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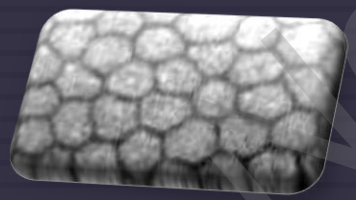
in conjunction with
the 5th Asia Orthokeratology and
Specialty Lens Conference (AOSLC 2016)
December 9 (Fri) - 11 (Sun), 2016
Sheraton Grande Walkerhill · Seoul, Korea



CORNEAL ENDOTHELIAL IMAGING & ANALYSIS

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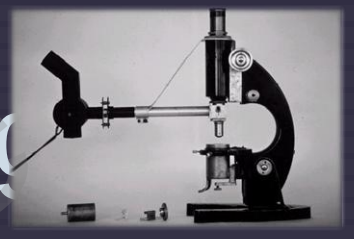
History of endothelial imaging

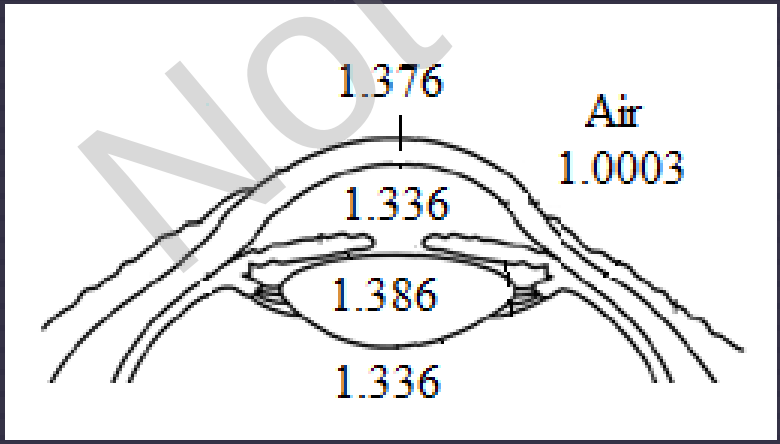


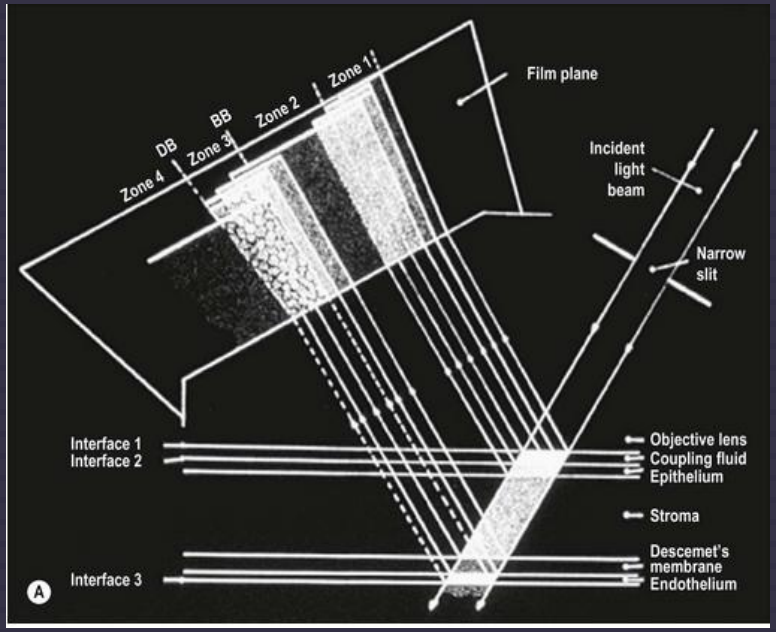
- 1918. Vogt A: direct visualization of the endothelium
- 1924. Graves B: description of Fuchs' endothelial dystrophy
- 1968. Maurice DM: first laboratory specular microscope
- 1975. Laing RA: in vivo photomicrography of the corneal endothelium in rabbits
- 1976. Bourne WM & Kaufman HE: specular microscopy of human corneal endothelium in vivo

Mannis MJ & Mannis AA, eds. *Corneal Transplantation: A History in Profiles,* Belgium, 1999

Optical background

- Specular reflection: light is reflected from the interfaces of materials with different indices of refraction.
  - mirrorlike way: angle of incidence = angle of reflection
- The difference in index of refraction between surfaces↑ ≈ the intensity of reflected light↑





Types of specular microscopes

CONTACT (CSM)

- Objective cone applanates the cornea
  - resulting in a flat surface (angle of incidence = angle of reflection)
- The cone may compress the precorneal tear film → the light passes through only the corneal layers

NONCONTACT (NCSM)

- Autofocus without changing the corneal surface
  - endothelial image is affected by the corneal curvature
- 2 additional refractive media may affect the refraction and the specular image

Endothelial cell morphometry

- Objective description of the features of a selected cluster
  - Cell Area ± SD (µm²)
  - Cell Density (cells/mm²) →
    - 2400-3000 cells/mm²
  - Polymegethism (CV) →
    - 0.25-0.31
  - Pleomorphism (% 6 sided)
    - 60-80%
- Central and peripheral cornea

$$\frac{\text{cells}}{\text{mm}^2} = \frac{1 \times 10^6 \ \frac{\mu\text{m}^2}{\text{mm}^2}}{\text{Mean cell area, } \mu\text{m}^2}$$

$$CV = \frac{SD_{\text{cell area}}}{\text{Mean cell area, } \mu\text{m}^2}$$

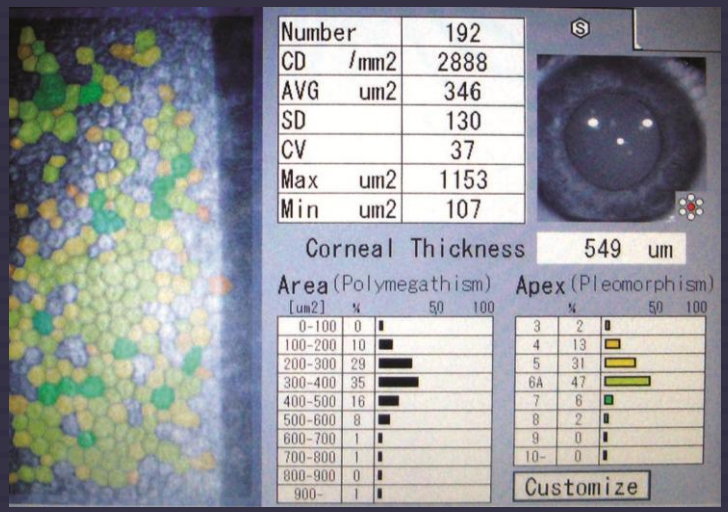
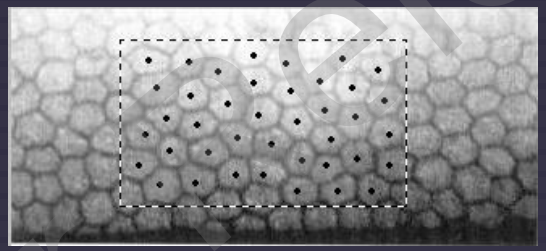
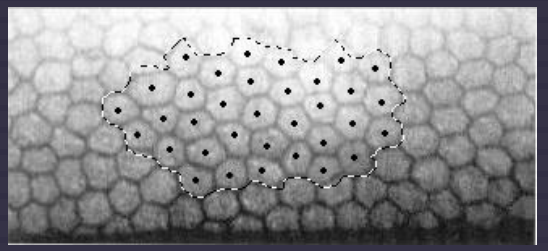


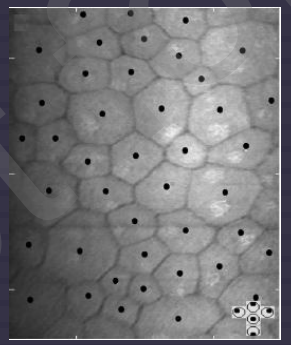
Image analysis I.

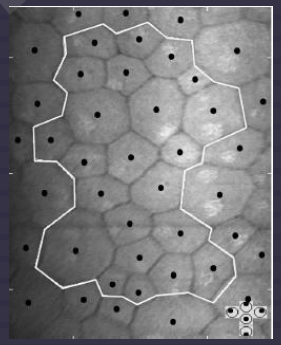
- Automated, semiautomated, manual
- Frame method:
  - Fixed frame
  - Variable frame

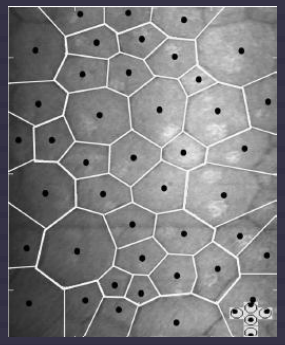


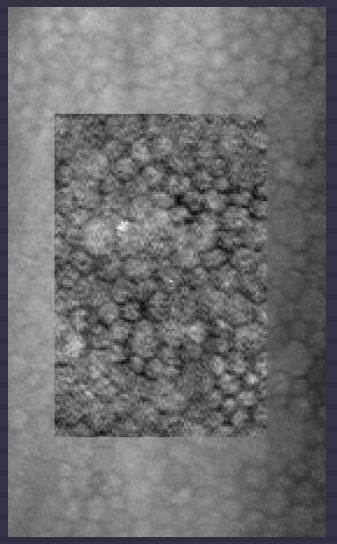


- Konan Inc.
  - Center
  - Flex-center
  - Corner





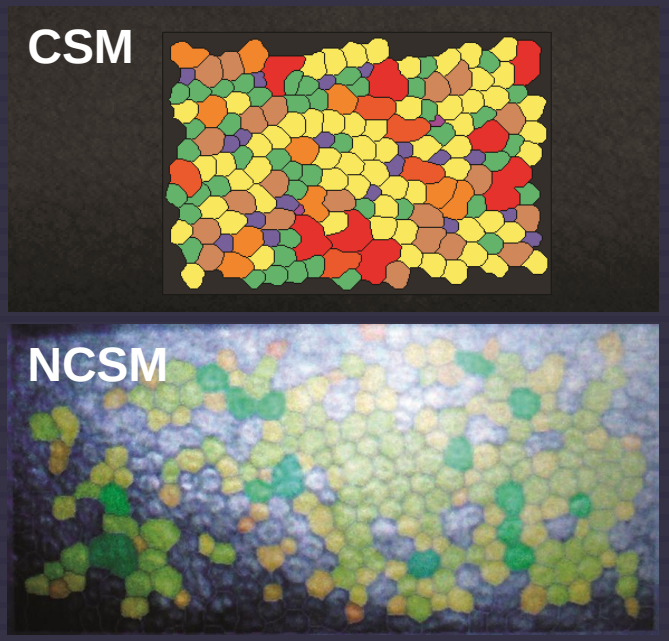




Patel SV et al. Cornea 2010;29:1042-7.

Image analysis II.

- The different software options may not equally identify the cell borders
  - a few cells are reported to have erroneously larger areas
  - result in higher cell area (and thus lower ECD) and higher CV values
- poor agreement between automated image analysis programs → not interchangeable



No: 173/192 cells, ECD: 2550 vs 2888 /mm², AVG: 389 vs 346 µm², CV: 48 vs 37

Szalai E et al. Cornea 2011;30:567–70.

Correction of cell count

- Magnification of CSM and NCSM changes with corneal thickness
  - a linear increase with increasing thickness
- In cases of NCSM, magnification also depends on corneal curvature

↓

- Use of conversion factors is suggested to correct ECD values as described in the literature or provided by the manufacturer.

Table 1. Normalized magnification.

	Specular microscope				
	Non-contact Anterior corneal radius/mm				Contact
CCT/microns	7	8	9	10	
300	0.9892	0.9862	0.9840	0.9822	0.9753
350	0.9931	0.9897	0.9870	0.9849	0.9809
400	0.9970	0.9931	0.9900	0.9876	0.9865
410	0.9978	0.9938	0.9906	0.9882	0.9876
420	0.9986	0.9945	0.9913	0.9887	0.9888
430	0.9994	0.9952	0.9919	0.9892	0.9899
440	1.0002	0.9958	0.9925	0.9898	0.9910
450	1.0010	0.9965	0.9931	0.9903	0.9921
460	1.0018	0.9972	0.9937	0.9909	0.9932
470	1.0026	0.9979	0.9943	0.9914	0.9944
480	1.0034	0.9986	0.9949	0.9920	0.9955
490	1.0042	0.9993	0.9955	0.9925	0.9966
500	1.0050	**1.0000**	0.9961	0.9931	0.9977
510	1.0058	1.0007	0.9968	0.9936	0.9989
520	1.0066	1.0014	0.9974	0.9942	**1.0000**
530	1.0074	1.0021	0.9980	0.9947	1.0011
540	1.0082	1.0028	0.9986	0.9953	1.0023
550	1.0090	1.0035	0.9992	0.9958	1.0034
560	1.0098	1.0042	0.9998	0.9964	1.0045
570	1.0106	1.0049	1.0005	0.9969	1.0056
580	1.0114	1.0056	1.0011	0.9975	1.0068
590	1.0122	1.0063	1.0017	0.9981	1.0079
600	1.0130	1.0070	1.0023	0.9986	1.0090
650	1.0171	1.0105	1.0054	1.0014	1.0147
700	1.0212	1.0140	1.0085	1.0042	1.0204

Wiffen et al. Cornea 2000;19:47-51.; Isager et al. Acta Ophthalmol Scand 1999;77:391-3.

Clinical applications – eye banking

- Screening grafts for keratoplasty
  - Transmission light microscopy, specular microscopy
- Minimum donor ECD: 2000 cells/mm²
- Overestimation of eye bank ECD (Campolmi et al., 2014)
- SM image quality classification:

Ophthalmology Volume 112, Number 3, March 2005

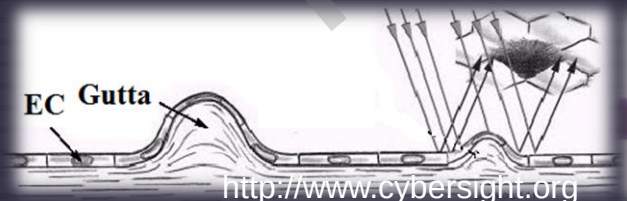
Table 1. Specular Microscopy Reading Center Image Quality Classification of the Corneal Endothelium

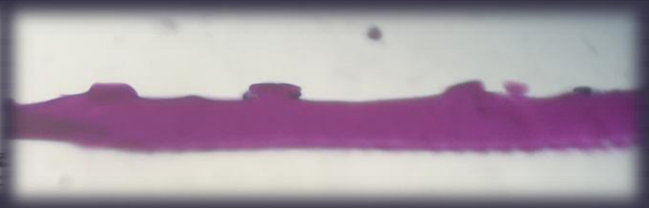
I. Analyzable image
- Excellent: All cell borders, boundaries, and centers across a single image of the endothelium are distinct excluding the peripheral edges of the image. The single image has a sufficient number of cells to count at least 50 and as many as 150 cells contiguous to each other.
- Good: A sufficient number of distinct cell borders, boundaries, and centers from a single image of the endothelium to count at least 50 and as many as 150 cells from variable frames encompassing a minimum of 15 cells contiguous to each other for each variable frame.
- Fair: A sufficient number of cell borders, boundaries, and centers from a single image of the endothelium to count at least 50 cells from variable frames encompassing a minimum of 15 cells contiguous to each other for each variable frame. The borders, boundaries, and centers of up to 25% of analyzed cells within the variable frames may be indistinct, but sufficient to estimate their location to conduct the analysis.

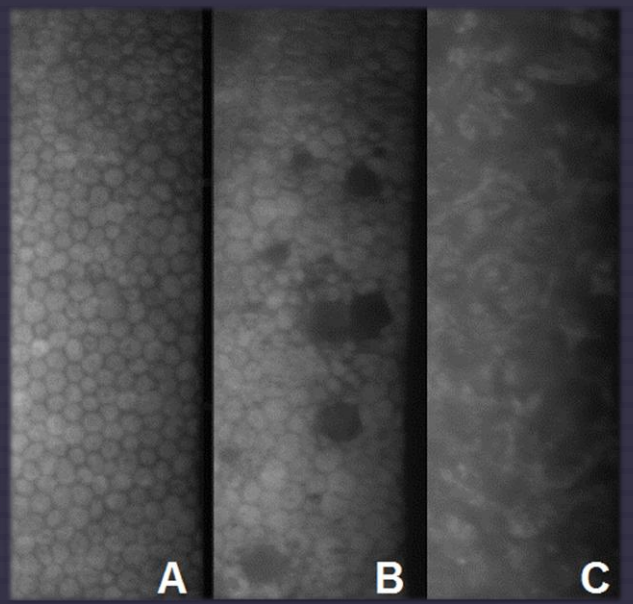
II. Unanalyzable image

Clinical applications – aging, dystrophy

- Corneal guttae: abnormal EC products form focal accumulations of collagen on the back surface of DM
- Fuchs' endothelial dystrophy (FECD): primary EC dysfunction → corneal swelling, collagen & ECM deposition
  - Role of SM: ECD loss follow-up, the safety of intraocular surgery
- FECD & cataract (Seitzman, 2005):
  - CCT<640 µm, no epithelial edema, ECD>1000/mm² → cataract surgery
  - Pachy>640 µm → triple procedure

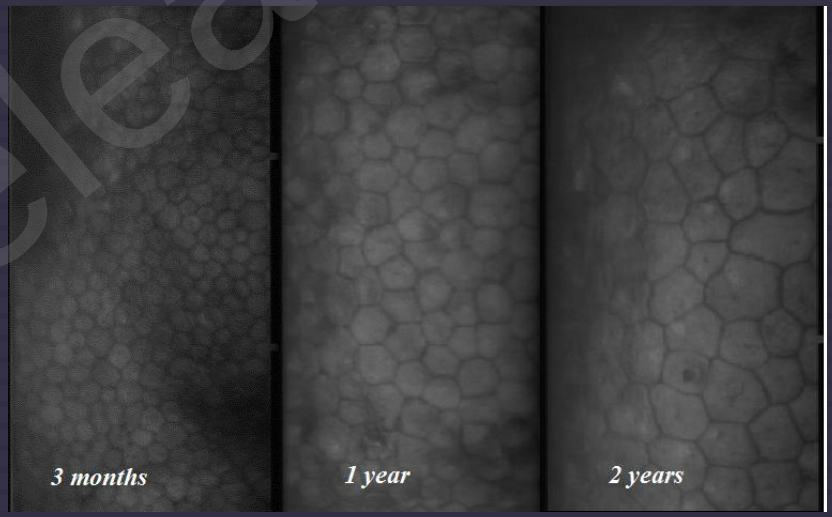


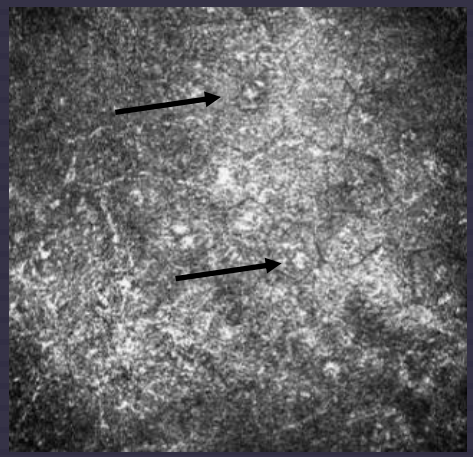




Clinical applications – keratoplasty

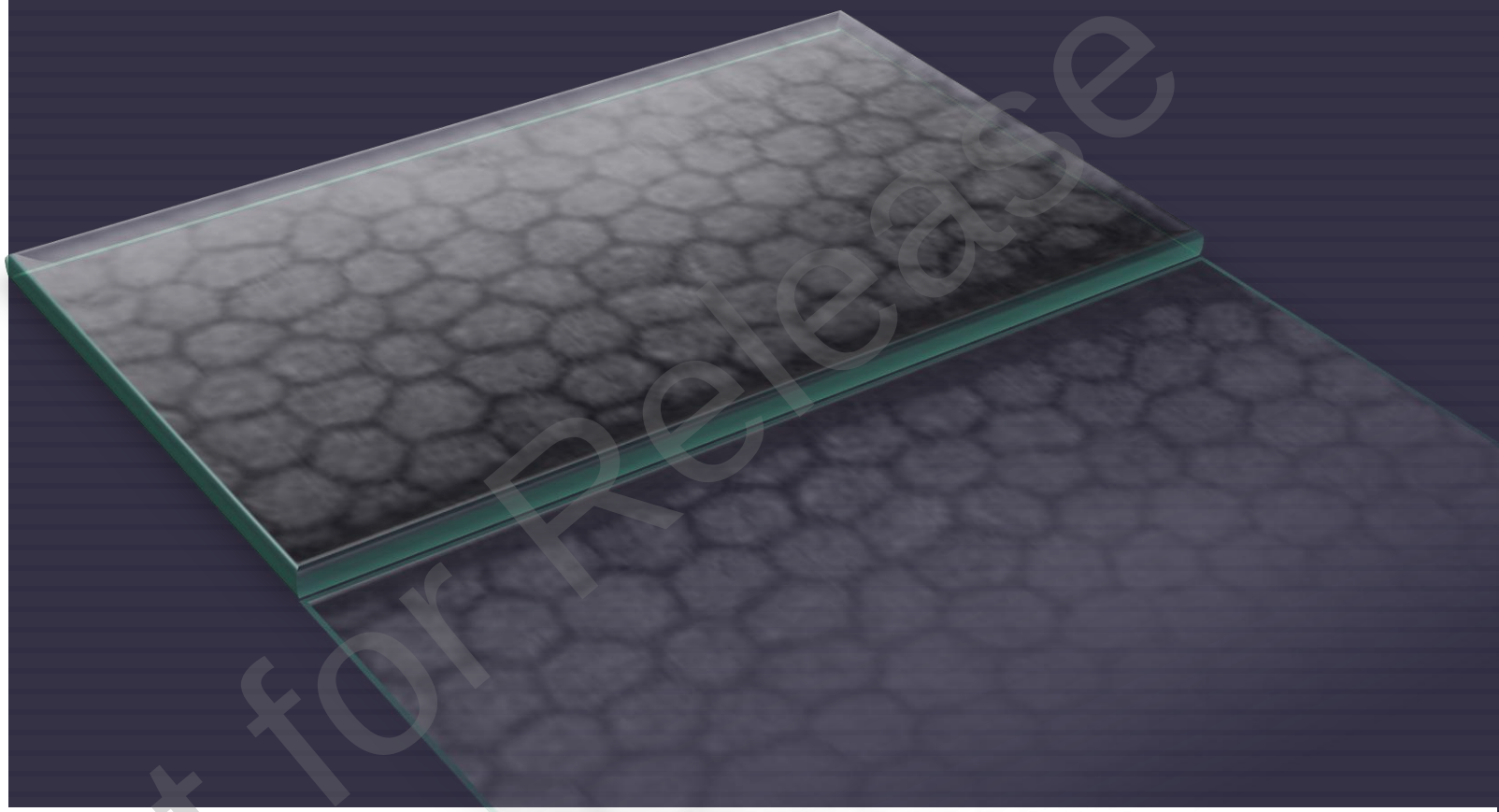
- PK: EC loss progresses 7x faster
- DSEK, DMEK:
  - Loss is higher than PK by 6 months
  - Decline is lower by 3-5 years
- *Surgical trauma, EC migration*
- Graft rejection:
  - PK: 15-30%, DS(A)EK: 5-12%; DMEK: 1-5%
- Quantitative/morphometry/qualitative
  - ic bright bodies, black inflammatory cells, KPs
- EC morphology may not return to normal → irreversible damage





General considerations

- Explain the procedure to the patient
- Image the region 3x at the same sitting
  - Use the average of 3 measurements for analysis
- Use the same analysis method during the follow-up
- To obtain maximum accuracy and minimize sampling error
  - At least 75 cells for the precise analysis (Doughty et al., 2000)
  - Count ≥100 adjacent cells (Inaba et al., 1985)
  - Count as many cells in the frame as possible (Binder et al., 1979)
- Variable frame method: the most cost-effective, reliable, reproducible method (Cornea Donor Study Group, 2005)



THANK YOU FOR YOUR KIND ATTENTION!

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