In vivo confocal microscopy in diagnosis of infectious keratitis

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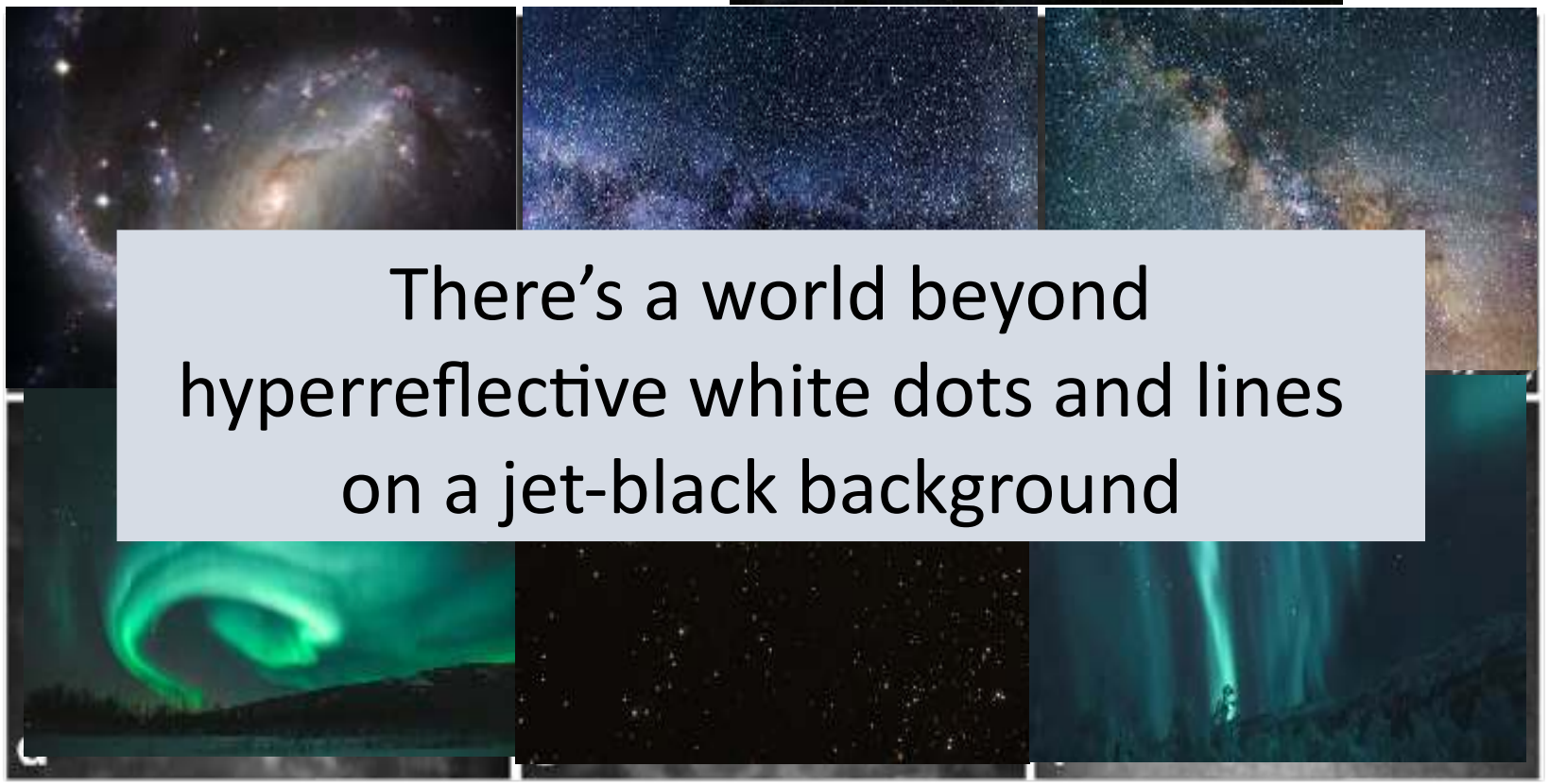
Is it sci-fi or real?



There’s a world beyond hyperreflective white dots and lines on a jet-black background

Principles of in vivo confocal microscopy (IVCM)

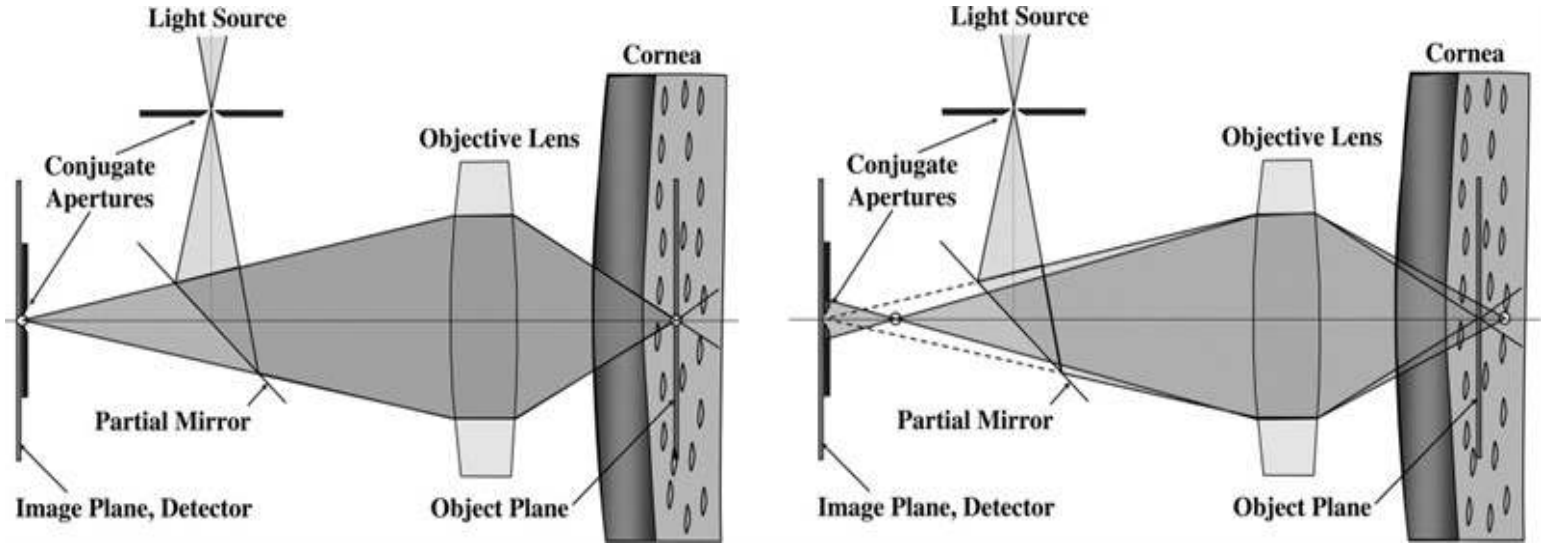
Light reflected **by the specimen** focal point is “co-focused” with the detection aperture(confocal).

Light reflected from tissue slightly in **front of or behind the specimen** focal point is “de-focused” at the observation aperture (not detected)

Enhanced X/Y (Transverse) 1-2 µm

\+ Z (Axial) 5-10 µm

En-face 3D images



Principles of in vivo confocal microscopy IVCM)

- The principle of confocal microscopy was first introduced by Marvin Minsky in 1955 and was initially utilized to study neural networks of the living brain (Petroll et al., 1996).
- Chew and colleagues first described the use of IVCM in the diagnosis of IK in 1992.

TSCM to examine live rabbit eyes infected with *Aspergillus* fungi.

S.J. Chew, et al. Early diagnosis of infectious keratitis with in vivo real time confocal microscopy. CLAO J, 18 (3) (1992), pp. 197-201.

Image size: 300 x 300 µm

400 x 400 µm

Advantages of IVCM

1. **Non-invasive in vivo imaging method to visualize cellular and subcellular corneal + eye lids details.**
2. **Z axis optical sectioning feature + sub-layer depth measurement & pachymetry.**
3. **Enhanced image contrast and resolution both axially and laterally**
4. **Ability to see through moderately opaque tissues (Corneal oedema & scarring)**
5. **Adaptable to real time observation of dynamic processes in the living eye**

Limitations of IVCM

1. **Involuntary movements result in significant blurring of the images, a problem that could be avoided by the use of rapid image capture.**

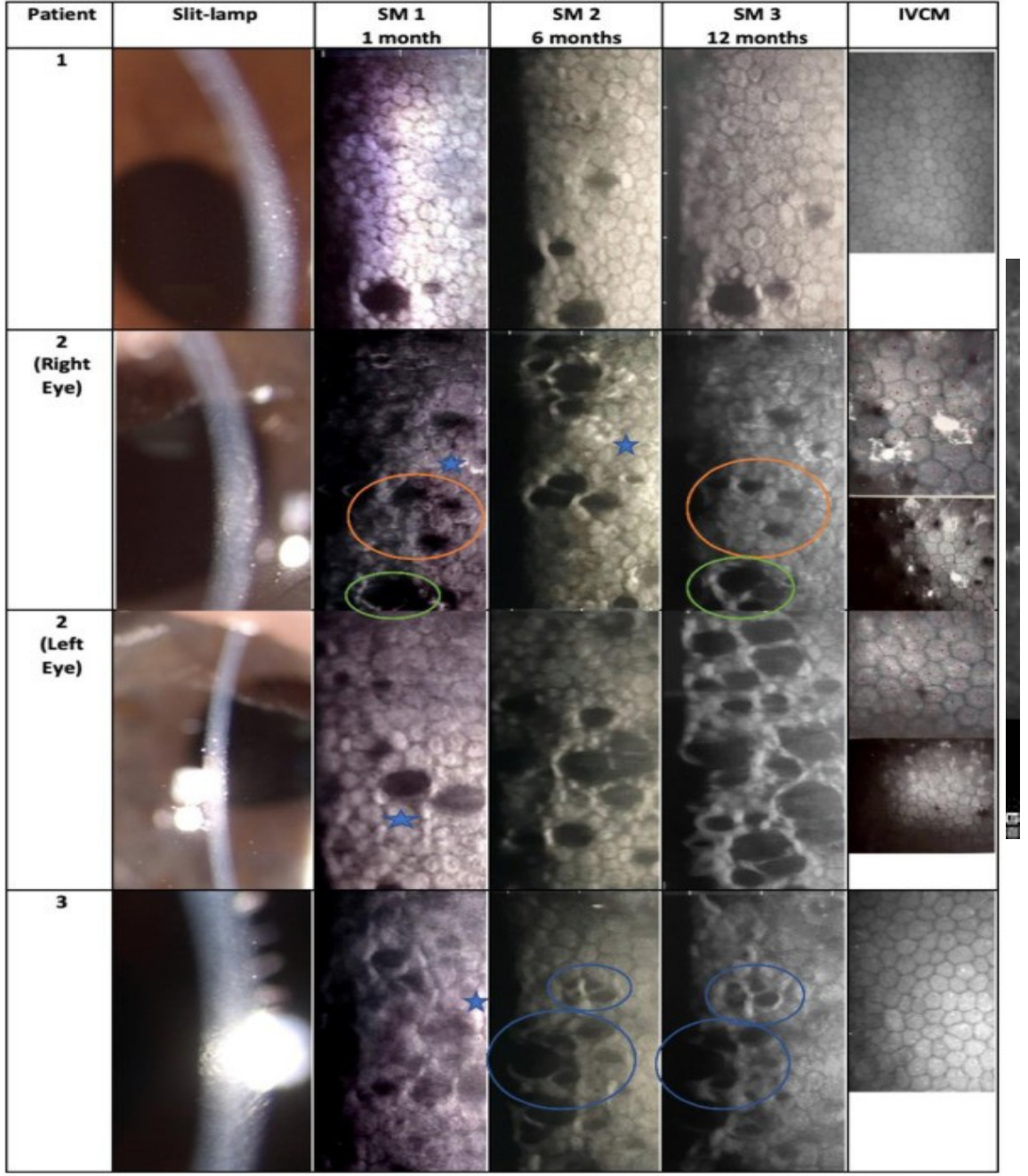
2. **It requires a good level of patient's compliance to efficiently perform the examination due to short working distance of the confocal microscope with the need to apply a coupling medium between the eye and the surface of the microscope objective lens.**

3. **Proper interpretation of the details seen on confocal images is a real challenge to clinicians in terms of diagnosis. Unless well correlated with cytological and histopathological work up (Masters and Böhnke, 2001).**

MASTERS, B. R. & BOHNKE, M. (2001a) Confocal Microscopy of the Hum an Cornea in vivo. I nternational Ophthalm ology, 23, 199-206.

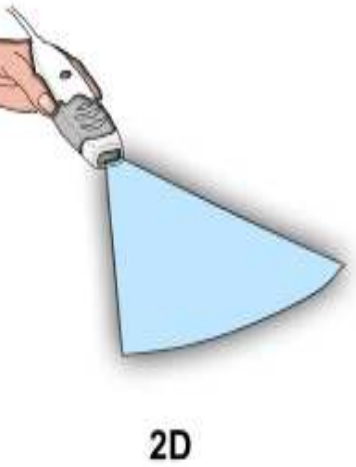
The uses

1. **Keratitis** (Fungal/Acanthamoeba/Bacterial/Viral)
2. **Monitor corneal blood vessels**
3. **Examine corneal nerves** (Diabetic neuropathy, post LASIK, DE
4. **Corneal dystrophies** (Granular, FED, MED, Lattice)
5. **Endothelial cell density**
6. **Ocular surface examination** (Conjunctivae/Lids)
7. **LSCD**
8. **Follow up corneal grafts** (PKP, DMEK, & CXL)

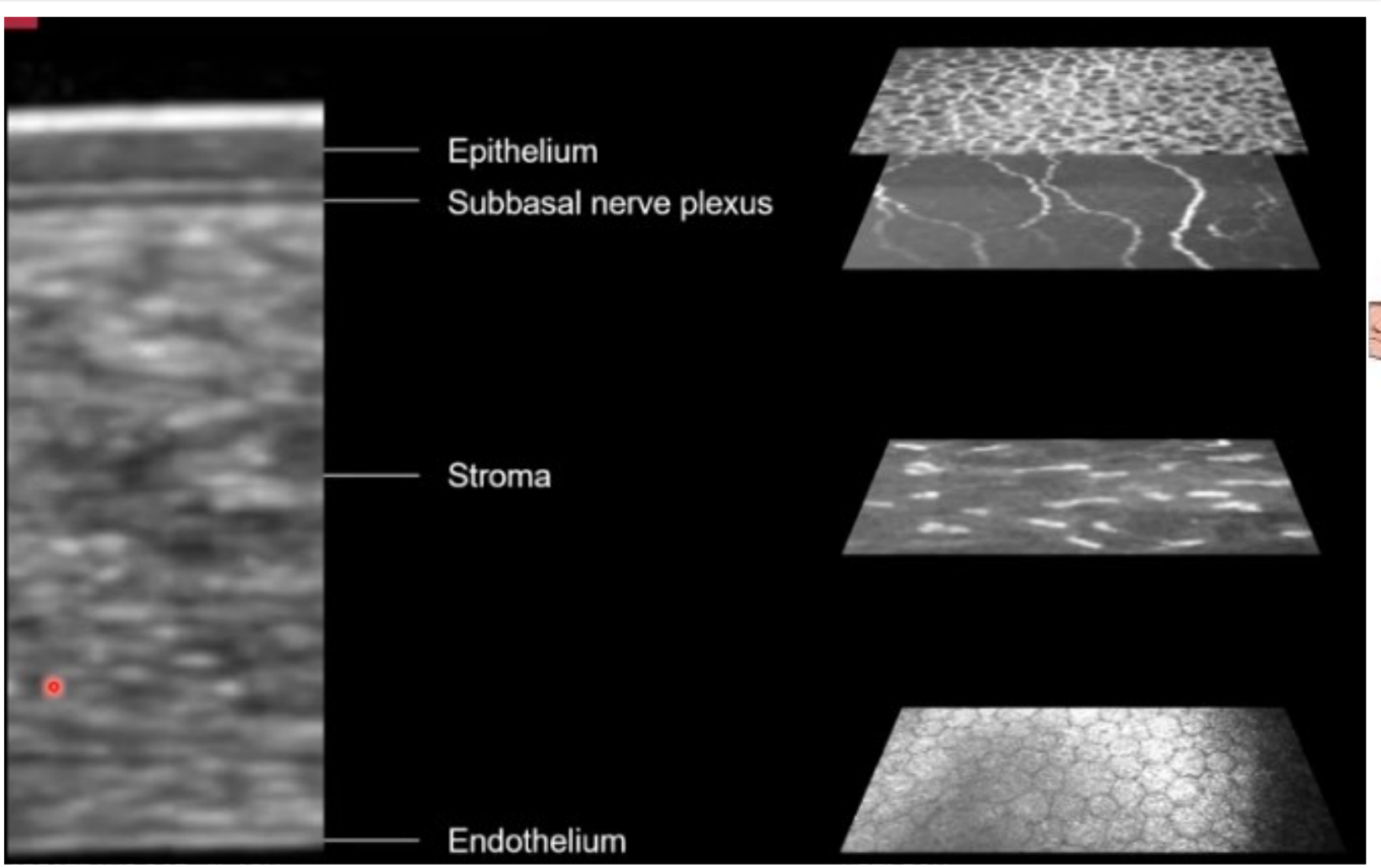


1.Guttae-like changes post Descemet membrane endothelial keratoplasty
Viola, Pietro et al.,Canadian Journal of Ophthalmology, Volume 56, Issue 6, e191 - e194

AS-OCT vs IVCM



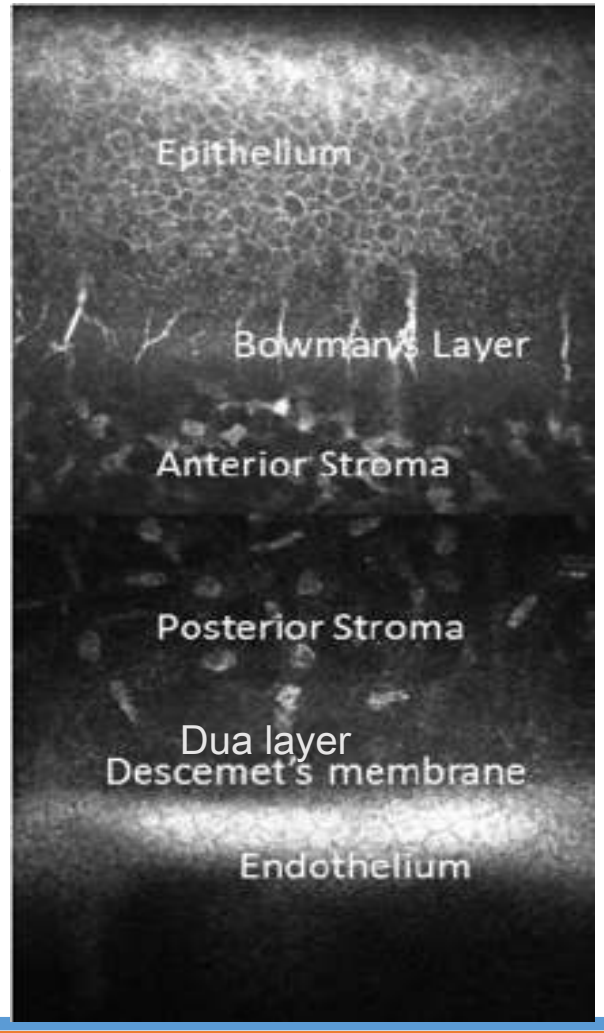
2D



3D

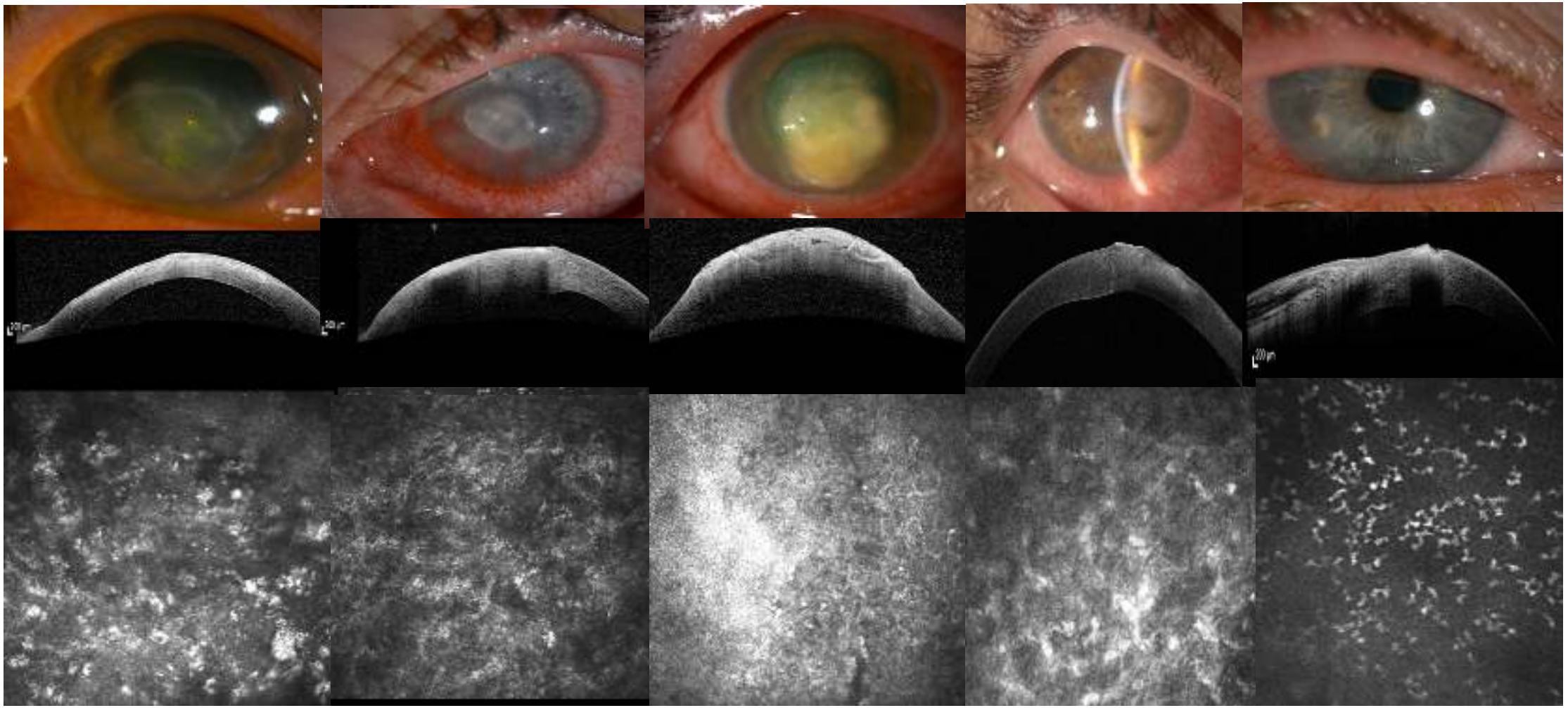
Examination of the corneal layers

1. **Epithelium – 30 µm in high detail (subdifferentiation of epithelial cell layers)**
2. **Corneal Sub-basal Nerve Plexus – 62 µm/Bowman's membrane**
3. **Stroma – starting at 300 µm (Superficial/Deep)**
4. **Dua layer (PDL)**
5. **DM**
6. **Endothelium – 572 µm**



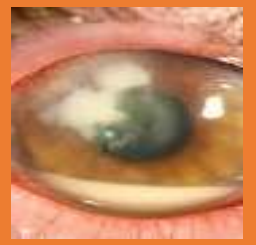
Dua HS, Faraj LA, Said DG, Gray T, Lowe J. Human corneal anatomy redefined: a novel pre-Descemet's layer (Dua's layer). *Ophthalmology*. 2013;120(9):1778-1785. doi:10.1016/j.ophtha.2013.01.018

IVCM IN IK



IK

AK



FK

BK

VK

Mixed K

FK

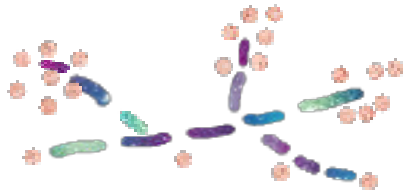
Filamentous

- ‘filaments’
‘hyphae’
‘elements’
‘lines’ or ‘linear’
’Structures’
‘highly’/’hyper-reflective
‘septate’ or ‘branching’

- Filament diameter between 3-8 µm
- The length up to 400 µm
- Hyphal branching angles to be 90° in **Fusarium spp**.
- 45° in **Aspergillus.**

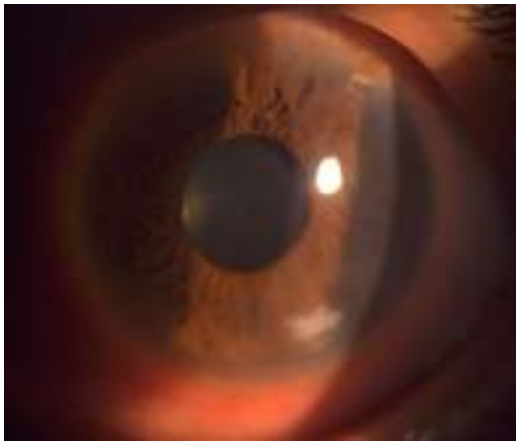
Yeast

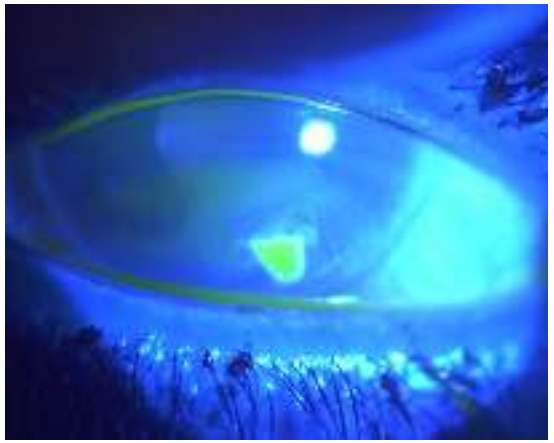
- **Candida parapsilosis :** small hyper-reflective round 3–5 µm structures
- **C. albicans**: pseudohyphae are 10–40 µm in length, and 5–10 µm in width
- Only a few of the reviewed studies investigated the use of IVCM in FK caused by yeast.

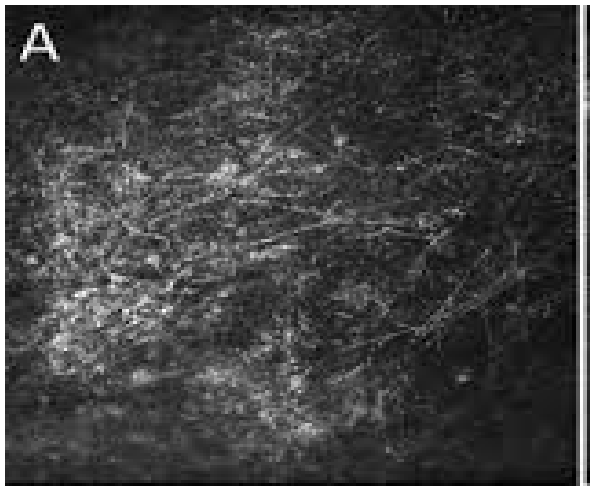


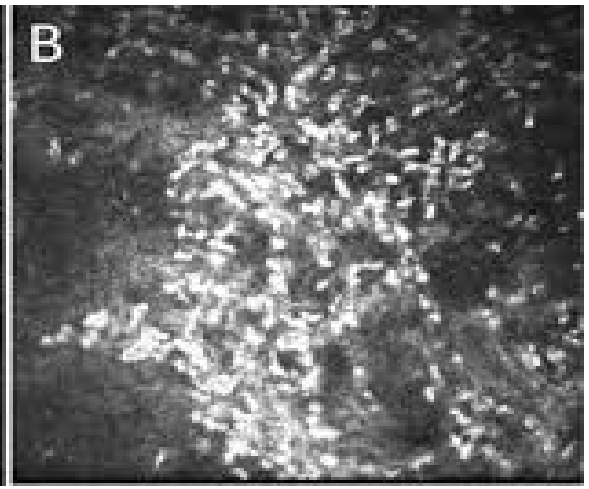
IVCM IN IK

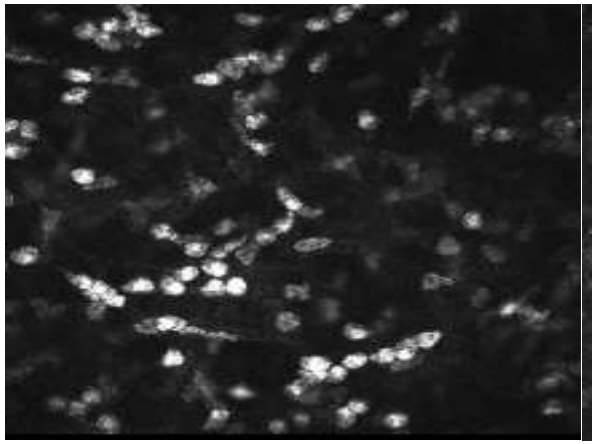
Yeast

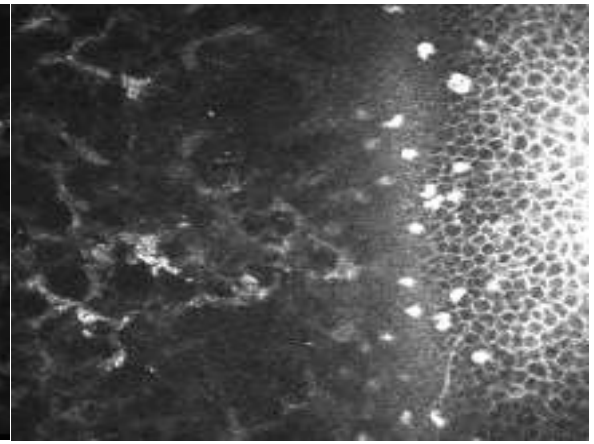




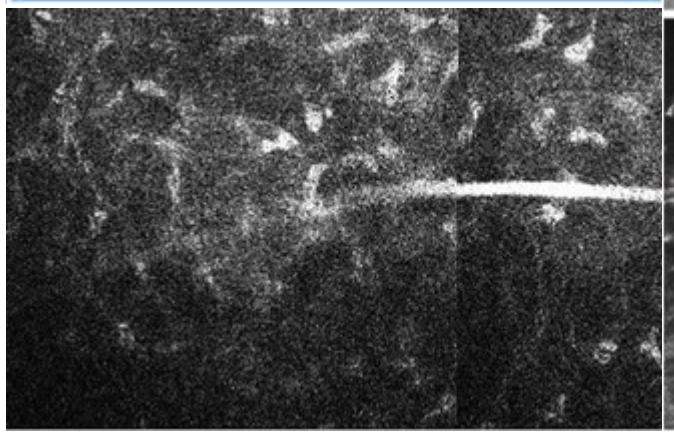


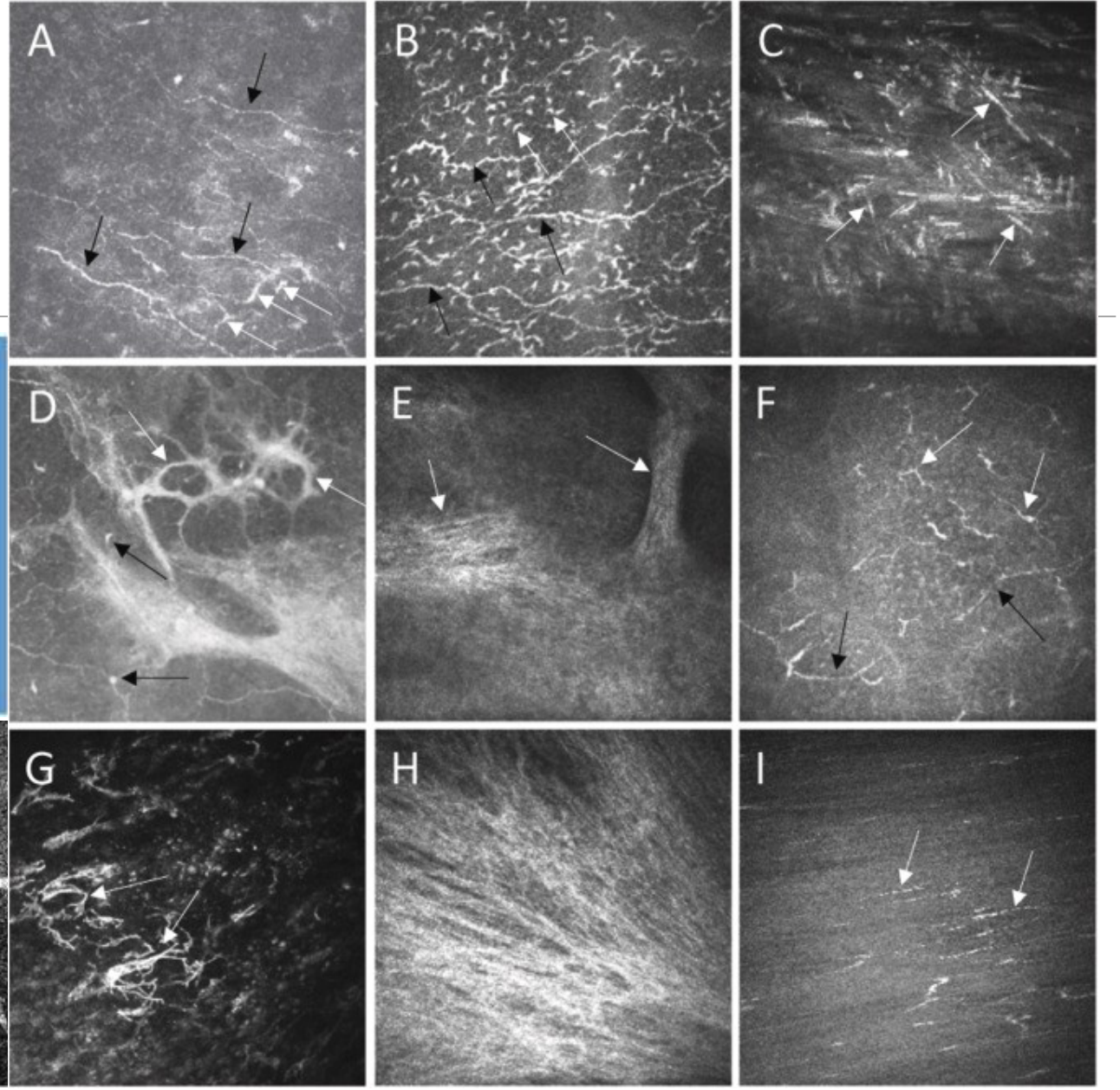






Filamentous





Bakken IM, Jackson CJ, Utheim TP, et al. The use of in vivo confocal microscopy in fungal keratitis - Progress and challenges. Ocul Surf. 2022;24:103-118.

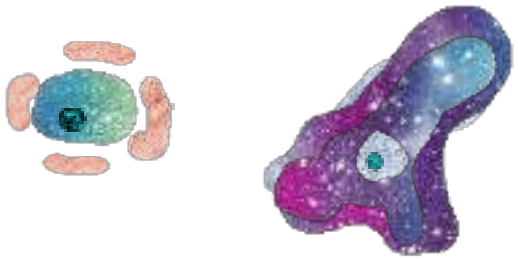
AK

Cysts/Trophozoites	Inflammatory cells

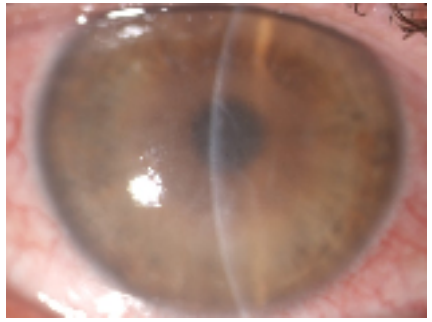
- Cysts (100%) 10–40 μm
- Bright spot
- Signet ring appearance
- Thick double-walled structure
- Linear arrangement of Acanthamoeba cysts

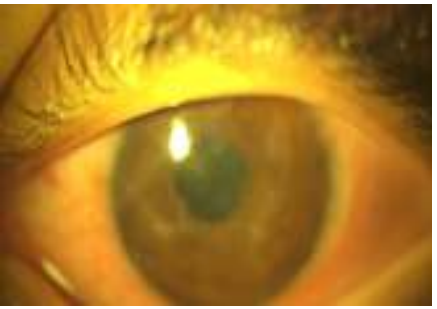
$\propto$

- Round inflammatory cells <10 μm
- Dendritic cells
- Inflammatory infiltrate

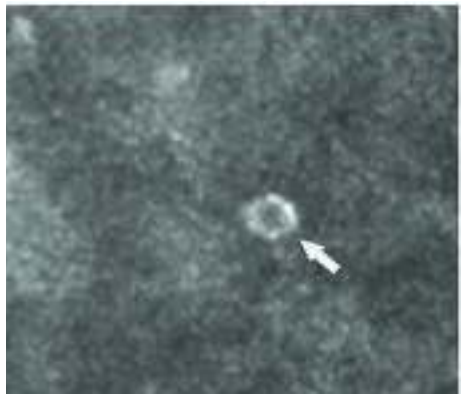


AK

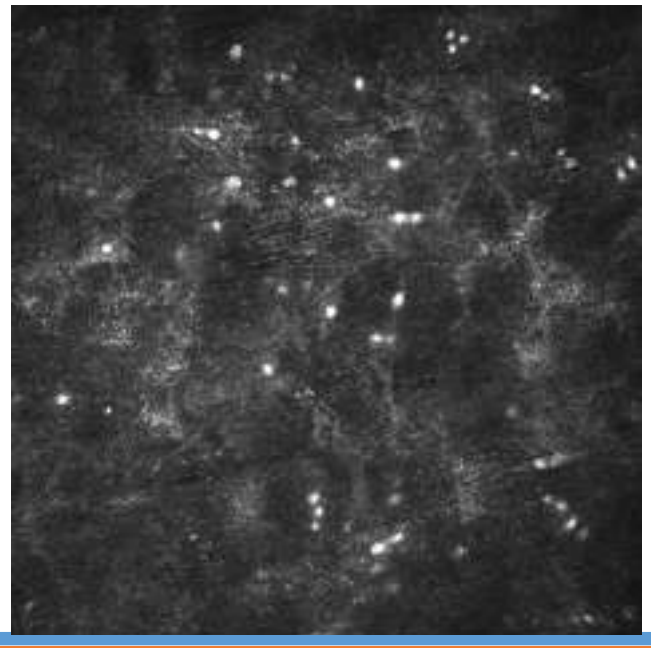


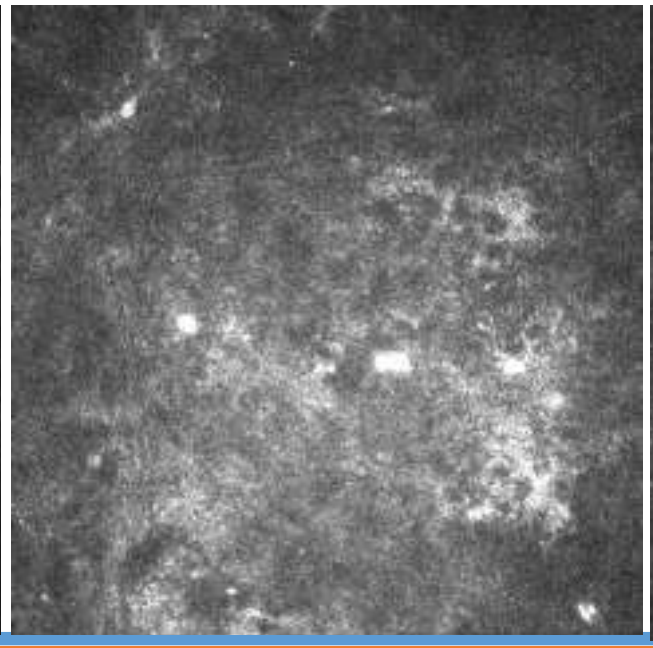


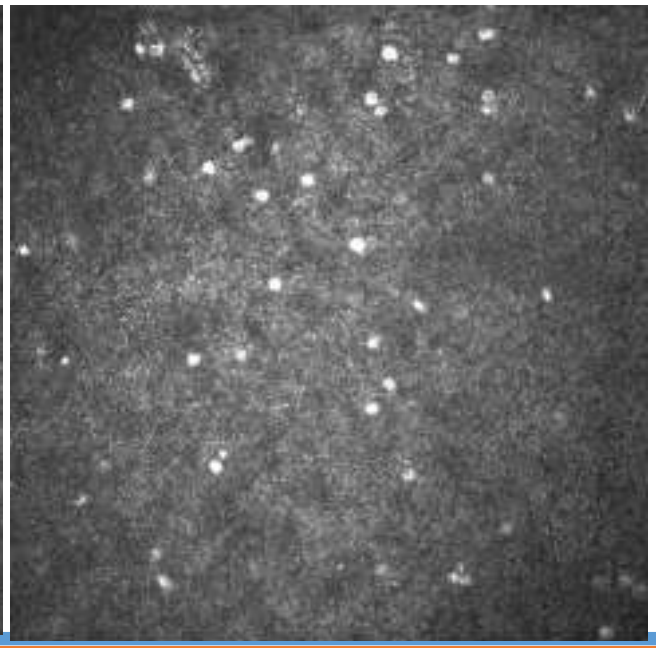
Cysts/Trophozoites

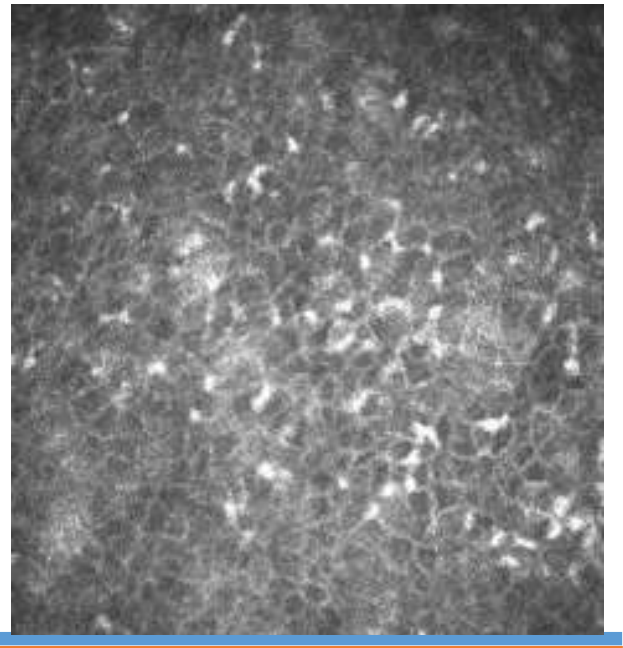


Inflammatory cells





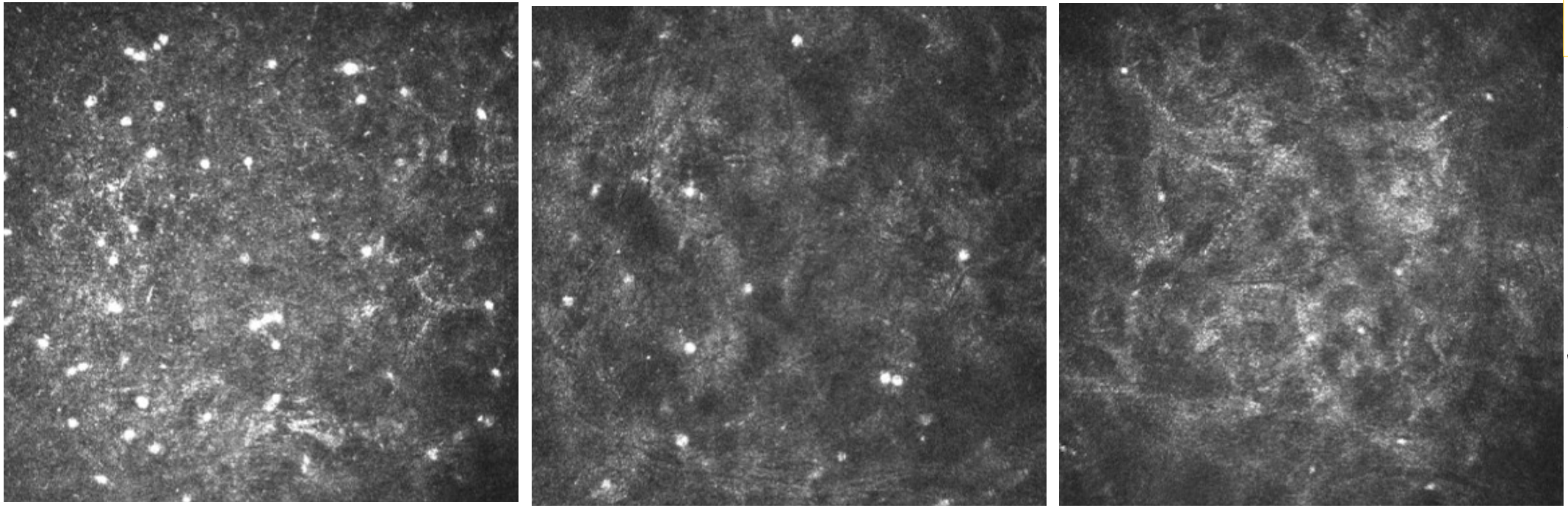




Huang et al. quantified the density and depth of *Acanthamoeba* cysts in the cornea in various stages of AK

Cruzat et al. found that invasion of *Acanthamoeba* cysts into Bowman's layer might be a useful predictor of a persistent clinical course.

Rx/ G/ PHMB 0.2%
G/ Brolene 0.1%



Huang P, Tepelus T, Vickers LA, et al. Quantitative analysis of depth, distribution, and density of cysts in *Acanthamoeba* keratitis using confocal microscopy. *Cornea*. 2017; 36(8): 927–932.

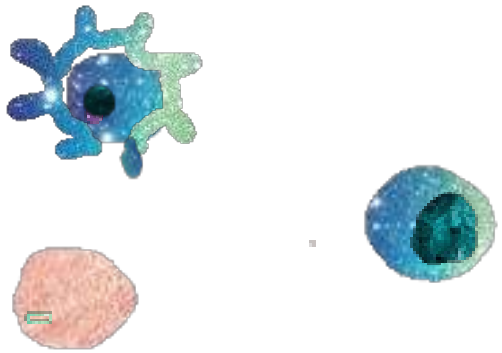
Cruzat A, Witkin D, Baniasadi N, et al. Inflammation and the nervous system: the connection in the cornea in patients with infectious keratitis. Invest Ophthalmol Vis Sci. 2011; 52(8): 5136–5143.

BK

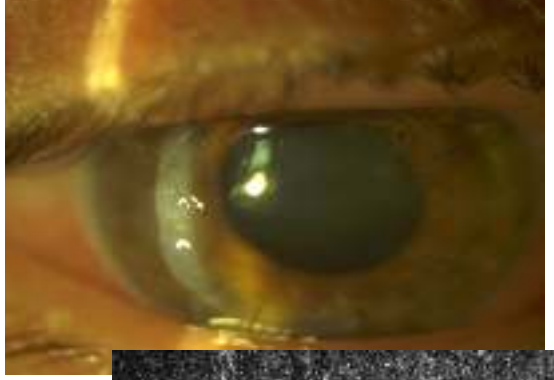
Abundance of inflammatory cells	Lack of atypical cells

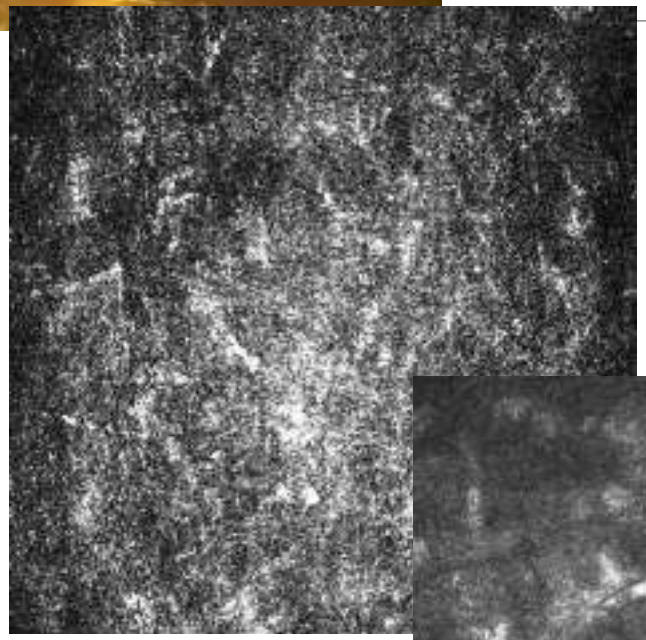
- Abundant dendritic cells (DC)
- Activated keratocytes
- Neutrophils and lymphocytes
- > in normal Corneas

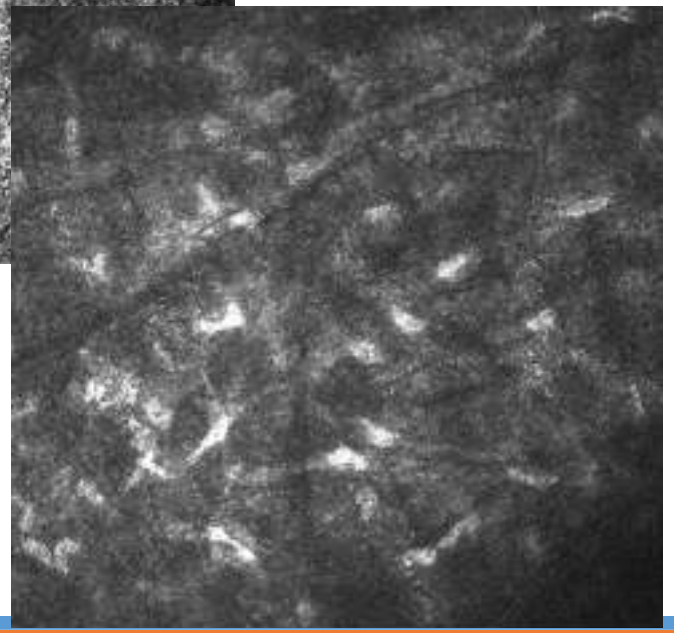
- NO visible Acanthamoeba cysts or trophozoites, fungal filaments or yeasts.

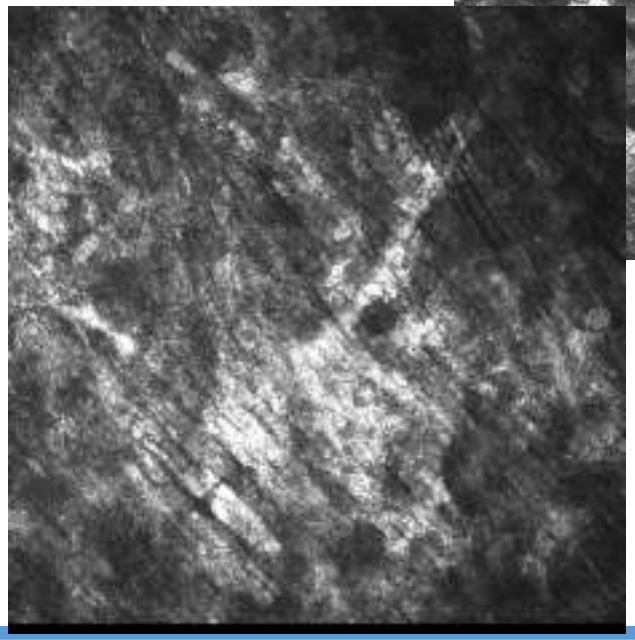


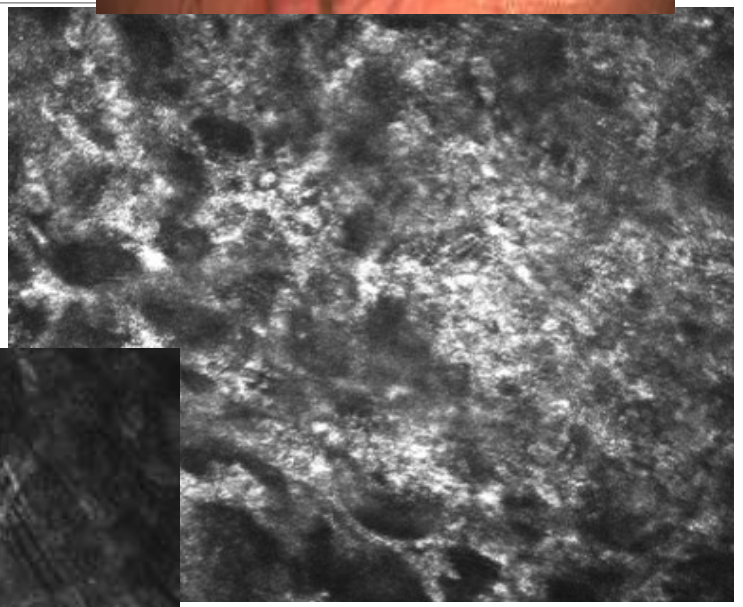
BK











VK

HZK HSK

Abundance of inflammatory cells

Lack of atypical cells

Clinical Judgement

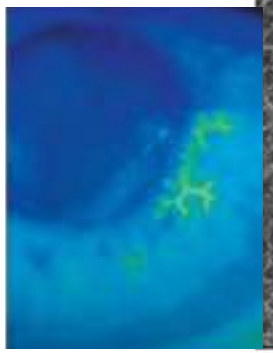
- Dendritic lesions/ linear lesions with branching, swollen terminal bulbs

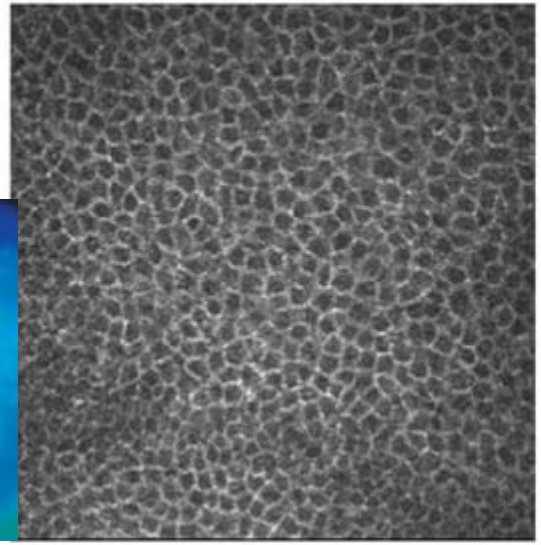
= Hyperreflective irregular epithelial cells, surrounded By elongated epithelial cells, swollen and necrotic epithelial cells.

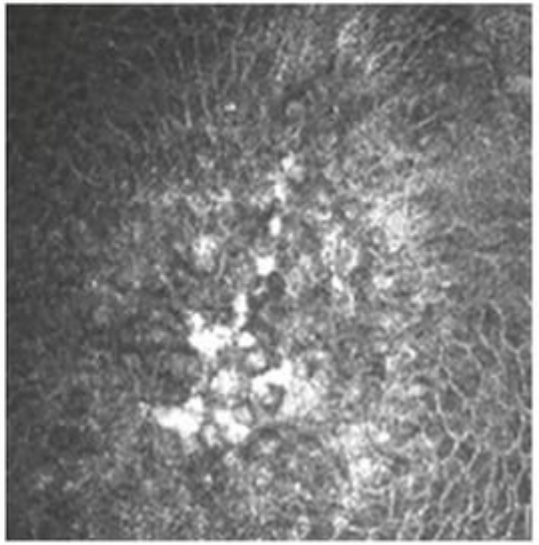
- -- Nerve
- Endothelial

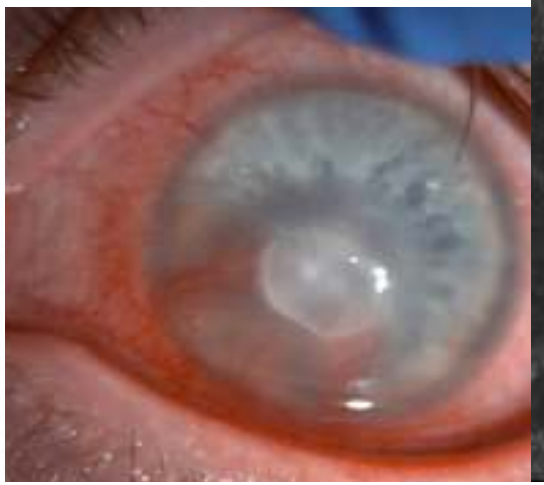
Cell changes

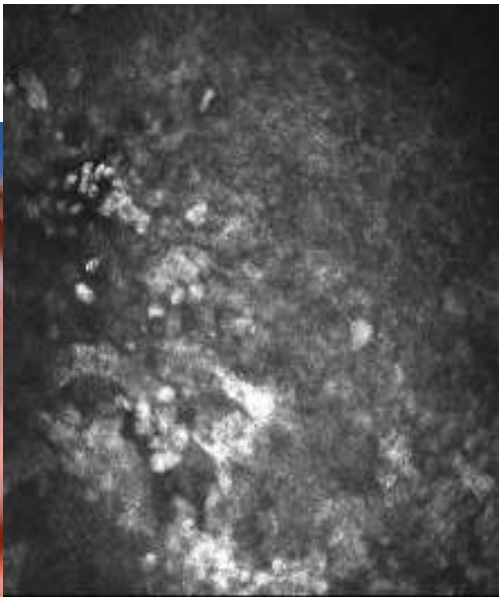
- NO visible Acanthamoeba cysts or trophozoites, fungal filaments or yeasts.











E

▸ Eye (Lond). 2021 N

10.1038/s41433-021

**Comparison
microscopy a
hospital use
diagnosis**

Jeremy J Hoffman [1]

[1,4], Nicole Carnt [1], G

▸ Author informatio

▸ Copyright and Lic

PMCID: PMC958191

Conclusions

Laser scanning IVCM performed with experienced confocal graders has high sensitivity, specificity, and test reproducibility for detecting fungal filaments and *Acanthamoeba* cysts in moderate to large corneal ulcers in India. This imaging modality was particularly useful for detecting organisms in deep ulcers in which culture and light microscopy results were negative.

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Acanthamoeba
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both IVCM and
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for each
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ıre, PCR or
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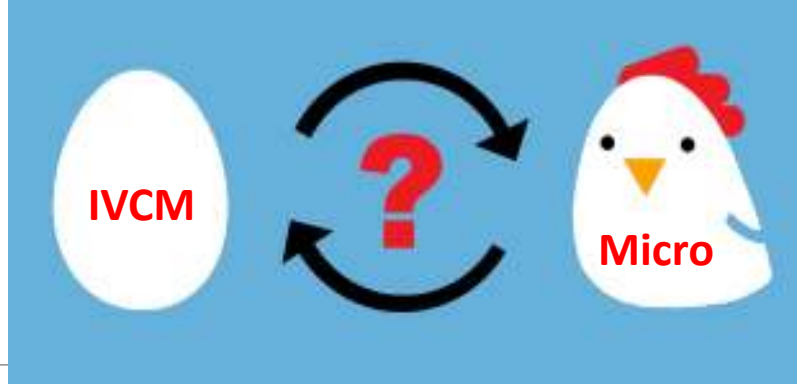
sitivities with

77.1% [62.7–
.6%], culture
M 81.8% [48.2–
.4%], culture
PCR 25.0%
[87.6–99.1%].

IVCM and/or Microbiology?



Microbiology: "Gold standard" diagnostic methods

Limitations

Corneal smear and culture:

Diagnostic insensitivity

Time-consuming nature.

Initial negative corneal scrapings (for fungi) deeper localization of stromal hyphae

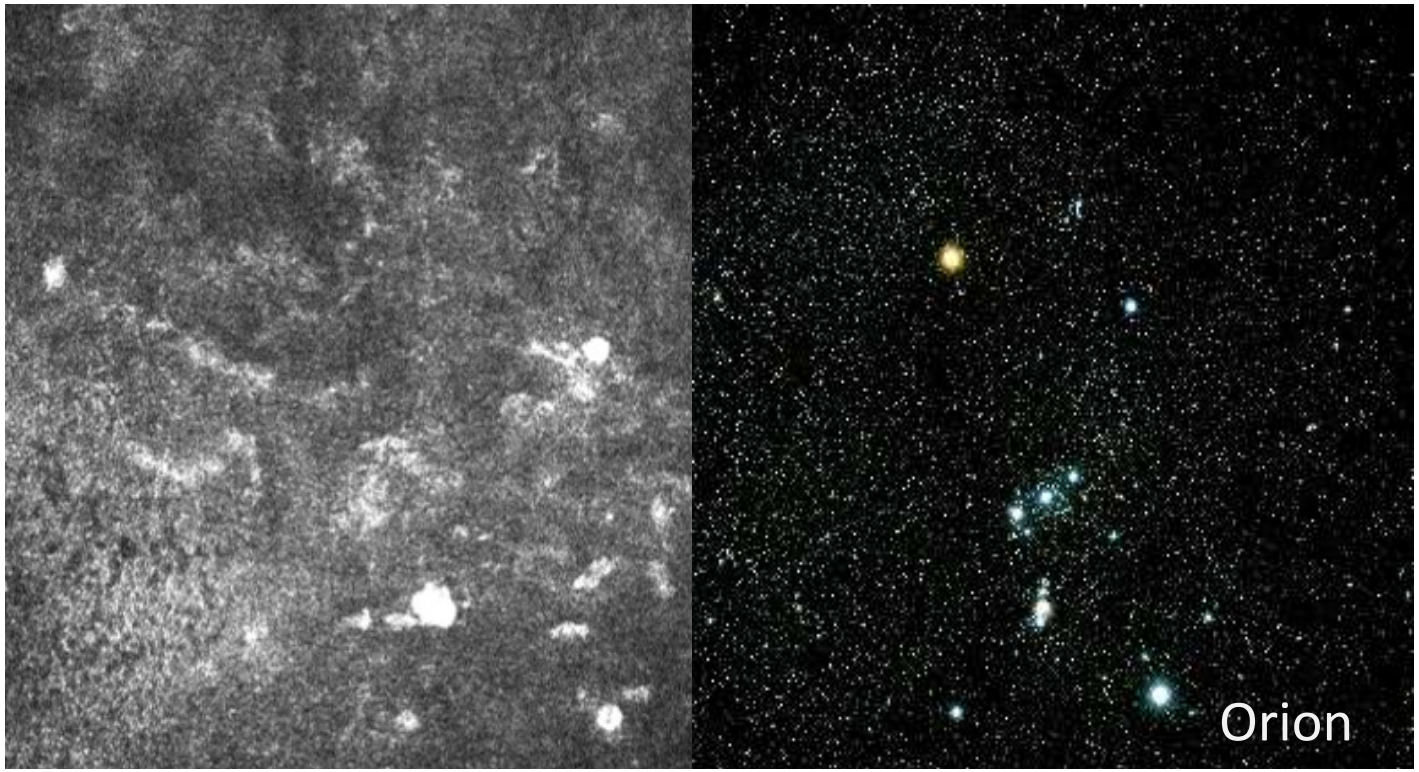
PCR (complementary in BK/FK) : sample-dependent.

In vivo confocal microscopy (IVCM) is a promising complementary diagnostic method

Allows non-invasive real-time direct visualization of FK/ AK pathogens

Conclusion:

- IVCM is a **non-invasive** imaging modality that can **rapidly** and **accurately** diagnose IK even for experienced corneal specialists. In complex cases of polymicrobial infection, IVCM may **guide** the correct clinical diagnosis and initiation of the appropriate treatment.



thank you