Corneal *In Vivo* Confocal Microscopy: Clinical Applications

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ABSTRACT

In vivo confocal microscopy (IVCM) has become a widely accepted imaging technique to study the human living cornea. It provides a unique opportunity to visualize the corneal tissue at the cellular level without damage and longitudinally observe its pathologic and normative changes. With rapidly evolving technology, there has been an abundance of interest in maximizing its potential to better understand the human cornea in health and disease. This is evidenced by a growing literature analyzing acquired and inherited corneal and also systemic diseases using corneal IVCM. This article provides a narrative review of IVCM and its applications.

KEYWORDS: *In vivo* confocal microscopy, cornea, infective keratitis, corneal dystrophy

INTRODUCTION

In vivo confocal microscopy (IVCM) is a non-invasive imaging tool that enables an *in vivo* examination of the cornea at greatly enhanced magnification. Recent advances have broadened its application in both research and clinical realms as they allow fast acquisition of high resolution images of living cornea and its microstructures. This article provides an overview of current research and clinical applications of this technology.

Optical principle of confocal microscopy

The main advantage of confocal microscopy is its ability to obtain images from selected depth by optical sectioning.[1] It is achieved by focusing a light source through a slit, or an aperture, on a small area of the tissue and analyzing the reflected light only from the selected focal plane. The light from the out of focus planes is attenuated. As the scan progresses serially through various depths of the cornea, multiple optical sections are acquired, creating an *en face* image of corneal layers and its microstructures such as Langerhan cells, sub-basal nerves, keratocytes and endothelial cells.[2] (**Figure 1**)

The concept of confocal microscopy was first patented by Marvin Minsky in 1957 to study the brain neural cells.[2] It was subsequently applied in various facets in ophthalmology, namely retinal and optic disc confocal scanning laser ophthalmoscopy. After numerous IVCM studies in *ex vivo* human eyes and *in vivo* rabbit eyes, the first *in vivo* images of the human cornea were obtained by Cavanaugh *et al.* in 1989. They demonstrated the confocal visualization of epithelium, basal lamina, Bowman's layer, stromal nerves, pre-Descemet's membrane and endothelium in living cornea.[3] More importantly, it portended a new paradigm to bridge histopathologic knowledge to the living tissue and study the dynamic nature of the living eye.

Thus far, three main commercial confocal systems have been developed for *in vivo* corneal imaging; the Tandem Scanning Confocal Microscope (TSCM), the Slit Scanning Confocal Microscope (SSCM) and the Laser Scanning Microscope (LSCM).[1,2,4] The first real-time TSCM was introduced in 1989. It was based on the modified Nipkow spinning disc technology which used a metal disc with multiple pinholes of 30 microns in size. The metal disc was rotated at high speed, allowing rapid acquisition. The small pinhole also provided a thin field of depth; however, it limited the light output that reached the detector to less than 1%.[3] Then, SSCM was introduced in 1994 with improved light output throughout and faster acquisition time; however, at the cost of axial resolution.[4] The axial resolution of SSCM ranged from 8 µm to 25 µm, in comparison to 9 µm to 12 µm in TSCM.[4] LSCM was first introduced in 2003, comprised of Heidelberg retina tomograph (HRT) and Rostock Corneal Module.[4] It rapidly scans a 670nm-diode laser beam and creates a high resolution image of 384 x 384 points in a 400 µm. This device provides a greater contrast than TSCM or SSCM with the superior axial resolution of 4 µm, but it is not as user-friendly as SSCM.[4] Unfortunately, the quantitative measurements between different types of machines are not comparable due to differences in contrast and sectioning thickness. TSCM is no longer commercially available.

Clinical application of IVCM

The role of corneal IVCM in the clinical setting has expanded over the last three decades. IVCM characteristics of acquired and inherited corneal pathologies such as infectious keratitis of many organisms and Fuchs' endothelial dystrophy have been extensively studied.[4,5] Further, the quantification of IVCM parameters, including cell densities (epithelium, keratocytes and endothelium), sub-basal nerve plexus density, number, length, tortuosity and reflectivity and anterior stromal backscatter were tirelessly pursued.[4,5] Through these

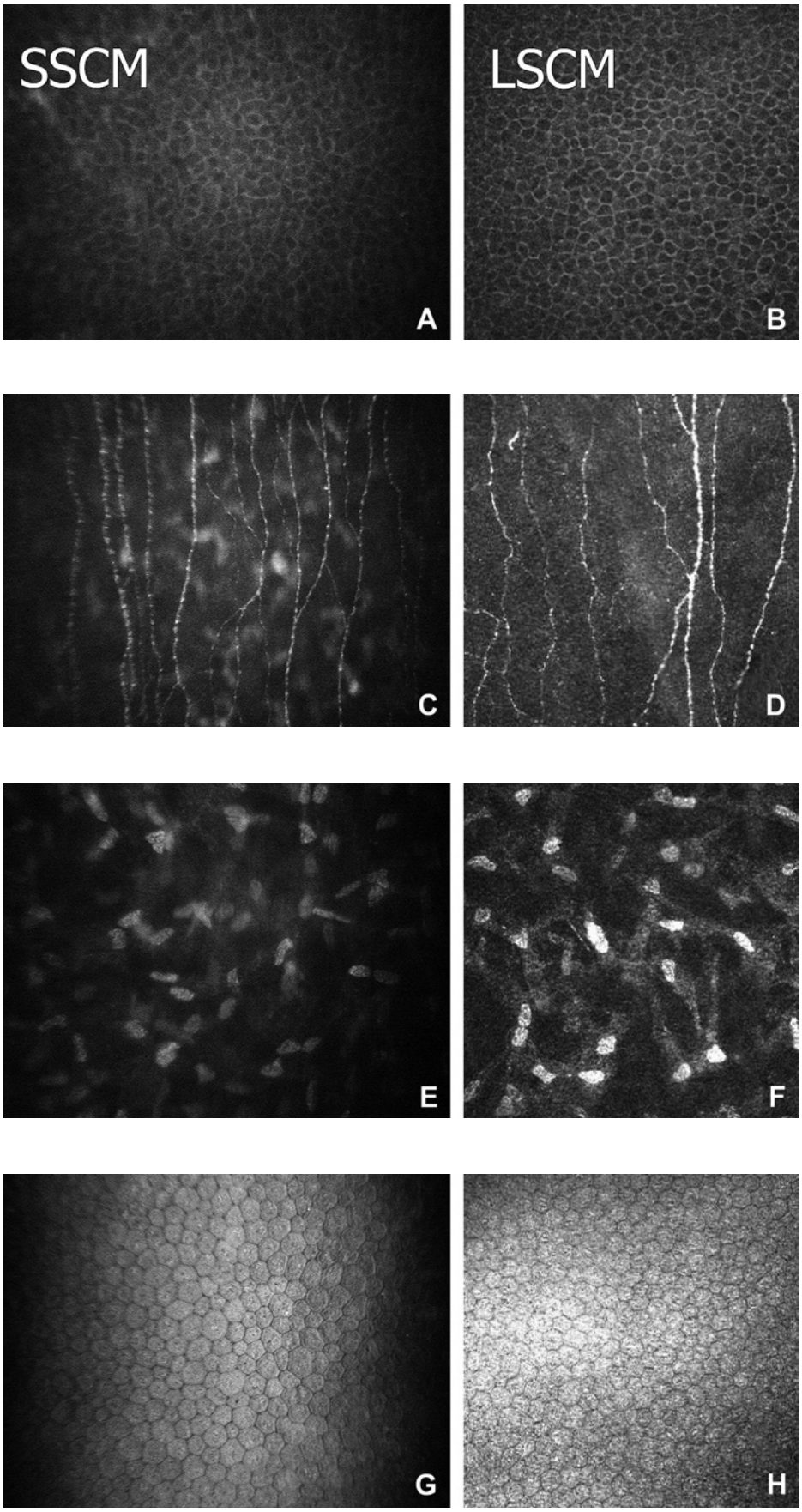

Figure 1. Representative IVCM images of normal cornea using SSCM (Confoscan 4, NIDEK, Gamagori, Japan) and LSCM (Heidelberg Retina Tomography II Rostock Corneal Module, Heidelberg Engineering, Heidelberg, Germany).

A and **B** demonstrate the corneal epithelium using SSCM and LSCM, respectively.

C and **D** show the sub-basal nerve plexus between basal epithelium and Bowman's layer.

E and **F** show the corneal stroma.

G and **H** show the corneal endothelium.

SSCM images are sized 460 x 345 mm, LSCM images are cropped to 345 x 345 mm.

(With permission. Niederer R, McGhee C. Clinical *in vivo* confocal microscopy of the human cornea in health and disease. *Progress in Retinal and Eye Res*. 2010;29:30-58)

endeavors, we have gained a useful insight into corneal infectious and inflammatory disease, dystrophy and wound healing.

Infectious keratitis

The role of IVCM in the clinical setting has been the most highlighted in the management of infectious keratitis. While microbiology diagnosis via corneal scrape or biopsy remains the gold standard, IVCM renders early diagnosis and initiation of targeted antimicrobial therapy.

This is particularly useful in challenging cases such as contact lens-related keratitis.[4,6,7] Acanthamoeba cysts measure 15–28 µm; the tropozoites measure 25–40 µm; fungal hyphae measures approximately 6 µm.[4] They can be visualized using IVCM directly. In a prospective, double-masked, observational study that included 103 microbiologically-proven Acanthamoeba and fungal keratitis cases, the sensitivity of IVCM in the identification of Acanthamoeba cysts and fungal elements was 88.3% and specificity was 91.1%.[7] This early diagnosis may have a profound impact on visual outcome.[8,9,10] The American Academy of Ophthalmology reported level II

evidence for the adjunctive role of IVCM in the diagnosis of Acanthamoeba keratitis.[11]

The sub-basal nerve and dendritic cell densities in herpetic simplex keratitis and herpes zoster ophthalmicus have also been studied extensively. The loss of sub-basal nerve density has been shown to be a prominent feature in IVCM along with increased dendritic cell density in the basal epithelium and squamous metaplasia.[12,13] The loss of sub-basal nerve correlates with the clinical loss of corneal sensation.[13] These characteristics can help diagnose herpetic keratitis in complex cases.

The sub-epithelial infiltrates in patients with epidemic keratoconjunctivitis has been associated with increased dendritic cell density in the basal epithelium and Bowman's layer.[4] On the other end, the use of IVCM in bacterial keratitis has been limited, as the organisms are generally beyond the resolution of IVCM.[4]

Post-surgical changes

IVCM has contributed greatly in understanding the healing process of the cornea after refractive surgeries. Using IVCM, we learned that it may take up to one month for epithelial architecture to return to normal following photorefractive keratectomy (PRK).[4] Also, the sub-basal nerve density is not fully recovered until two years after PRK and five years after laser-assisted in situ keratomileusis (LASIK).[14] Additionally, IVCM has been instrumental in demonstrating the efficacy of using mitomycin-C with PRK.[15] Post-PRK haze is correlated to increased cellular reflectivity due to activated keratocytes. Gambato et al. performed a randomized controlled study of corneal wound healing following PRK and observed a reduction in the IVCM appearance of activated keratocytes and clinical corneal haze with application of mitomycin C in high myopic patients.[12]

Most of our current knowledge in cellular changes after corneal transplant come from animal models or *ex vivo* issues. Real-time observation of wound healing process after various keratoplasty techniques has become possible with IVCM. A longitudinal study found that only 53% of subjects re-innervated with anterior stromal nerves in the central cornea at 12 months following penetrating keratoplasty and the regenerated nerves have abnormal morphology such as increased tortuosity.[16]

IVCM has also been implicated as a potential tool to monitor subclinical cellular changes after corneal transplant.[17] A prospective observation study monitored the keratocyte counts in the anterior, middle and posterior stroma for two years following penetrating keratoplasty and demonstrated that the increase in the AK counts was seen two months before the clinical diagnosis of rejection. This was followed by the normalization of keratocyte count after intensive anti-rejection regimen, comparable to the group that did not have clinical signs of the graft rejection.[17] The ability of IVCM to detect signs of rejection prior to clinical signs will allow early diagnoses, treatment of corneal rejection and successful corneal transplants.

Corneal dystrophy

The use of IVCM has enabled us to visualize the pathophysiology of many corneal dystrophies *in vivo*. While *ex vivo* specimen allowed the studies of end-stage disease, we are now able to follow the changes occurring over the course of the disease. We are also able to screen the affected family members for some inheritable corneal dystrophies.[4] IVCM is also being used to newly classify the severity of corneal dystrophy. A good example is seen in Fuchs' endothelial dystrophy.[18] The traditional grading, based on the extent of guttata and corneal edema, is inadequate in the era of DSAEK and DMEK. IVCM has demonstrated early factors associated with increased anterior corneal backscatter, abnormal sub-basal and stromal nerve density and decreased anterior keratocyte densities.[18] These parameters are being studied with the goal of achieving an objective method of assessing disease severity.

Additionally, in complexes cases of corneal dystrophy, IVCM is increasingly utilized to diagnose one or more diseases.[19]

Peripheral neuropathy

The cornea is the most densely innervated part of the human body. It has emerged as a promising region to study systemic peripheral neuropathies.[2] Using IVCM, we can quantify subbasal nerve damage as a surrogate for the severity of peripheral neuropathy.[2] This allows early detection, diagnosis and treatment response. Its possible role in detecting and managing nerve damage has been studied in diabetes, Parkinson's disease, amyotrophic lateral sclerosis and chemotherapy-induced peripheral neuropathy.[20] Studies have shown that decreased subbasal nerve density is associated with symptoms of peripheral neuropathy. Further, the loss in corneal subbasal nerves precede any clinical signs or symptoms of neuropathy, retinopathy and nephropathy.[20] Interestingly, IVCM has been shown to detect early nerve regeneration after pancreas transplantation in patients with type I diabetes. This is an especially exciting application of IVCM, as it can potentially benefit a large number of patients.

FUTURE DIRECTION

IVCM has broadened our understanding of the cornea in a profound way, offering a unique window to examine *in vivo* cornea tissue in health and disease and quantify corneal pathology. It has yet to reach its peak. Advancing technology in software and engineering, standardized acquisition, establishment of baseline values and development of software for automated analysis are some of the areas that will increase its accessibility and application in both research and clinical arenas. Regardless, with rapidly evolving technology, it continues to be a powerful clinical tool in vision science.

References

1. Girkin CA. "Principles of Confocal Scanning Laser Ophthalmoscopy for the Clinician" in The Essential HRT Primer. M Fingeret, JG Flanagan, JM Liebmann. San Ramon, CA. 2005;1-5.
2. Tavakoli M, Hossain P, Malik R. Clinical applications of corneal confocal microscopy. Clinical Ophthalmology. 2008;2(2):435-445.
3. Cavanagh H, Jester J, Essepian J, Shields W, Lemp M. Confocal microscopy of the living eye. CLAO. 1990;16(1):65-73.
4. Niederer R, McGhee C. Clinical in vivo confocal microscopy of the human cornea in health and disease. Progress in Retinal and Eye Research. 2010;29:30-58.
5. Amin S, Baratz K, McLaren J, Patel S. Corneal abnormalities early in the course of Fuchs' endothelial dystrophy. Ophthalmology. 2014;121:2325-2333.
6. Kobayashi A, Ihibashi Y, Oikawa Y, Yokogawa H, Sugiyama K. In vivo and ex vivo laser confocal microscopy findings in patients with early-stage acanthamoeba keratitis. Cornea. 2008;27(4):439-445.
7. Vaddavalli P, Sharma S, Sanwan V, Rao G, Thomas R. Role of confocal microscopy in diagnosis of fungal and acanthamoeba keraitits. Ophthalmology. 2011;118:29-35.
8. Nielsen E, Heegaard S, Ivarsen A, Mortensen KL, Hjortdal J. Fungal keratitis- improving diagnostics by confocal microscopy. Case Rep Ophthalmol. 2013;4(3): 303–310.
9. Brasnu E, Bourcier T, Dupas B, Degorge S, Rodallec T, Laroche L, Borderie V, Baudouin C. In vivo confocal microscopy in fungal keratitis. Br J Ophthalmol. 2007;91(5):588-591.
10. Nakano E, Oliveira M, Portellinha W, Freitas D, Kozo N. Confocal microscopy in early diagnosis of Acanthamoeba keratitis. J Refract Surg. 2004;20(5):S737-S740.
11. Kaufman S, Musch D, Belin M, Cohen E, Meisler D, Reinhart W, Udell I, Van Meter W. Confocal microscopy: a report by the American Academy of Ophthalmology. Ophthalmology. 2004;111:396-406.
12. Cruzat A, Witkin D, Baniasadi N, Zhung L, Ciolino J, Jurkunas U, Chodosh J, Pavan-Langston D, Dana R, Hamrah P. Inflammation and the nervous system: the connection in the cornea in patients with infectious keratitis. Invest Ophthalmol Vis Sci. 2011;52:5136-5143.
13. Hamrah P, Sahin A, Dastjerdi M, Shahatit B, Bayhan H, Dana R, Pavan-Langston D. Cellular changes of the corneal epithelium and stroma in herpes simplex keratitis: an in vivo confocal microscopy study. Ophthalmology. 2012;119:1791-1797.
14. Erie J, McLaren J, Hodge D, Bourne W. Recovery of corneal subbasal nerve density after PRK and LASIK. Am J Ophthalmol. 2005;140(6):1059-1064.
15. Gabato C, Ghirlando A, Moretto E. Mitomycin C modulation of corneal wound healing after photorefractive keratectomy in highly myopic eyes. Ophthalmology. 2005;112(2):208-218.
16. Niederer RL, Perumal D, Sherwin T, McGhee C. Corneal innervation and cellular changes after corneal transplantation: an in vivo confocal microscopy study. Invest Ophthalmol Vis Sci. 2007;48:621-626
17. Kocaba V, Colica C, Rabilloud M, Burillon C. Predicting corneal graft rejection by confocal microscopy. Cornea. 2015;34(S10):S61-4.
18. Amin S, Baatz K, McLaren J, Patel S. Corneal abnormalities early in the course of Fuchs' endothelial dystrophy. Ophthalmology. 2014;121:2325-2333.
19. John T, Pullos A. Combined granular and Fuchs' corneal dystrophy diagnosed by confocal microscopy after total anterior lamellar keratoplasty. Ann Ophthalmol. 2009;41(3-4):179-83.
20. Wang E, Misra S, Patel D. In vivo confocal microscopy of the human cornea in the assessment of peripheral neuropathy and systemic diseases. BioMed Research International. 2015:2015;1-11.

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Disclosures

None

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