Cornea: Confocal microscopy

Jill Fishbaugh

University of Iowa

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The confocal microscope opens a whole new window in early diagnosis of ocular conditions. Previously, details at the cellular level could only be viewed with conventional microscopes in a laboratory setting. By using confocal microscopy, results can now be obtained instantaneously in the living human eye. This non-invasive high magnification technique provides real-time images of cornea morphology.

What's this hot new topic of discussion in cornea called confocal microscopy? And why are so many people excited about its potential? Recently approved by the Food and Drug Administration (FDA), the confocal microscope opens a whole new window in early diagnosis of ocular conditions (Figure 1). Previously, details at the cellular level could only be viewed with conventional microscopes in a laboratory setting. This involved a time-consuming fixative and staining process with a significant turn around period. In addition, a biopsied specimen was required causing discomfort and a minor risk to the patient. By using confocal microscopy, results can now be obtained instantaneously in the living human eye. This non-invasive high magnification technique provides real-time images of cornea morphology such as the tear film, ocular surface infections, corneal dystrophies, and other diseases.

Magnification
The degree to which normal and diseased cells can be magnified with a confocal microscope is far greater than with a conventional slit lamp. With variable objectives, the confocal microscope has the ability to provide the observer with a wide range in magnification powers. A conventional slit lamp has a maximum magnification capability of 40X. The most commonly used applanating objective on the confocal microscope provides a magnification of 400X.

Optical sectioning of the corneal layers is possible at these high magnifications. The minute focal plane is a mere 6-10 microns in depth. Any thing above or below this range will be out of focus. To illustrate the strength of magnification, notice how clearly the nuclei can be seen in the hexagonal shaped corneal epithelial cells (Figure 2). This more in-depth view can be most beneficial in examining corneal pathology and the outcomes of medical management.

For example, patients with acanthamoeba keratitis (a corneal disease that often eludes a definitive diagnosis without corneal biopsy) can more quickly be diagnosed noninvasively with the confocal microscope (Winchester, Mathers, Sutphin, & Daley, 1994). The cysts of this organism can be easily detected by the experienced ophthalmologist (Figure 3). Patients do much better with early medical treatment of this potentially blinding condition. The minor cellular changes that occur with the progression of the disease or with the help of the medical treatment instituted can more accurately be monitored. Adjustments in drug dosages can then be increased or decreased accordingly.

Types
There are several types of confocal microscopes being used for research and in clinical practice throughout the country. The two most popular are the slit scanning and the Nipkow-disc-based scanning. Slit scanning confocal works much like a specular micro-
scope, whereas Nipkow-disc-based scanning confocal operates through a rotating disc with pinholes using either a single or a dual light path system and an applanating objective.

The Nipkow-disc-based confocal microscope was originally developed in 1967 by Petran and Hadravsky in Czechoslovakia, with its first application on the living human eye in 1989 (Cavanagh, Petroll, Alizadeh, He, McCulley, & Jester, 1993). To understand the Nipkow-disc-based system in greater detail, see Figure 4 showing the pathway of light. A mercury vapor lamp is used as the intense light source needed to illuminate the eye. A glass and chrome rotating Nipkow disc with 35,000 symmetric pinholes, each pinhole a diameter of 20 microns, spins at a speed of 900 rpm, transmitting light through the beam splitter and objective onto the corneal surface.

Since the eye is a moving target, a drop of hydroxymethylcellulose is used with a 24X applanating objective to stabilize the eye and to provide a protective non-refracting medium between the objective tip and the corneal surface. Light is reflected back from the cornea through the objective to a mirror and onto a detector pinhole exactly 180 degrees from where the light was transmitted through the Nipkow disc. The image is then collected and recorded on a video camera that collects real-time images at a rate of thirty frames per second for instantaneous observation.

The most common style of Nipkow-disc-based scanning confocal is made by the Tandem Scanning Corporation. There are about fifteen of this type being used worldwide to diagnose corneal disease. Dr. William Mathers, M.D., Director of the Cornea and External Disease Clinic at the University of Iowa, Department of Ophthalmology describes its impact on his clinical practice as, "The resolution attainable with confocal microscopy allows the researcher to investigate corneal diseases in a fundamentally new way. Hence, we don't yet know what disease looks like at this cellular level and every patient examined presents a chance to explore the eye and learn something new."

Currently, this new technology is cost prohibitive for most general clinical practice settings, but hopefully it will continue to be developed into an instrument that could be incorporated into many private practice settings in the near future. It promises to be an important diagnostic tool in the field of ophthalmology.

This column focuses on different subspecialty practices and has excellent review information for ophthalmic nurses in every practice setting. If you have topics you would like to see covered, contact the author by writing to: Jill Fishbaugh, R.N., CRNO, Cornea Center, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

Jill Fishbaugh is the Cornea Center Clinic Coordinator for the Department of Ophthalmology, University of Iowa Hospitals and Clinics in Iowa City IA. She is on the INSIGHT Editorial Board and has been a member of ASORN since 1986.
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