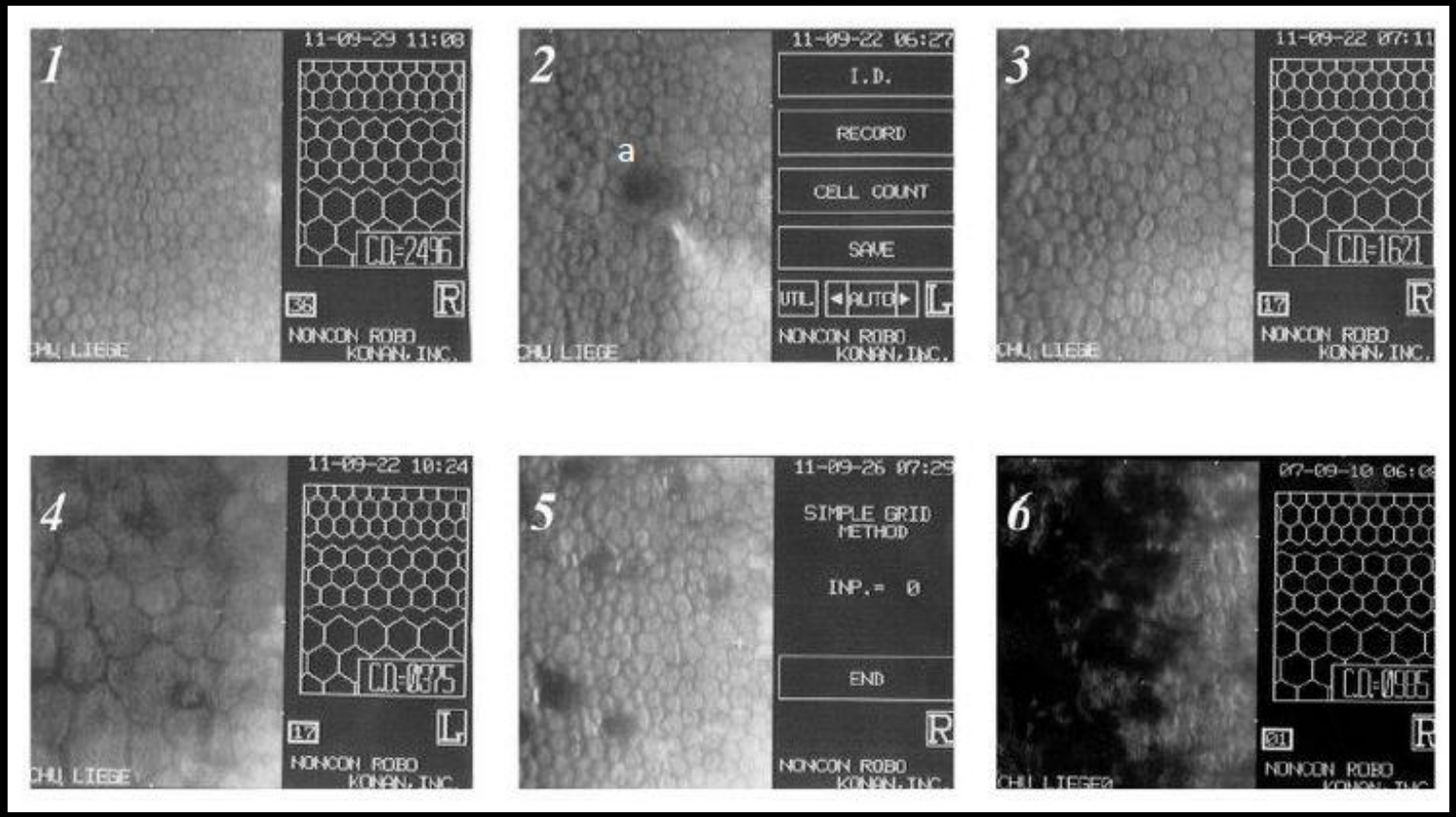
Quality control – qualification of the donation

1. Microbiological examination
2. Sterility of the conservation and transport medium
3. Graft quality:
   - Endothelial cell density
   - transparency

Limited control parameters,

in particular for stromal characteristics

Endothelial cell density - microscopy



1 & 2 – acceptable density (> 2200 cells/mm²) - a: dead cell

3 & 4 – rejected cell density

5 & 6 – cornea guttata

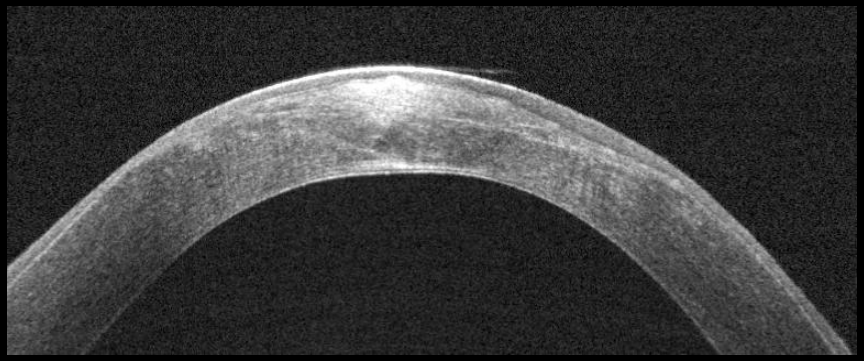
Eye banks – current process (cornea)

- Success rate of corneal grafts procedures: 60 – 90%
- Studies in Eye Banks show significant errors in the measurement of endothelial cell density (from 40 % undervaluation to 82% overvaluation) source: EFS statistics 2010 - France
- Rejection rate in the cornea qualification process rises up to 57% source: EFS statistics 2010 - France

Significant room for understanding/improvement of current procedures (requalification of initially rejected samples, rejection of initially qualified samples)

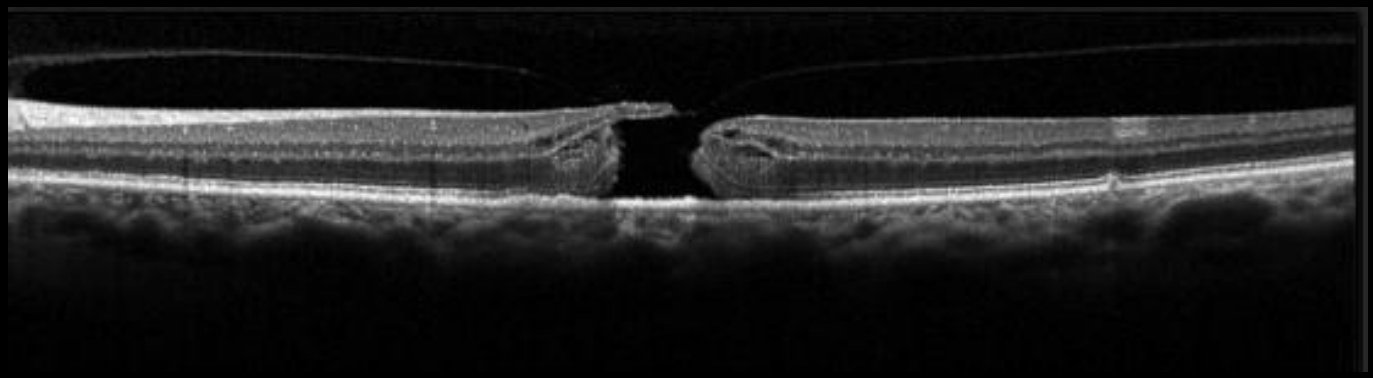
Ophthalmic OCT

Conventional ophthalmic OCT fails to image cellular morphology

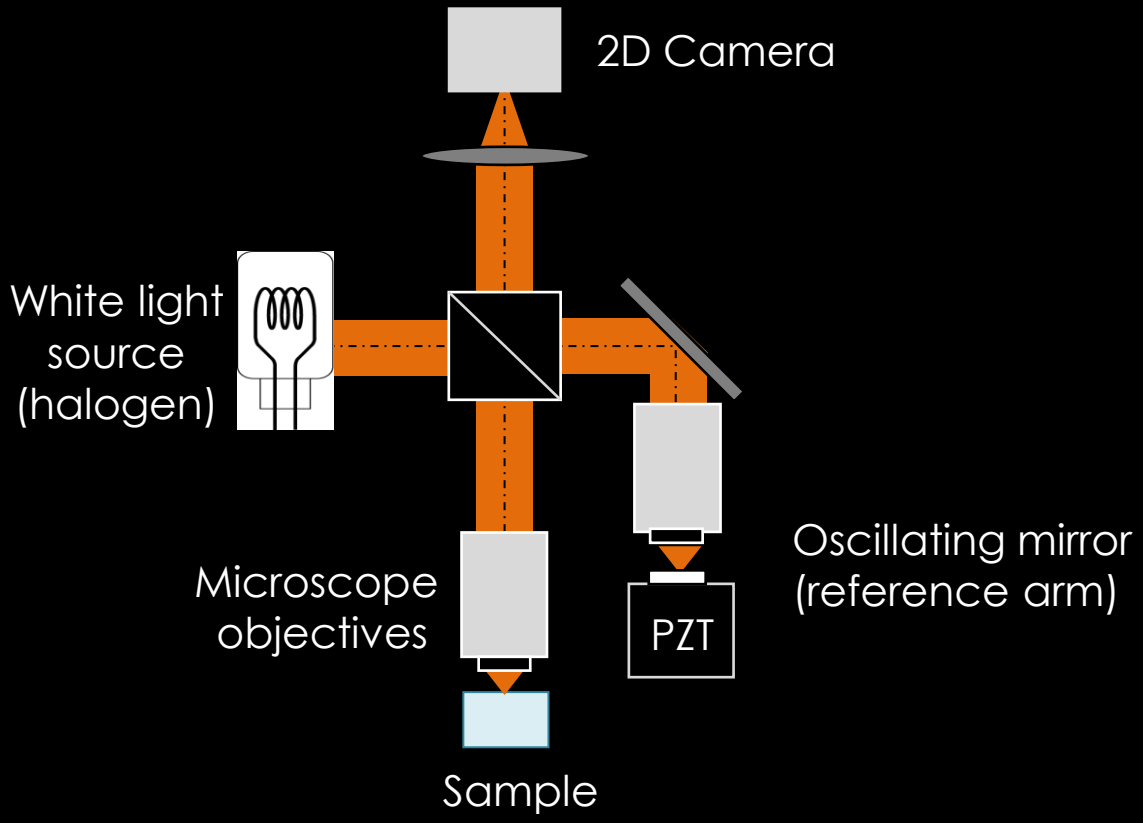


Keratoconus

Macular hole



Full Field Optical Coherence Tomography



- High-resolution en face (C-scan) imaging = similar to pathology slides
- Usually based on a Linnik interferometer
- ~ 1 µm isotropic 3D resolution
- Large fields possible (unitary field of view 800 x 800 µm)
- 5x5mm in 2 minutes

A. Dubois, K. Grieve, G. Moneron, R. Lecaque, L. Vabre, C. Boccara
Appl Opt 2004;43:2874-83.

Commercial FFOCT microscope

Light-CT™ scanner,

LLTech, France

- resolution: 1.5µm transverse, 1µm axial
- non-invasive, non-destructive
- 70Hz max tomographic frame rate – 1M voxels
- penetration depth 200µm – 1mm depending on tissue
- 3D DICOM viewer : 3D reconstruction, MPR navigation
- 25mm diameter max. sample size

Pilot study – material and methods

Histology:

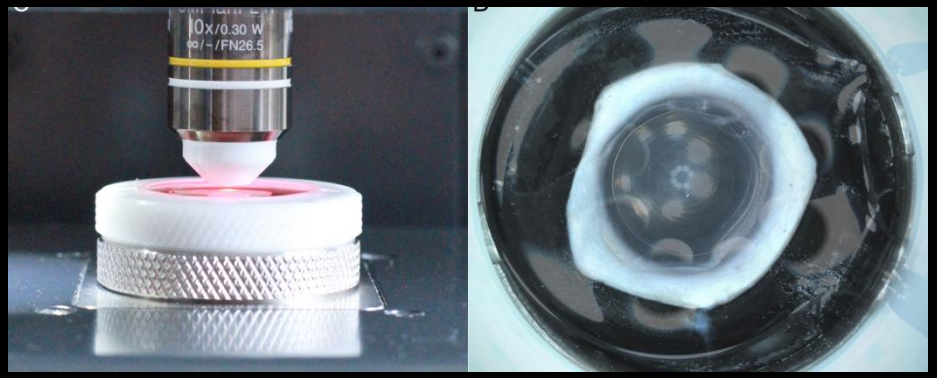
- Conventional histology process
- formaldehyde fixation (10%)
- paraffin inclusion
- 4µm slices (microtome)
- HES/PAS coloration
- Microscopic examination

Spectral domain OCT:

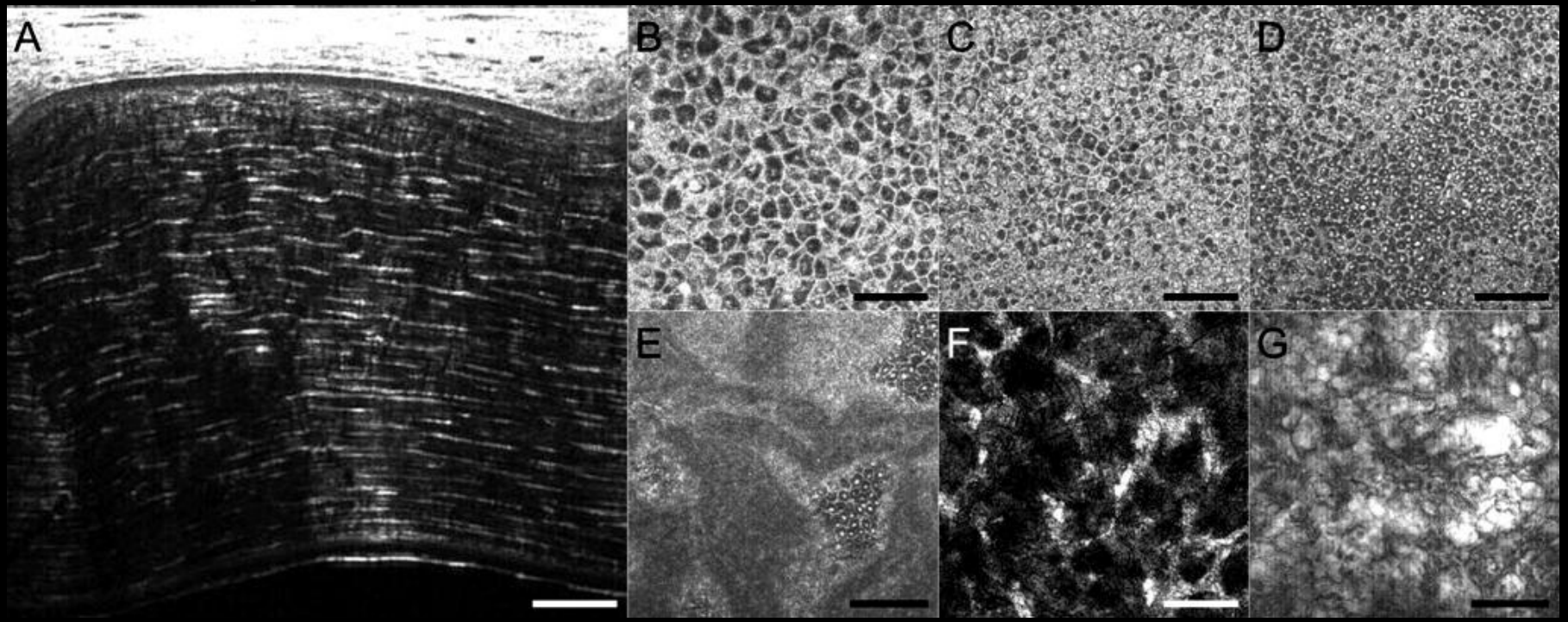
- SD-OCT Optovue
- transverse resolution 8µm
- axial resolution 5µm
- non-contact imaging

FFOCT:

- Encapsulation into sample holder
- immersion microscope

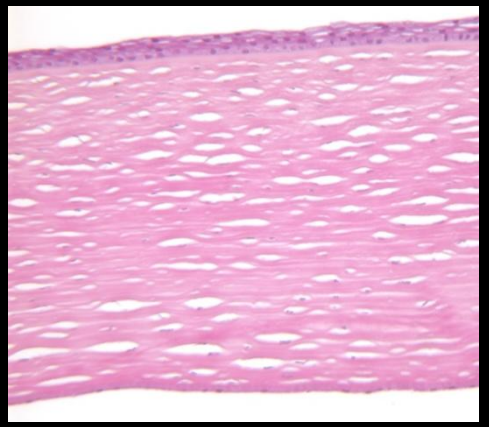


Results – ‘normal’cornea



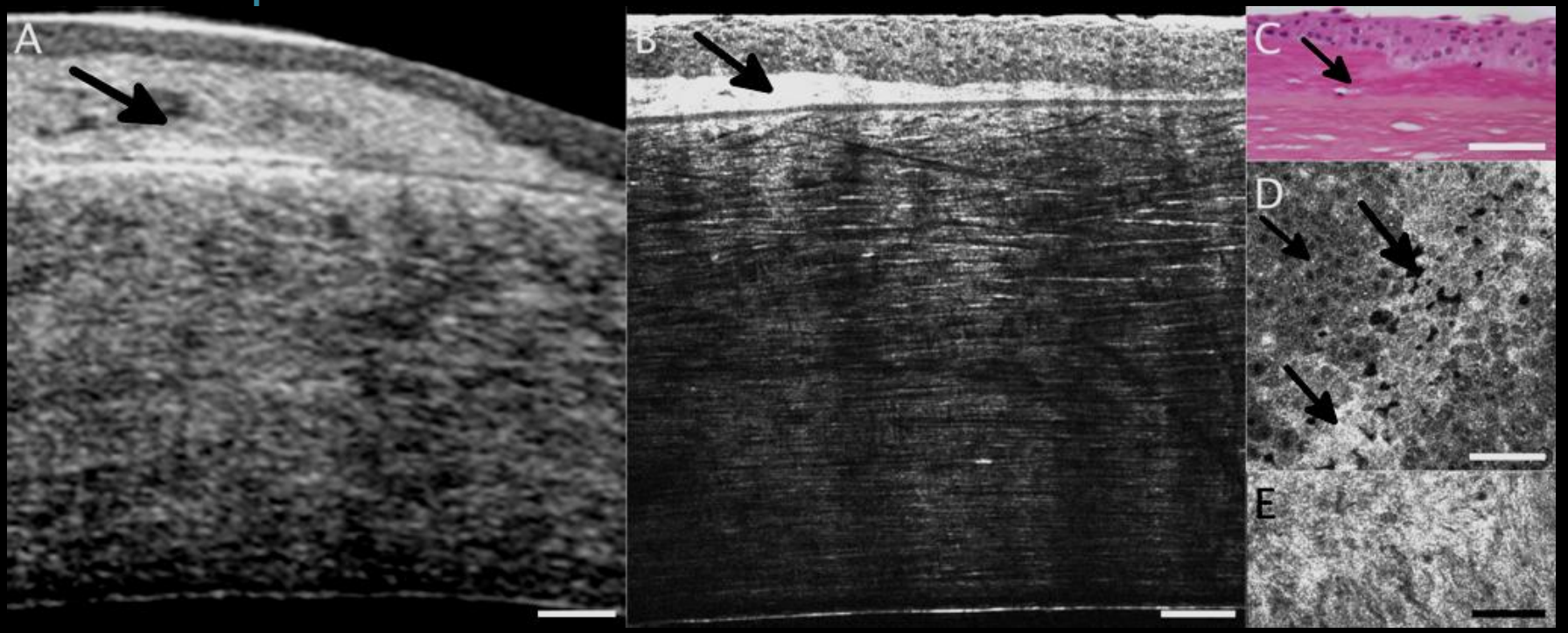
A: cross sectional slice,
B surface epithelium,
C intermediary epithelial layer,
D basal epithelial layer,

E Bowman’s membrane,
F stromal keratocytes,
G endothelial cells revealed by decreasing axial resolution (sum of ten 1 µm thick slices)



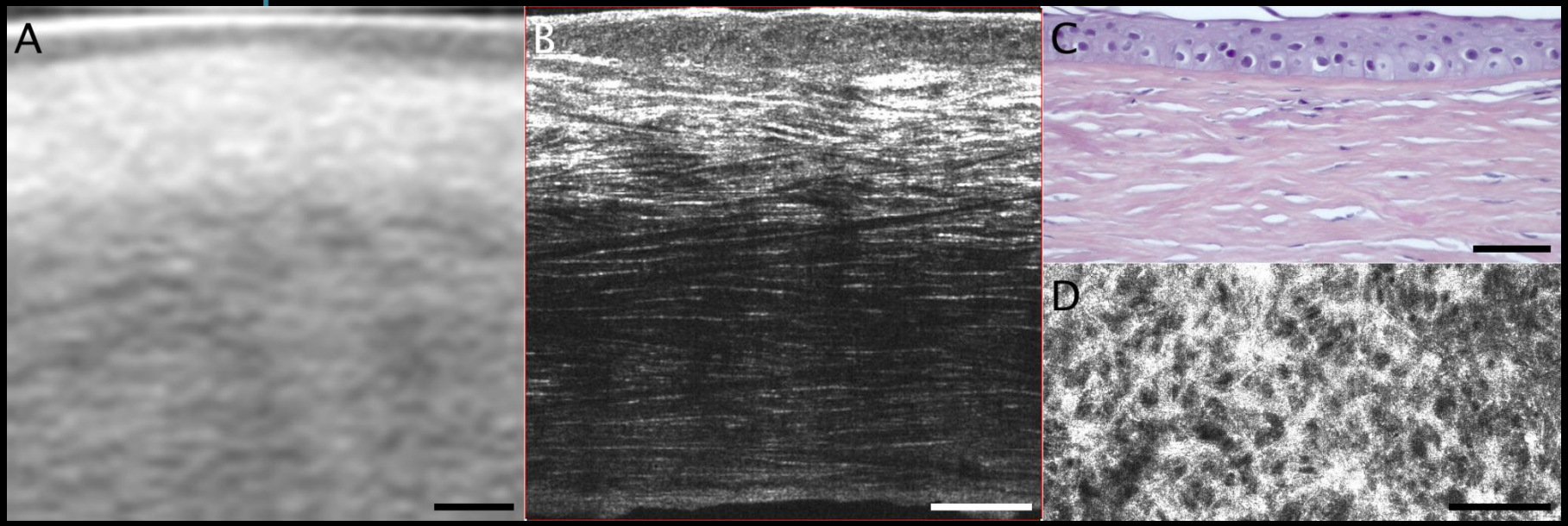
W. Ghouali, K. Grieve, L. Laroche, F. Harms, M. Paques, V. Borderie
IOVS, E-abstract 2013

Bullous keratopathy



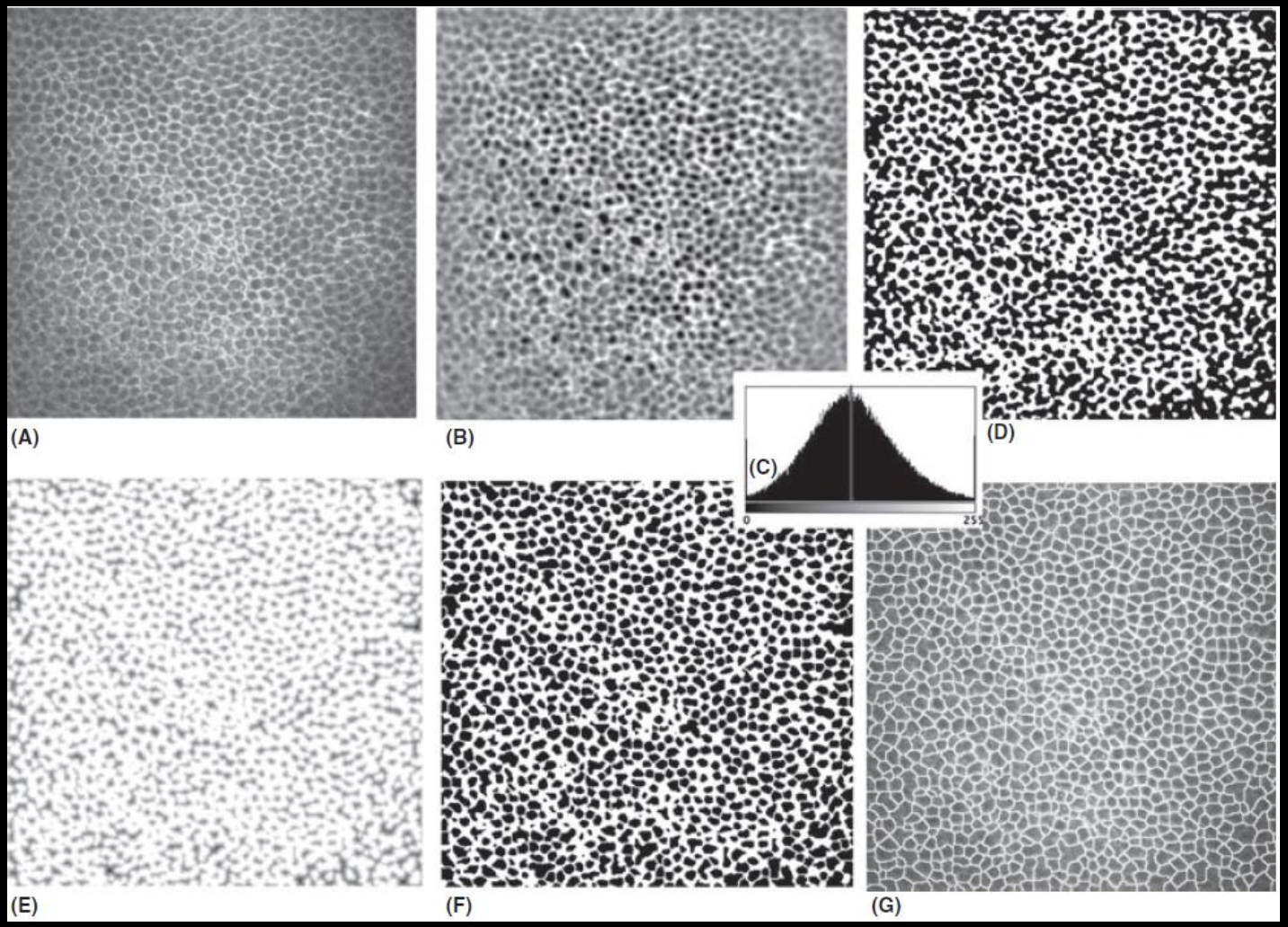
A : portion of a spectral domain OCT image of the cornea before keratoplasty.
B : corresponding FF-OCT view of this area ex vivo. Arrows in A, B and C : sub-epithelial fibrosis. Bowman's layer is clearly seen under the area of sub-epithelial fibrosis.
C : corresponding histology.
D : "en face" view in the upper epithelium. Arrows : intraepithelial edema which is seen as dark spaces (top arrow), grey wing cell layer (middle arrow) and bright surface epithelial cells (lower arrow).
E : "en face" view located in the area of subepithelial fibrosis (bright white area).
Scale bars show 200um (A, B) and 100um (C, D, E)

Lattice corneal dystrophy



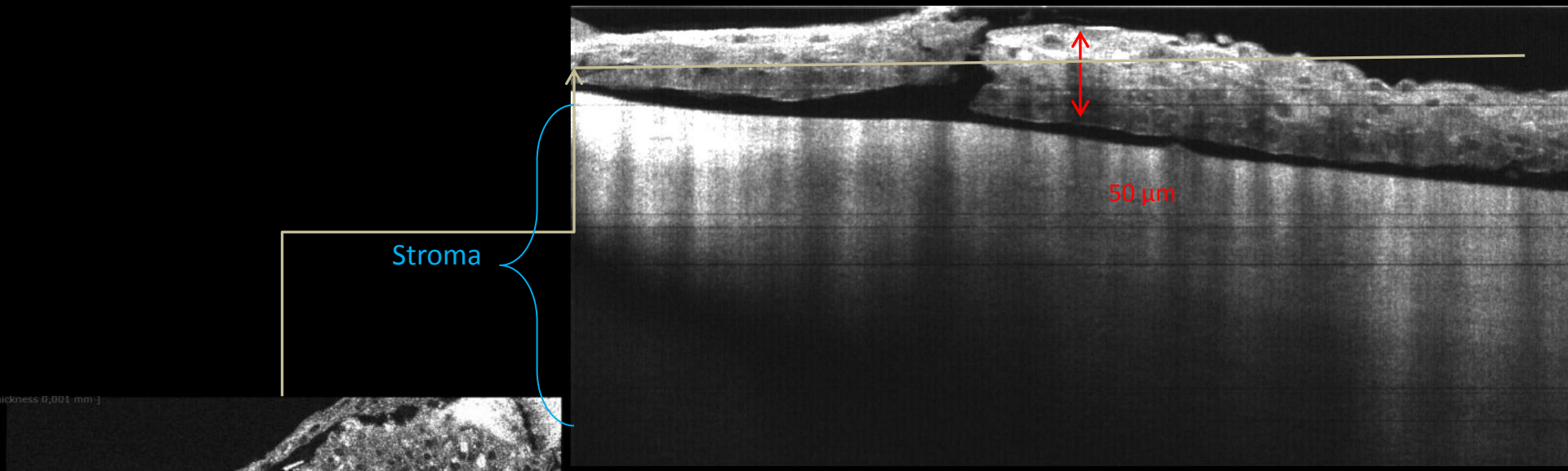
A : portion of a spectral domain OCT image before keratoplasty.
B : corresponding FF-OCT cross section in the *ex vivo* cornea.
C : corresponding HES histology.
The high density of keratocytes in the upper stroma can be seen in cross sectional (B) and "en face" (D) views. The **filament deposits** below the epithelium can be seen in D.
Scale bars : 200um.

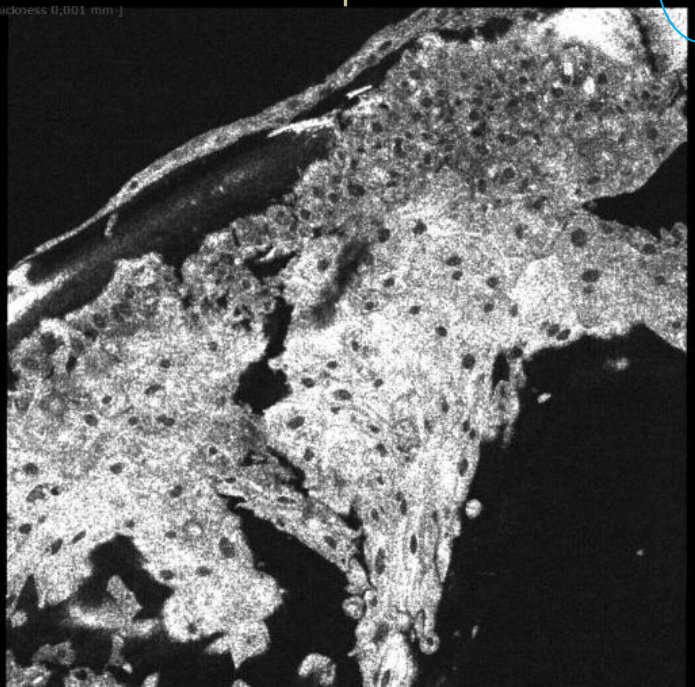
Automated cell segmentation



J. Bullet, T. Gaujoux, V. Borderie, I. Bloch, L. Laroche
Acta Ophthalmol.2013 doi: 10.1111/aos.12304

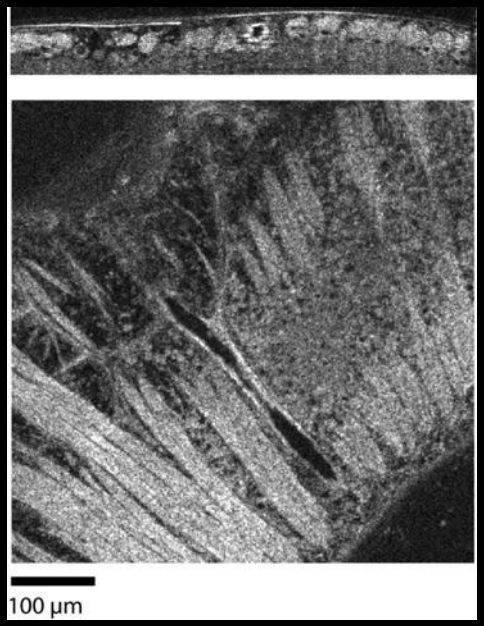
Bioengineered cornea



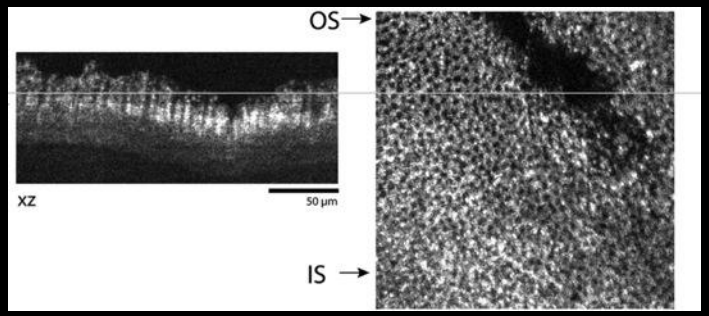


D Ghoubay-Benallaoua, L Laroche, V Borderie et al
PlosOne 2013
Curr Eye Res 2012
Mol Vis 2011

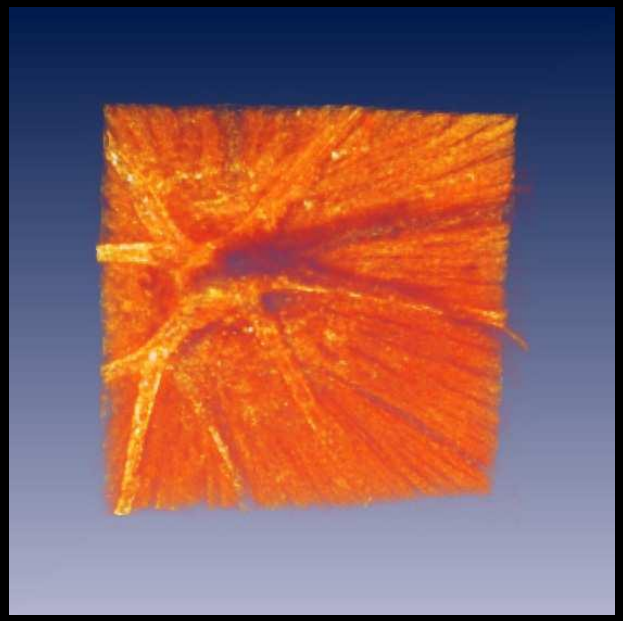
Retina



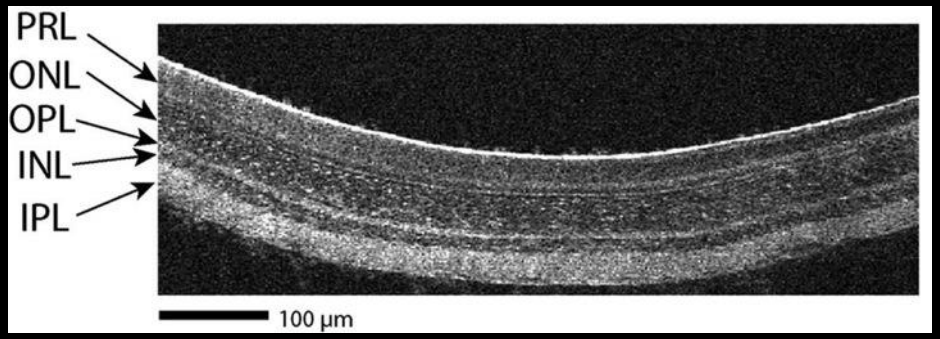
Nerve fiber layer - rat



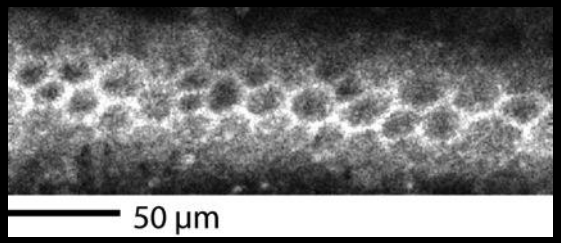
Photoreceptors - pig



Optic Nerve - pig



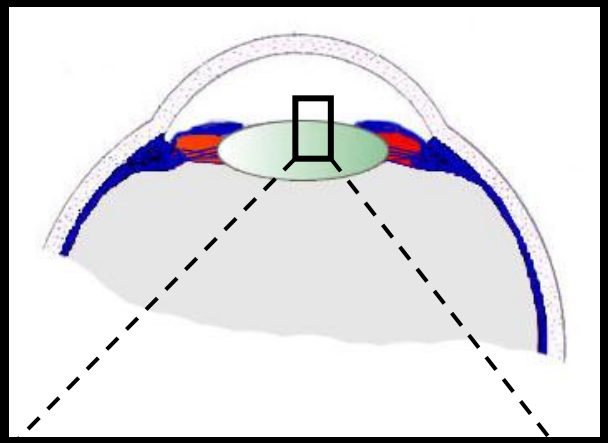
Retina - rat

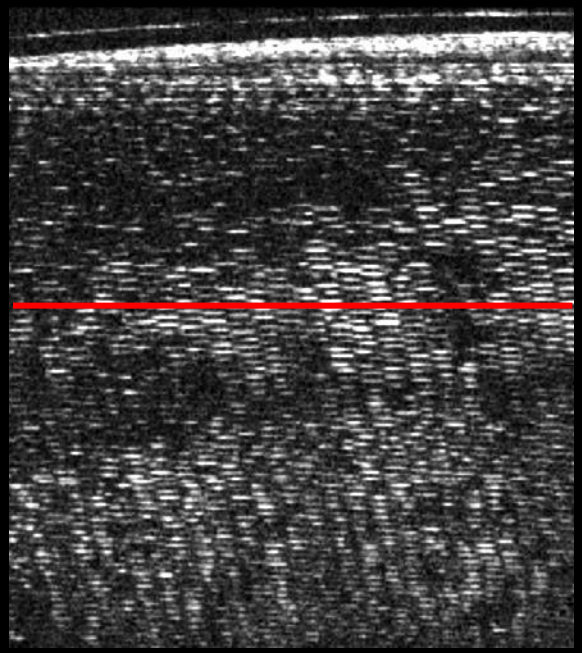


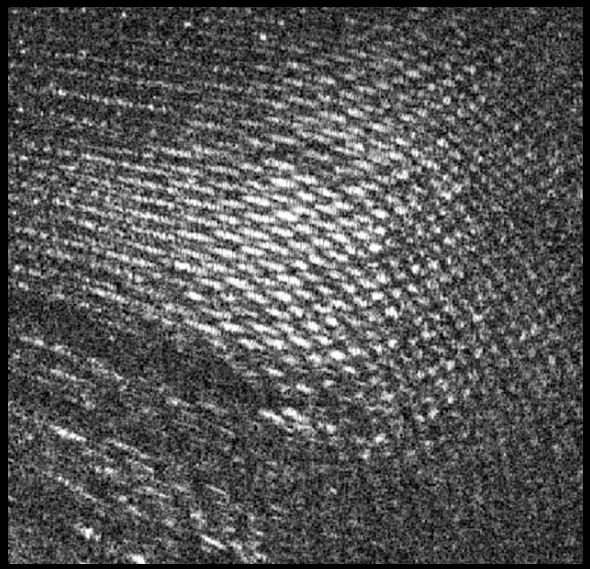
Retinal pigment epithelium - pig

K Grieve, M Paques et al *IOVS 2004 Nov;45(11):4126-31.*

Lens







MicroEye: perspectives

1. Provide a new means of evaluating graft tissues leading to improved graft success and donor acceptance rate

2. Provide a means to evaluate bioengineered corneas

3. Histology-like imaging of surgically removed tissues enables
   - Evaluation of surgical accuracy and success
   - Basic knowledge of 3D structure of tissues (eg lens: cataractogenesis)

INSTITUT DE LA VISION PARIS

EFS ÉTABLISSEMENT FRANÇAIS DU SANG

QUINZE-VINGTS CENTRE HOSPITALIER NATIONAL D'OPHTALMOLOGIE