Confocal Microscopy Findings of *Acanthamoeba* Keratitis

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• PURPOSE: Tandem scanning confocal microscopy was performed on two patients with *Acanthamoeba* keratitis to provide images detailing characteristic findings of the disease. Although tandem scanning confocal microscopy of *Acanthamoeba* has been described in previous reports, *Acanthamoeba* keratitis has not been fully characterized with this instrument. In vivo confocal micrographs showed the double-walled structure of the *Acanthamoeba* cyst and associated radial keratoneuritis (perineuritis).

• METHODS: We reviewed the records of two patients with a clinical diagnosis of *Acanthamoeba* keratitis, one with culture-proven *Acanthamoeba* and the other with a suspected *Acanthamoeba* infection. Slit-lamp biomicroscopy and in vivo tandem scanning confocal microscopy were performed. The images obtained were compared with images from patients without corneal disease.

• RESULTS: High-contrast round bodies suggestive of *Acanthamoeba* cysts, as previously described, and irregular forms suggestive of *Acanthamoeba* trophozoites were found by tandem scanning confocal microscopy. Additionally, we showed conclusively that under certain circumstances (that is, corneal scarring) tandem scanning confocal microscopy can resolve the double-walled structure of the *Acanthamoeba* ectocyst surrounding the endocyst. Furthermore, radial keratoneuritis was demonstrated, consisting of an irregularly swollen nerve fiber with probable amoebic infiltration.

• CONCLUSIONS: Confocal microscopy can be a useful, noninvasive imaging technique helpful in the study, diagnosis, and treatment of *Acanthamoeba* keratitis.

ACANTHAMOEBA IS A UBIQUITOUS, FREE-LIVING amoeba found in water and soil, but *Acanthamoeba* keratitis is a relatively newly recognized entity, with the first reported case in 1975.[1] Although it remains a relatively uncommon origin of infectious keratitis, a growing number of cases have been reported, especially in association with contact lens wear.[2-6] *Acanthamoeba* keratitis is a painful, potentially blinding condition that, like any infectious keratitis, benefits from early detection and treatment. To detect the organism early, several diagnostic tools, including calcofluor white,[7] a nonspecific stain, and an indirect fluorescent antibody stain[8] have been described, but these methods require invasive techniques, frequently including biopsy, to obtain tissue to stain.

See also pp. 129 and 207.

More recently, investigators have reported the use of confocal microscopy for rapid, noninvasive, in vivo detection of *Acanthamoeba*.[9-12] In this study, we examined two patients with *Acanthamoeba* keratitis who underwent both clinical examination and in vivo tandem scanning confocal microscopy. The results of this study provide additional support for the use of in vivo confocal microscopy in the rapid diagnosis and treatment of *Acanthamoeba* keratitis. Furthermore, we add two findings to the study of

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Acanthamoeba keratitis by in vivo tandem scanning confocal microscopy. First, we definitively demonstrate with tandem scanning confocal microscopy the double-walled nature of the *Acanthamoeba* ectocyst surrounding the endocyst, previously illustrated by light microscopy[13,14] and electron microscopy[15] in specimens and suggested by an in vivo scanning slit confocal microscopy image published by Auran and associates.[11] Auran and associates described a "cyst-like structure . . . with indentations . . . suggestive of the double-walled *Acanthamoeba* cyst," but they were unable to demonstrate the ectocyst surrounding the endocyst. Second, we show a confocal micrograph of perineuritis, a biomicroscopy finding possibly pathognomonic for *Acanthamoeba* keratitis.

PATIENTS AND METHODS

TWO PATIENTS WERE IDENTIFIED. THE PATIENT DEscribed in Case 1 was a follow-up patient with culture-proven *Acanthamoeba* keratitis; and the patient described in Case 2 underwent initial examination for suspected infection with *Acanthamoeba*. The clinical records of these patients were reviewed, and they underwent an examination with both biomicroscopy and in vivo tandem scanning confocal microscopy.

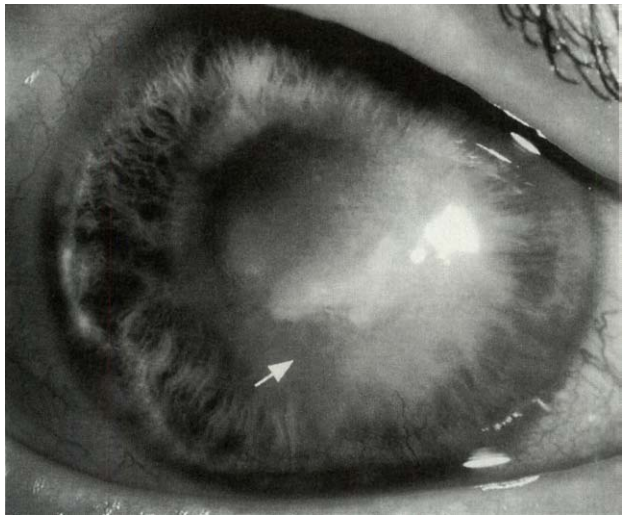

Fig. 1 (Pfister and associates). Case 1. Right eye showing extensive stromal scarring, 40% paracentral thinning (arrow), moderate stromal haze, paracentral stromal edema, endothelial plaque, and fine keratic precipitates.

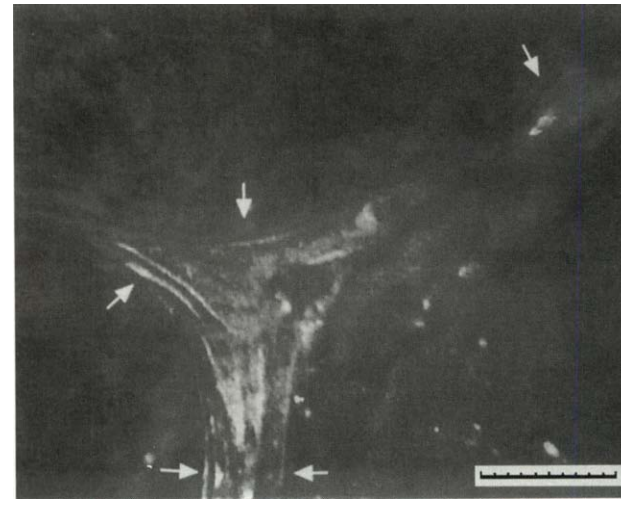

Fig. 2 (Pfister and associates). Case 1. Confocal micrograph of the anterior corneal stroma showing high-contrast linear and curvilinear striations (arrows) consistent with extensive stromal scar formation (marker = 100 μm).

After topical administration of proparacaine, tandem scanning confocal microscopy (Tandem Scanning Corporation, Reston, Virginia) was performed on the eye of interest by using methyl cellulose as a coupling agent between the objective and the surface of the cornea, as previously described.[10,16] A 24×, 0.6 numeric aperture objective lens (Tandem Scanning Corporation, Reston, Virginia) with a working distance of 1.5 mm and a separate electronic depth controller for z-axis changes was used to obtain images. Currently, the tandem scanning confocal microscopy unit manufactured by the Tandem Scanning Corporation is the only confocal microscope approved by the Food and Drug Administration for general clinical use.

To visualize and record the confocal microscopy findings, a low-light application Hamamatsu-C2400 SIT video camera connected to both an S-VHS video recorder and a black-and-white video monitor was used. Single-image frames were captured and digitized from the videotape, by using a computer video display board, onto a Macintosh computer with IPLab Spectrum software (version 2.5.7, Signal Analytics Corp., Vienna, Virginia). Images were further processed,

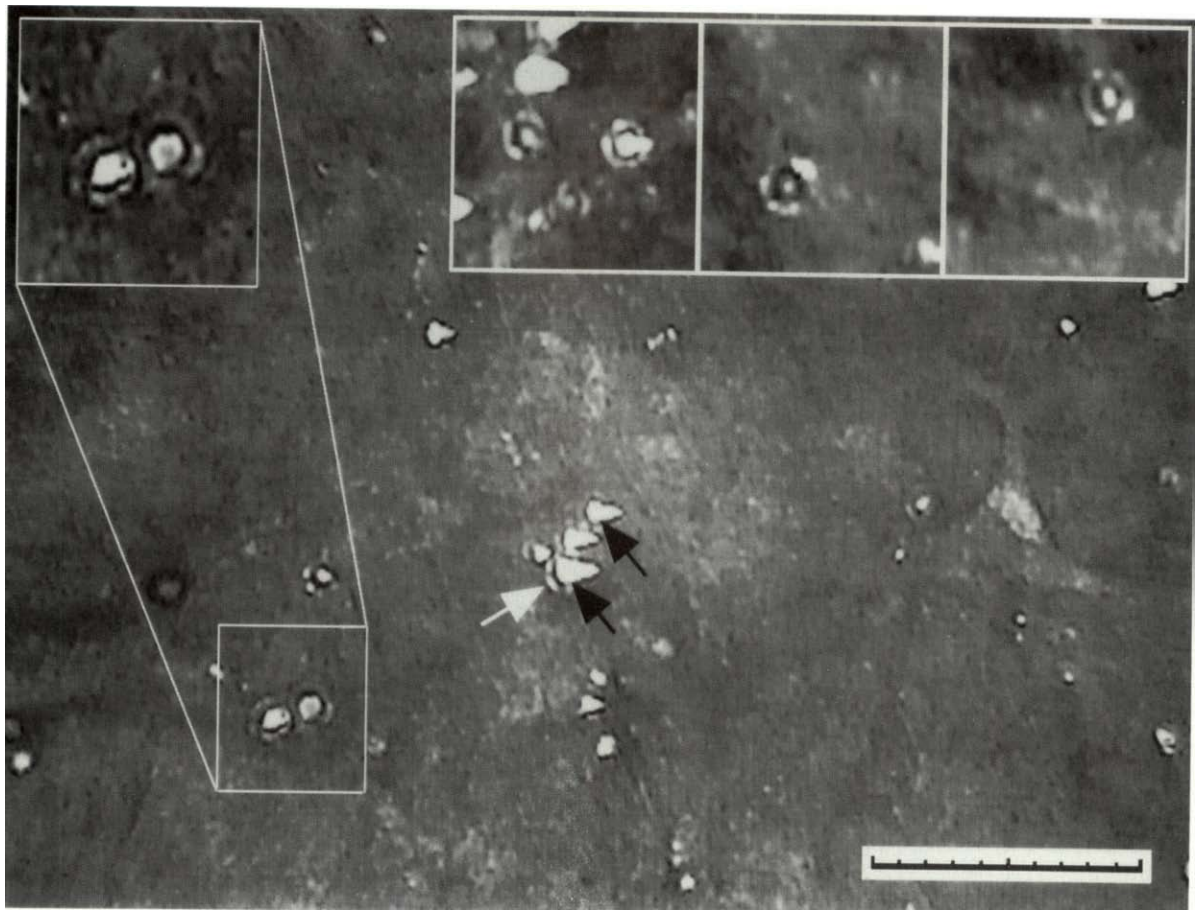

Fig. 3 (Pfister and associates). Case 1. Confocal micrograph of scarred corneal stroma, with several pear-shaped *Acanthamoeba* cysts (black arrows), some with evidence of a surrounding ectocyst (white arrow) (marker = 100 μm). Upper left inset, enlargement of the cysts outlined in the main image shows the double-wall nature of the cysts. Upper right three insets, Further illustrations of the ectocyst encasing the endocyst, with some cysts showing fine gray spokelike structures radiating between the inner and outer cyst.

including signal averaging, by using Adobe Photoshop (version 3.0, Mountain View, California), and final images were obtained from a digital continuous-tone printer. The tandem scanning confocal microscopy images obtained from the two patients with keratitis were compared with images from control patients without corneal disease, images obtained from previous rabbit studies, as well as previously published *Acanthamoeba* keratitis images.

CASE REPORTS

• CASE 1: Herpes simplex keratitis was initially diagnosed in the right eye of a 51-year-old woman and treated with topical vidarabine twice a day, dexamethasone sodium phosphate every three hours, and atropine 1% twice a day; nevertheless, both her symptoms and clinical course continued to worsen. She was referred to the University of Minnesota eight weeks after her initial symptoms. She used extended-wear contact lenses and had episodes of severe pain. Her visual acuity was R.E.: hand movements and L.E: 20/20, and her right eye showed an ovoid epithelial defect, stromal edema, few keratic precipitates, and a ring infiltrate. Initial fluorescent antibody stain and cultures were negative for *Acanthamoeba*, bacteria, and viruses. A clinical diagnosis of *Acanthamoeba* was established, and topical polyhexamethylene biguanide 0.02% every hour with atropine once a day

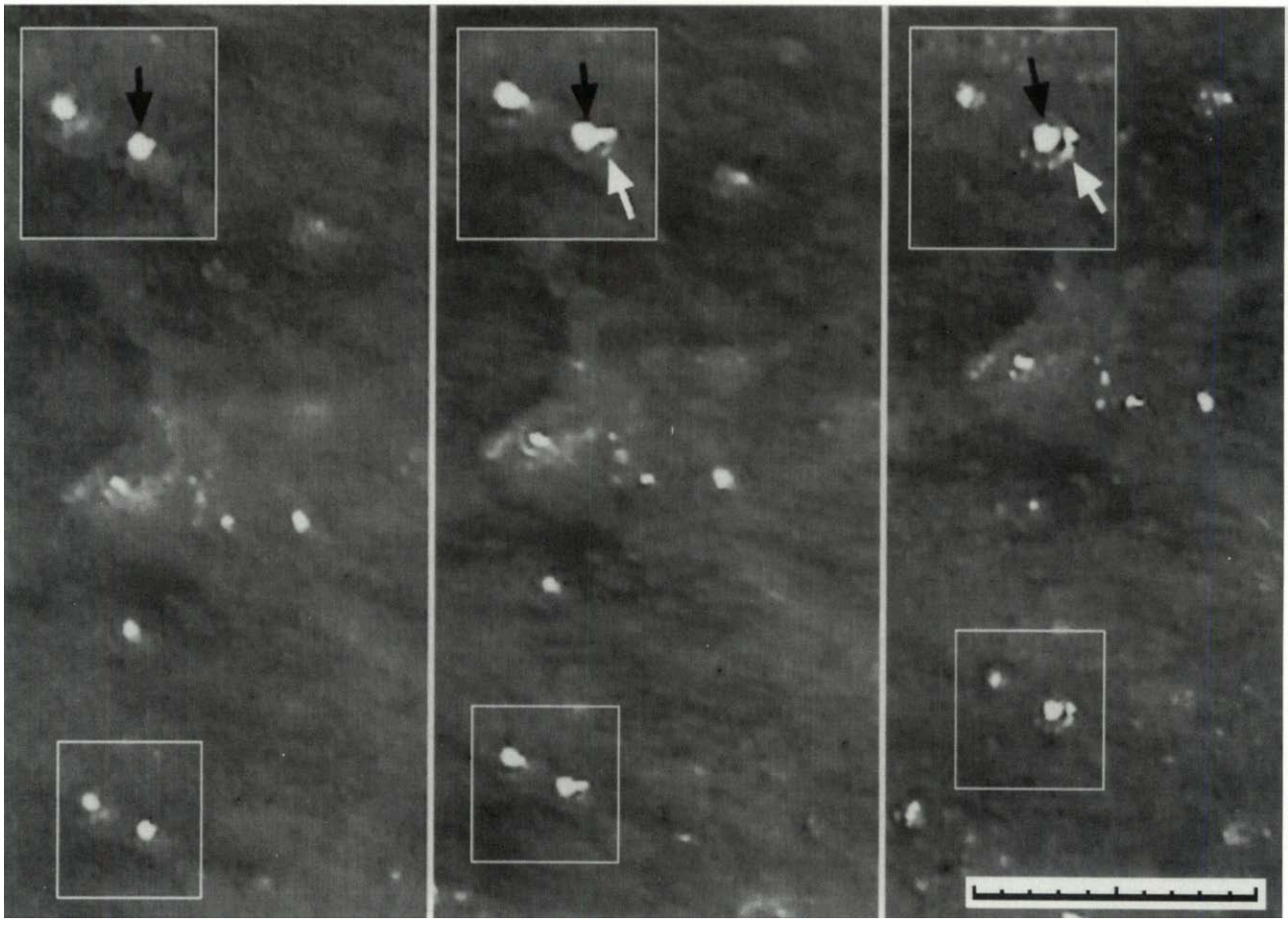

Fig. 4 (Pfister and associates). Case 1. Confocal micrograph of corneal stroma showing three panels of the same cysts scanned at slightly different depths (marker = 100 μm). The upper inset in each panel is an enlargement of the outlined area showing the endocyst (black arrows) surrounded by the ectocyst (white arrows). Left, Bright round cysts only; middle, pear-shaped body with suggestion of ectocyst; and right, endocyst with ectocyst clearly resolved surrounding one half of the endocyst.

was started in the right eye while culture results were pending. Polymyxin B, neomycin, and gramicidin; clotrimazole 1%; and propamidine isethionate were added later. Her ocular condition waxed and waned despite intensive multidrug treatment. After numerous attempts, including several corneal biopsies, *Acanthamoeba* was cultured six months after starting treatment.

After 17 months of treatment, her visual acuity was R.E.: hand movements. Extensive stromal scarring with 40% paracentral thinning, moderate stromal haze, paracentral stromal edema, an endothelial plaque, and fine keratic precipitates were present (Fig. 1). During this follow-up examination, tandem scanning confocal microscopy was performed. Extensive stromal scarring (Fig. 2) and numerous round, high-contrast, double-walled cysts were easily detected in the stroma (Figs. 3 and 4), confirming the presence of persistent *Acanthamoeba* organisms. The cysts ranged between 14 and 17 μm, averaging 15.4 μm. Multidrug therapy was continued.

• CASE 2: An 18-year-old woman who had a history of daily-wear soft contact lens use was referred to us for examination of the left eye with a three-week history of increasing pain, redness, lacrimation, and severe photophobia. She had been treated initially with ofloxacin, without improvement. Trifluridine

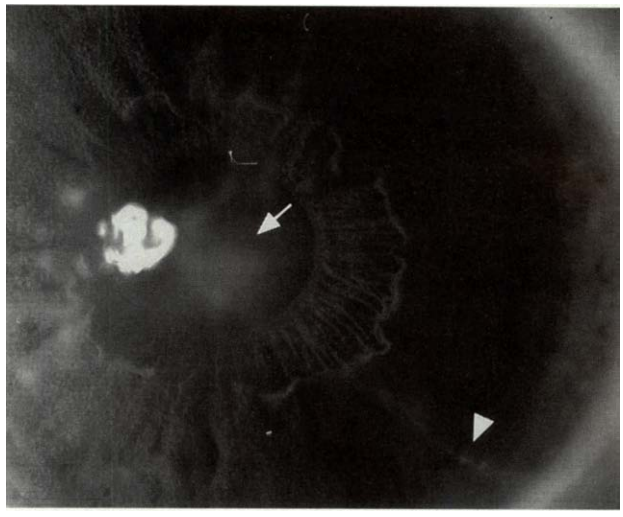

Fig. 5 (Pfister and associates). Case 2. Left eye with central epitheliopathy (arrow) and radial keratoneuritis (arrowhead).

was subsequently added, but the symptoms worsened. Her visual acuity was R.E.: 20/20 and L.E.: 20/40. A mild to moderate ciliary injection, thickened and irregular central corneal epithelium, several subepithelial infiltrates, and radial keratoneuritis were present in the left eye (Fig. 5).

Numerous high-contrast round *Acanthamoeba* cysts with several irregular trophozoites were visible by tandem scanning confocal microscopy in the mid and deep epithelium (Fig. 6). The *Acanthamoeba* cysts measured between 11 and 15 μm, with an average of 13.3 μm. *Acanthamoeba* trophozoites were more variable in size and shape than were the cysts and measured from 23 to 25 μm × 11 to 19 μm, averaging 23.6 × 15.2 μm. No cysts were visible in either the superficial epithelium or the anterior stroma below Bowman's layer; however, the anterior stroma just beneath Bowman's layer was markedly abnormal, exhibiting irregular, high-contrast septae surrounding lucent areas (Fig. 7). This abnormal stromal appearance was similar to the stromal edema that we observed by tandem scanning confocal microscopy in rabbits after perforating corneal wounds (compare Fig. 7 main image with upper right inset). The highest contrast elements seen in the anterior stroma are probably keratocyte nuclei. Also, an irregularly thickened stromal nerve was seen, consistent with radial keratoneuritis (Fig. 8). A diagnosis of *Acanthamoeba* keratitis in the left eye was established on the basis of the clinical appearance of the irregular epithelium and perineuritis, as confirmed by tandem scanning confocal microscopy, and the patient was treated with propamidine isethionate (Brolene); polyhexamethylene biguanide 0.02%; polymyxin B, neomycin, and gramicidin (Neosporin); and miconazole 1% every hour in the left eye. Over the next few weeks, her symptoms resolved. At her last visit, five months after diagnosis, her examination disclosed visual acuity of L.E.: 20/25, no conjunctival injection, smooth and intact epithelium, mild subepithelial scarring, and no radial keratoneuritis. The miconazole was discontinued, and the propamidine isethionate; polyhexamethylene biguanide; and polymyxin B, neomycin, and gramicidin were tapered to four times a day.

DISCUSSION

EARLY DIAGNOSIS AND TREATMENT OF *ACANTHAMOEBA* keratitis yields a better outcome,[17] likely because early in the course, the organism is in the superficial cornea. Early clinical signs include an irregular epithelium[6,18] that breaks down recurrently, an epithelial dendritiform pattern,[19] a raised, gelatinous epitheliopathy,[20] subepithelial infiltrates,[21] and radial keratoneuritis (perineuritis).[6,22] Late signs indicating deeper penetration include a stromal ring infiltrate,[6,20] corneal ulceration, and stromal edema with inflammation. Subepithelial infiltrates can also be a late finding related to an immunologic reaction, but they may not indicate active disease.[23]

In the past, definitive diagnosis of *Acanthamoeba* keratitis required invasive diagnostic techniques. *Acanthamoeba* must be cultured on nonnutrient agar overlaid with *Escherichia coli* or *Enterobacter*, and this culture can require between one and nine days to grow and may not recover the organism. Culturing the parasite also necessitates performing a corneal scraping to obtain superficial tissue and often requires a more invasive corneal biopsy. Likewise, special stains, including calcofluor white[7] and indirect fluorescent antibodies,[8] can be used; however, these staining methods also require a tissue scraping or corneal biopsy, a fluorescence microscope, and expertise with these methods.

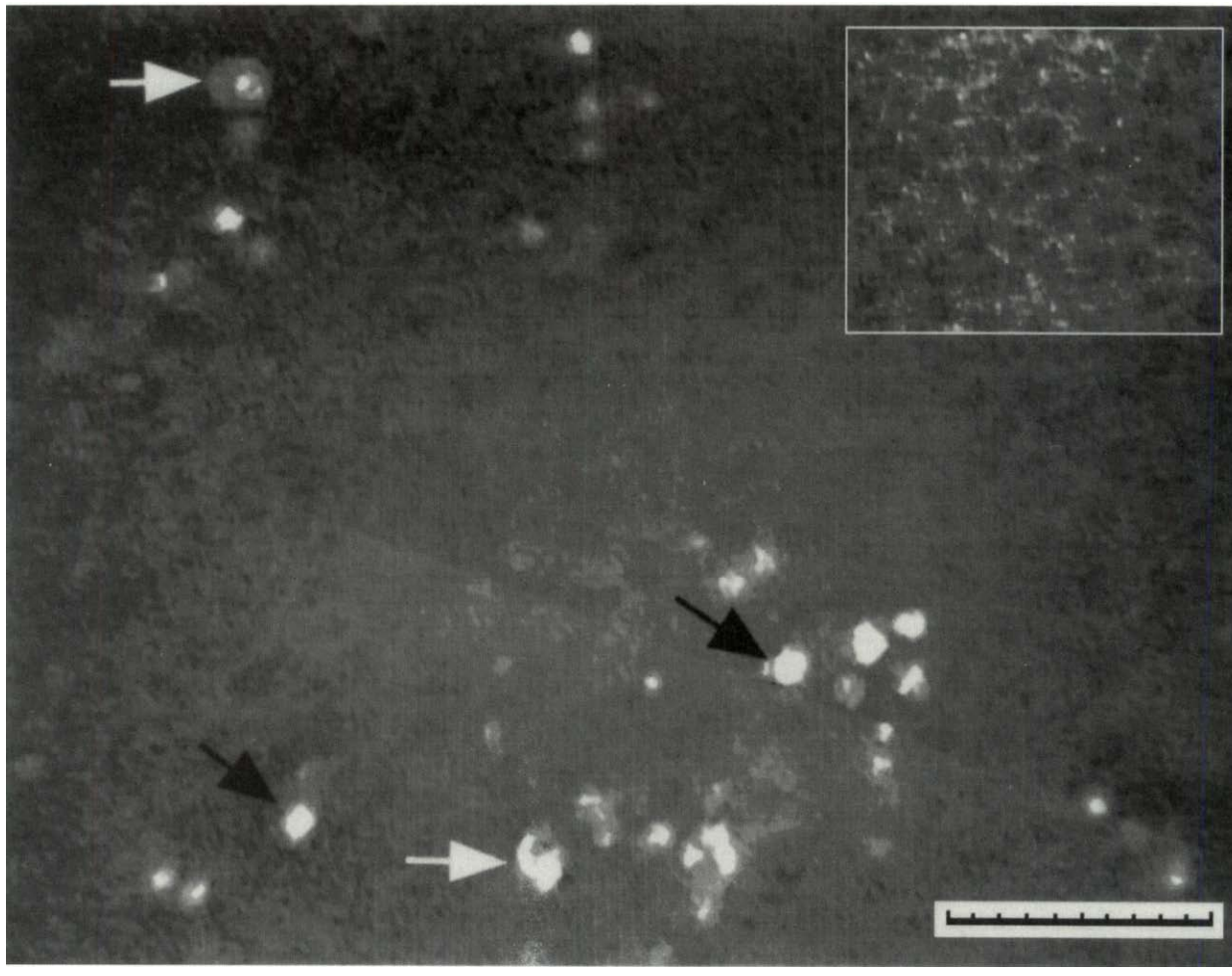

Fig. 6 (Pfister and associates). Case 2. Confocal micrograph of basal epithelium showing bright round bodies consistent with *Acanthamoeba* cysts (black arrows) and larger ovoid or irregularly shaped cells consistent with the trophozoite form (white arrows). Note the cell indicated by the lower white arrow appears to be extending a pseudopod above the level of the arrow. Inset, Normal human basal epithelium shows tessellated cell borders without internal cellular details (marker = 100 μm, image and inset).

Confocal microscopy is capable of providing noninvasive, high-contrast, in vivo images of the cornea at different depths (a change in the z-axis) from epithelium to endothelium. Magnifications of 240× or more can be obtained (approximately 380× on a video monitor), depending on the objective and screen display used, and this amount of magnification is great enough to see individual cells, including *Acanthamoeba*, in the cornea.

Conventional light microscopy is achieved by imaging thin histologic sections to minimize interference and light scatter (that is, loss of resolution) by tissue above or below the plane of focus. It follows that ideal histologic specimens are two dimensional; no light is scattered by extraneous tissue planes. Clearly, this procedure is not clinically useful unless a pathologic specimen is obtained. The magnification available through slit-lamp biomicroscopy is more severely limited by the interference from tissue in planes other than the one of interest. Only under certain circumstances are individual cells discernible (for example, as are endothelial cells viewed by specular reflection). Using slit-lamp biomicroscopy, cellular detail is minimal at best and limited to the high-contrast borders between cells or guttata.

Tandem scanning confocal microscopy is based on

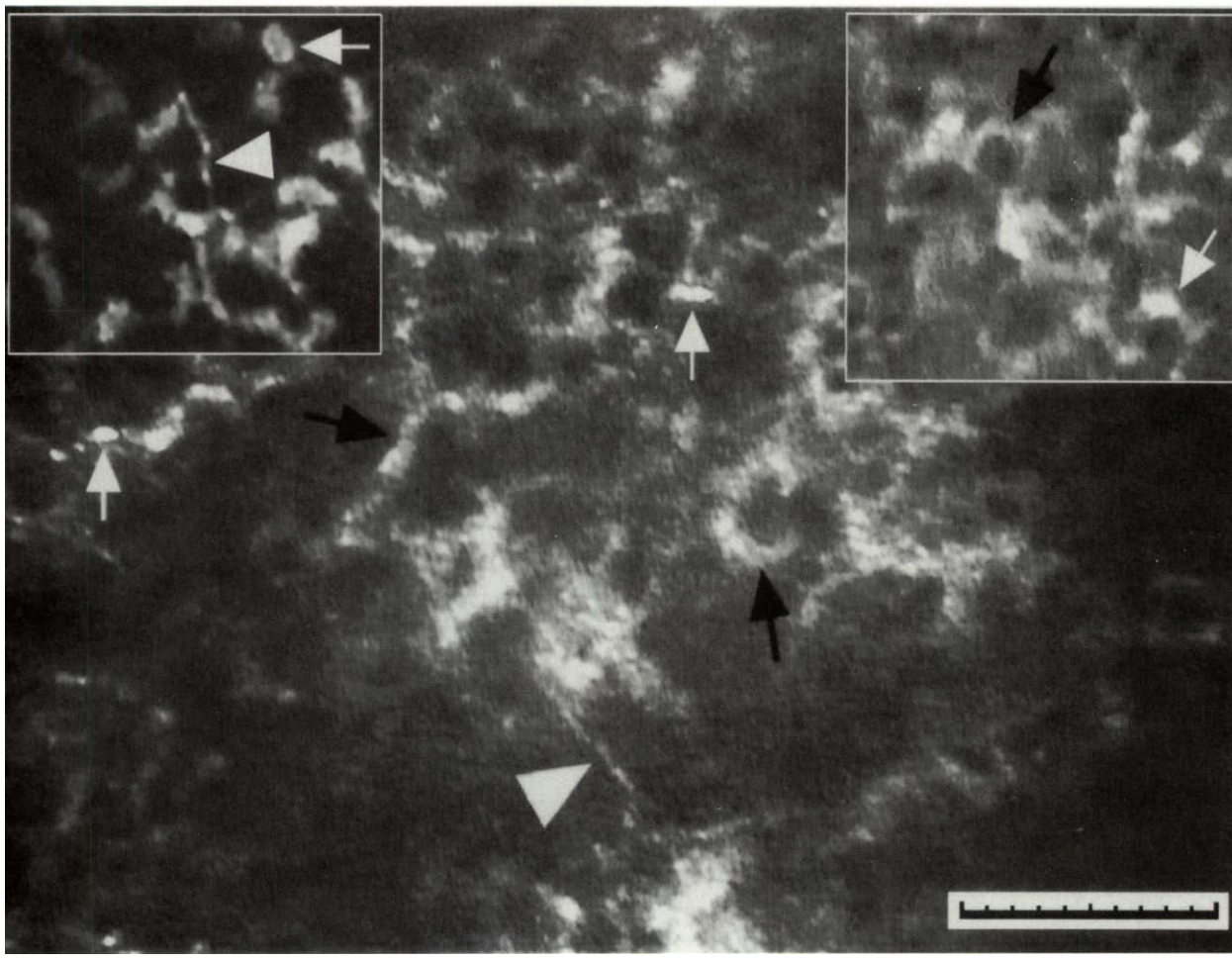

Fig. 7 (Pfister and associates). Case 2. Confocal micrograph of anterior stromal haze directly beneath Bowman's layer showing bright, irregular septae (black arrows) surrounding lucent areas, small high-contrast bodies suggestive of keratocyte nuclei (white arrows), and a fine linear structure consistent with a small corneal nerve (arrowhead). Upper left inset, Normal human anterior corneal stroma at the same level shows keratocytes (white arrow) and part of a small nerve (arrowhead). Upper right inset, Rabbit cornea with stromal edema for comparison with the human corneal stroma shown in the main image. Confocal microscopy of the edematous rabbit corneal stroma (upper right inset) shows irregular septae (black arrow) and bright nuclei (white arrow). Note the similar appearance of the edematous rabbit corneal stroma to the human corneal stroma of Case 2 shown in the main image. Both show irregular septae surrounding lucent areas and bright nuclei (marker = 100 μm, image and insets).

optics described by Minsky[24] in 1961, which detailed simultaneous focus of both the illuminating source and the objective lens on a single point of tissue. In such a system, both the illumination source and objective have the same focal point and are, therefore, confocal. Pinpoint illumination and focus create high-resolution images but produce only a minute field of view. However, this limitation is obviated by building a larger image from the rapid summation of many pinpoint fields of view acquired in an ordered, gridlike manner.[16] A similar concept of building an image one point at a time is used in both scanning electron microscopy and television. The Tandem Scanning unit uses a rotating disk (Nipkow disk) with numerous precisely arranged 20-μm holes, so that as the disk rotates, different pinpoint areas of a plane are illuminated and imaged in rapid succession to build the composite picture. Real-time video rates of 30 frames/second are achieved with this system. When compared to conventional histologic sections, the

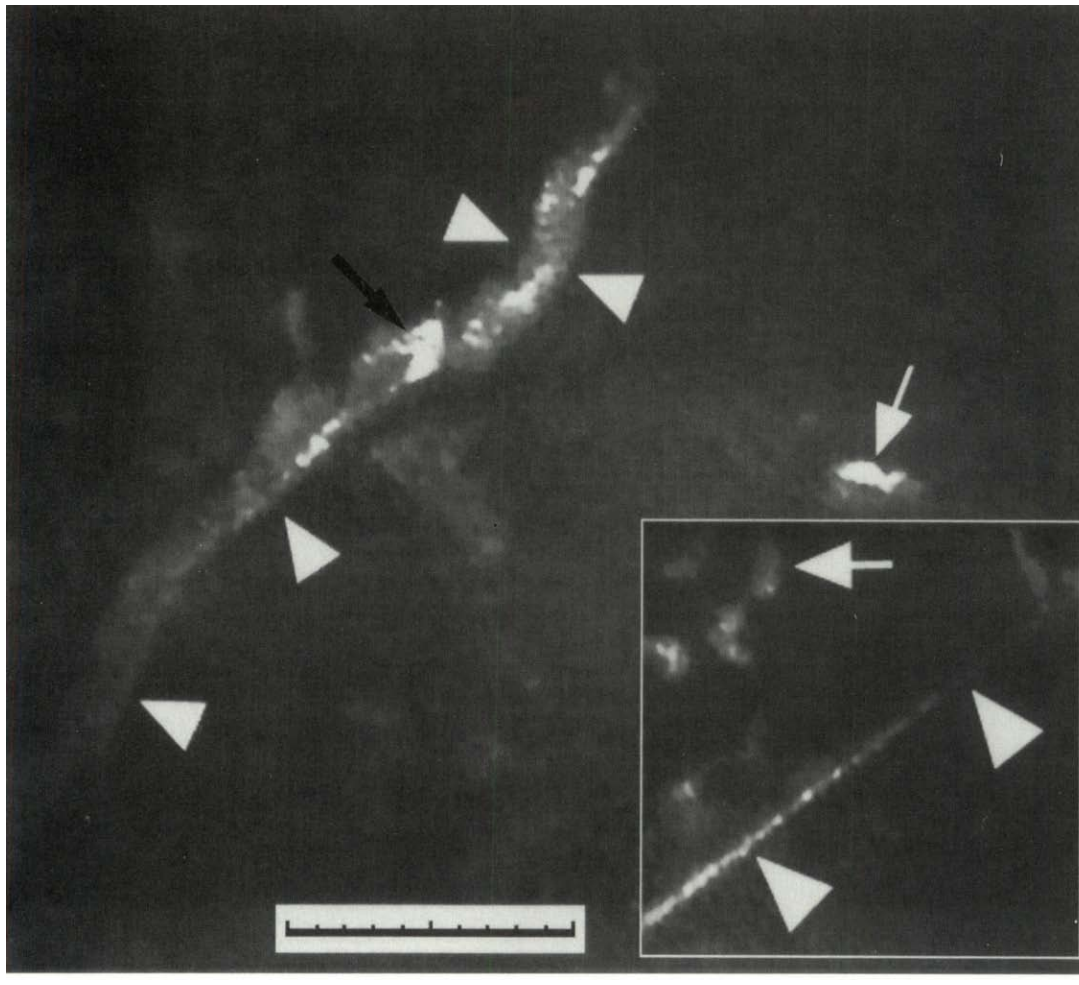

Fig. 8 (Pfister and associates). Case 2. Confocal micrograph of the anterior to midcorneal stroma showing a swollen nerve (arrowheads) consistent with radial keratoneuritis, a bright irregular body on the nerve (black arrow) consistent with an *Acanthamoeba* trophozoite extending pseudopods and migrating along the surface of the nerve, and a stromal keratocyte (white arrow). Inset, Normal human stromal nerve (arrowheads) and stromal keratocytes (arrow). Note the difference in caliber and irregularity between the two nerves shown, as well as the ragged appearance of the nerve to the left of the trophozoite (marker = 100 μm, image and inset).

view is of two-dimensional sections in the coronal (x,y) plane, in contrast to the customary anterior to posterior (sagittal or oblique) orientation in both slit-lamp and light microscopy. Changing the depth of the focal point (z-axis) within the tissue allows different layers of the cornea to be imaged with little interference from layers outside the plane of interest, which enhances resolution.

Lateral resolution of the confocal microscope in the x,y plane is 1 μm or less, and z-axis resolution is approximately 6 μm.[25] In contrast to a slit lamp where the maximum magnification is between 16× and 40×, confocal microscopy, with screen magnification of about 380×, allows the detection of individual cells and some internal cellular structures. Additionally, the approximate depth of focus within the tissue can be determined by using the electronic z-axis control of the z-axis controller, but in vivo, this procedure is cumbersome.

Acanthamoeba cysts can be visualized as high-contrast, round structures measuring between 10 and 25 μm,[26] well above the resolution limits of the instrument, and the measurements we obtained for cysts of 15.4 and 13.3 μm in Cases 1 and 2, respectively, agreed with these data. In certain circumstances, such as Case 1, perhaps because of the

extensive corneal scarring, the double-walled nature of the cysts is apparent by tandem scanning confocal microscopy (Figs. 3 and 4). In slightly different planes, the organisms can appear as bright, round structures (Fig. 4, left) or as obviously double-walled organisms (Fig. 4, right), and between these planes they may appear pear or egg shaped, where the bright endocyst is not differentiated from one side of the surrounding ectocyst (Fig. 4, center). This observation may contribute to the variability seen in measuring the diameter of the cysts by tandem scanning confocal microscopy. Also apparent within some of these cysts are spokelike structures running between the ectocyst and the endocyst (Fig. 3, top right three insets). Anatomically, these spokes correspond to pores or ostioles between the ectocyst and endocyst.[14] Trophozoites extending pseudopodia may also be detected by tandem scanning confocal microscopy, but they appear to be more variable in size and shape than cysts (Fig. 6).

Figure 8 from Case 2 shows what is presumably inflammation of a corneal nerve in the anterior to midcorneal stroma, which is a characteristic slit-lamp finding in *Acanthamoeba* keratitis. The nerve appears irregular, thickened, and has a shaggy border compared with the normal nerve in the inset. The highest contrast structure on the nerve appears to be an *Acanthamoeba* trophozoite extending pseudopodia along the nerve. The size of this structure is consistent with our observations of other trophozoites and measures 23.0 × 14.0 μm. The most irregular portion of the nerve, just to the left of the trophozoite, suggests that the organism may be disrupting the neural membrane. If this is the case, perhaps the swollen appearance of the nerve is caused by hydration resulting from the disruption. Clinically, the severe pain associated with *Acanthamoeba* keratitis may be caused by both the invasion of the neural membrane by the trophozoite and the resulting swelling of the nerve fiber. Additionally, we postulate that the organism tracks along and feeds on ocular neural tissue, for *Acanthamoeba* infections of the central nervous system are known to occur from certain species of amoeba.[5,27]

In summary, we obtained images of the double-walled nature of *Acanthamoeba* cysts in vivo and from patients with radial keratoneuritis with amoebic infiltration. Tandem scanning confocal microscopy can be useful clinically as a noninvasive imaging technique for rapid diagnosis and subsequent treatment, as well as in the study of the pathophysiologic characteristics, of this disease.

REFERENCES

1. Jones DB, Visvesvara GS, Robinson NM. *Acanthamoeba polyphaga* keratitis and *Acanthamoeba* uveitis associated with fatal meningoencephalitis. Trans Ophthalmol Soc U K 1975;95:221–32.
2. Auran JD, Starr MB, Jakobiec FA. *Acanthamoeba* keratitis: a review of the literature. Cornea 1987;6:2–26.
3. Dornic DI, Wolf T, Dillon WH, Christensen B, Deem CD. *Acanthamoeba* keratitis in soft contact lens wearers. J Am Optom Assoc 1987;58:482–6.
4. Johns KJ, Parrish CM, Seal MR, Jerkins GW, Berrie WR, Litchford DW, et al. *Acanthamoeba* keratitis in Tennessee: a growing problem in patients wearing contact lenses. J Tenn Med Assoc 1989;82:584–8.
5. Ma P, Visvesvara GS, Martinez AJ, Theodore FH, Daggett PM, Sawyer TK. *Naegleria* and *Acanthamoeba* infections: review. Rev Infect Dis 1990;12:490–513.
6. Bacon AS, Frazer DG, Dart JK, Matheson M, Ficker LA, Wright P. A review of 72 consecutive cases of *Acanthamoeba* keratitis, 1984–1992. Eye 1993;7:719–25.
7. Wilhelmus KR, Osato MS, Font RL, Robinson NM, Jones DB. Rapid diagnosis of *Acanthamoeba* keratitis using calcofluor white. Arch Ophthalmol 1986;104:1309–12.
8. Epstein RJ, Wilson LA, Visvesvara GS, Plourde EG Jr. Rapid diagnosis of *Acanthamoeba* keratitis from corneal scrapings using indirect fluorescent antibody staining. Arch Ophthalmol 1986;104:1318–21.
9. Chew SJ, Beuerman RW, Assouline M, Kaufman HE, Barron BA, Hill JM. Early diagnosis of infectious keratitis with in vivo real time confocal microscopy. CLAO J 1992;18:197–201.
10. Cavanagh HD, Petroll WM, Alizadeh H, He YG, McCulley JP, Jester JV. Clinical and diagnostic use of in vivo confocal microscopy in patients with corneal disease. Ophthalmology 1993;100:1444–54.
11. Auran JD, Starr MB, Koester CJ, LaBombardi VJ. In vivo scanning slit confocal microscopy of *Acanthamoeba* keratitis: a case report. Cornea 1994;13:183–5.
12. Winchester K, Mathers WD, Sutphin JE, Daley TE. Diagnosis of *Acanthamoeba* keratitis in vivo with confocal microscopy. Cornea 1995;14:10–7.
13. Ishibashi Y, Matsumoto Y, Kabata T, Watanabe R, Hommura S, Yasuraoka K, et al. Oral itraconazole and topical miconazole with debridement for *Acanthamoeba* keratitis. Am J Ophthalmol 1990;109:121–6.
14. Visvesvara GS. Classification of *Acanthamoeba*. Rev Infect Dis 1991;13Suppl 5:S369–72.
15. Ubelaker JE, Moore MB, Martin JH, Silveny R, Dougherty JM, Meyer DR, et al. In vitro intercellular adherence of *Acanthamoeba castellanii*: a scanning and transmission electron microscopy study. Cornea 1991;10:299–304.
16. Cavanagh HD, Jester JV, Essepian J, Shields W, Lemp MA. Confocal microscopy of the living eye. CLAO J 1990; 16:65–73.
17. Bacon AS, Dart JK, Ficker LA, Matheson MM, Wright P.

Acanthamoeba keratitis: the value of early diagnosis. Ophthalmology 1993;100:1238–43.
18. Jones DB. *Acanthamoeba*—the ultimate opportunist? [editorial] Am J Ophthalmol 1986;102:527–30.
19. Lindquist TD, Sher NA, Doughman DJ. Clinical signs and medical therapy of early *Acanthamoeba* keratitis. Arch Ophthalmol 1988;106:73–7.
20. Florakis GJ, Folberg R, Krachmer JH, Tse DT, Roussel TJ, Vrabec MP. Elevated corneal epithelial lines in *Acanthamoeba* keratitis. Arch Ophthalmol 1988;106:1202–6.
21. Beattie AM, Slomovic AR, Rootman DS, Hunter WS. *Acanthamoeba* keratitis with two species of *Acanthamoeba*. Can J Ophthalmol 1990;25:260–2.
22. Insler MS, Portnoy SL, Beuerman RW, Kaufman HE. Radial intrastromal lines in *Acanthamoeba* keratitis: case report. Arch Ophthalmol 1988;106:883.
23. Holland EJ, Alul IH, Meisler DM, Epstein RJ, Rotkis WM, Nathenson AL, et al. Subepithelial infiltrates in *Acanthamoeba* keratitis. Am J Ophthalmol 1991;112:414–8.
24. Minsky M. U.S. patent 3013467, microscopy apparatus. December 19, 1961.
25. User manual for the model 165A Tandem Scanning Confocal Microscope. Reston, Virginia: Tandem Scanning Corporation, 1994.
26. Hirst LW, Green WR, Merz W, Kaufmann C, Visvesvara GS, Jensen A, et al. Management of *Acanthamoeba* keratitis: a case report and review of the literature. Ophthalmology 1984;91:1105–11.
27. Visvesvara GS, Stehr-Green JK. Epidemiology of free-living ameba infections. J Protozool 1990;37:25S–33S.

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