High-Resolution Ultrasonic Imaging and Characterization of the Ciliary Body

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PURPOSE. To develop a means for noninvasive in vivo visualization of the ciliary processes using very-high-frequency (50 MHz) ultrasound and to develop quantitative morphologic descriptors that may relate to physiologic function.

METHODS. The region of the ciliary body was scanned with very-high-frequency ultrasound, both in rabbits and in normal human subjects. Data were acquired in a series of planes so that the spacing between them was less than the beam width of the transducer in its focal plane. Three-dimensional perspective images were constructed, representing the anatomy of the angle region, including the ciliary processes. The automatically detected boundaries of the ciliary processes were analyzed to compute their periphery, area, shape factor, and fractal dimension. These measures were compared between the human and the rabbit eye and analyzed for periodicities related to the spacing of successive processes.

RESULTS. Three-dimensional images allowed visualization of the radial arrangement of the processes. All biometric descriptors were significantly different between the rabbit and human eye and showed periodicities consistent with spacing between processes.

CONCLUSIONS. The methods described in this report are sensitive descriptors of the state of the ciliary processes. These techniques may be of value in measurement of changes in the ciliary body associated with disease, medical therapy, and aging. (Invest Ophthalmol Vis Sci. 2001;42:885–894)

The ciliary processes, the site of aqueous fluid production, are largely inaccessible to direct visualization because of their location posterior to the optically opaque iris and sclera. Intraoperative viewing of the ciliary processes is now possible (invasively) using fine endoscopic systems. In this report we describe noninvasive visualization of the ciliary body with very-high-frequency ultrasound (VHFU). We also demonstrate quantitative morphologic descriptors of the ciliary processes that may relate to function.

Lizzi et al.1 and Pavlin et al.2,3 introduced VHFU and ultrasound biomicroscopy (UBM) in the early 1990s for ocular studies. These techniques, although differing in certain aspects of signal processing, both use 50-MHz polyvinylidene fluoride (PVDF) transducers. Ultrasound allows imaging of tissues that are located behind optically opaque structures. At the high frequencies used in these techniques, ultrasound is necessarily limited to study of the anterior segment of the eye because of the exponential increase in acoustic attenuation that occurs with frequency. With resolution on the order of 35 μm axially by 65 μm laterally, visualization of the ciliary processes can be accomplished. By scanning in a series of parallel planes, high-resolution, three-dimensional (3-D) perspective images can be produced as well.4 However, unless scan planes (and pulse-echo vectors within planes) are within a beam’s width of each other, 3-D images will not obtain the resolution to which they are entitled. The major factor that tends to mitigate against increasing scan plane density is time: The longer the acquisition process, the more likely motion-induced blurring or distortion will occur. We have recently demonstrated, however, that this problem can be superseded both in animal and human subjects.5

Since their inception, VHFU and UBM systems have been used in many clinical and preclinical studies related to glaucoma. As the site of aqueous fluid production, the ciliary body is also the site of medical intervention intended to reduce intraocular pressure. The ability to study drug action on the morphology of the ciliary body itself may offer useful insights regarding mechanisms of action.

A number of UBM studies of ciliary body morphometrics have been conducted. Several reports have described the effects of pharmacologic agents on ciliary body thickness.6–8 Gentile et al.9 measured ciliary body cross-sectional area in a series of patients with uveitis. Frieling and Dembinsky10 measured ciliary body length and thickness in relation to axial length. Gohdo et al.11 examined ciliary body thickness in normal eyes with narrow angles. Several studies have been made of ciliary body anatomic changes that occur during accommodation.12–14

In these studies, the ciliary body was treated as a unit—that is, the epithelial and muscular tissues were not distinguished for biometric purposes. In general, overall ciliary body thickness was treated as the measure of interest. None of these reports attempted to describe 3-D anatomy of the ciliary body or to distinguish the ciliary processes from muscle. In this report we describe the 3-D anatomy of the ciliary processes in rabbits and in normal human subjects. We describe quantitative methods for characterizing the processes, including surface area, volume, and fractal dimension. To validate these measures, we demonstrate that their values differ between human and rabbit ciliary processes and that in each species, these parameters show periodicities that correspond to the spacing between successive processes.

METHODS

The transducer used in these studies consists of a spherical PVDF section with an aperture of 6 mm and focal length of 12 mm.
reflectance spectrum from a glass plate aligned perpendicular to the beam axis in the focal plane reveals a 38-MHz center frequency and a -15-dB bandwidth extending from 10 to 55 MHz. The scanning system consisted of two orthogonal linear stages with computer-controlled stepper motors providing a positional resolution of 10 μm. We used a digitizing oscilloscope to store echo data, which were subsequently transferred to the computer hard drive.

Each scan sequence consisted of series of parallel scan planes spaced at 40-μm intervals, and each plane consisted of 128 pulse-echo vectors spaced 40 μm apart (less than the 65-μm lateral resolution). Vectors consisted of 2048 samples of radio frequency echo data, acquired at a sample rate of 250 MHz. Thus, the 3-D data comprised a block 5.1 mm in length by 6.4 mm in depth by 3 to 4 mm in width (depending on the number of planes acquired). Once acquired, we determined the envelope of the echo data and generated a series of B-mode images.

The experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Dutchbelt rabbits were used in these studies. To scan rabbits, we first induced general anesthesia with intramuscular injection of xylazine (5 mg/kg) and ketamine (35 mg/kg). The eye was then gently proptosed and placed through a hole in a rubber membrane. This allowed formation of a normal saline water bath that provided acoustic coupling between the transducer and the eye. We obtained four sets of scans on two rabbit eyes.

Experiments on human subjects were performed in accordance with the Declaration of Helsinki after the purpose and the risks of the protocol had been explained and written consent obtained from the subjects. In human subjects, we formed a reservoir around the eye using a sterile drape (1020 Steridrape; 3M Health Care, St. Paul, MN), which provides an adhesive ring around a central aperture. After administering a few drops of topical proparacaine HCl (0.5%), we inserted a lid speculum to prevent blinking. We then filled the reservoir to a depth of roughly 2 cm. We provided a fixation target visible to the subject’s other eye. The subject’s task was to maintain constant gaze (with the other eye) on the target during the 1 to 2 minutes required to acquire the many scan planes. We twice scanned one eye on each of two healthy human subjects, a 49-year-old man and a 30-year-old woman. Neither subject had a history of glaucoma or used glaucoma medications.

The 3-D volume-rendered images were generated from the B-mode image series using a computer workstation (VoxelView software; Vital Images, Fairfield, IA). The 3-D images could be oriented for viewing from a variety of perspectives, and we could examine individual planes cut either orthogonally to the original scan plane or at arbitrary orientations through the data set. This provided a qualitative evaluation of the ciliary body and adjacent structures.

Biometric analysis of the ciliary processes was accomplished in a semiautomated manner using image analysis software (PhotoShop, ver. 5.0; Adobe Systems, San Jose, CA) as illustrated in Figure 1. We analyzed one complete scan set each of a human eye (90 planes) and a rabbit eye (80 planes). In both cases, planes were oriented parallel to the processes. In each scan plane, we manually delineated and selected the region of the ciliary processes. This was easily accomplished in the rabbit eye, where the ciliary muscles are quite diminutive in comparison with the prominent processes. In the human eye, we carefully delineated the boundary between the muscle and epithelium, which appeared as a thin anechoic line between the two tissues in the VHFU images (Fig. 2). We reduced speckle by use of a median filter (span, 1 pixel) and used a thresholding function to form a binary image consisting of a black background corresponding to regions with little or no echo signal (vitreous, aqueous, or other) versus echogenic solid tissues (the ciliary processes) in white. We then used an edge filter to outline the borders of the detected region. The only manual step in this process was the initial circling of the area of interest.

We determined the area and perimeter of the ciliary processes by counting the number of pixels (and using the appropriate scaling factor) in the filled binary and edge-detected images, respectively. We
divided the periphery by the area to obtain a ratio that we term the shape factor. A circle has the smallest shape factor for an object of a given size. For a circle, the shape factor is defined as $\frac{2\pi r}{\pi r^2} = \frac{2}{r}$.

Note that this ratio of circumference to area decreases as the size of a circle increases. Finally, we converted periphery and area measurements to surface area and volume units by multiplying by the interplane interval.

Fractal dimension denotes a concept related to certain shapes in the natural world and in mathematics. Such shapes exhibit a property called self-similarity, in which form is invariant over changes in scale. Examples of this are coastlines, snowflakes, and river networks. The measured length of these objects depends on the length of the yardstick used for making measurements. As the yardstick gets smaller, we are able to measure finer and finer features, and the measured overall length increases. Fractal dimension is a function of the relationship between length and yardstick size. A straight line is one-dimensional, and fractal objects have a dimension greater than 1.0 but less than 2.0. We determined the fractal dimension of the ciliary process boundary directly from the edge-detected image using specially designed software (Fractal Dimension Calculator, ver. 1.5 (Paul Bourke, Auckland...
University School of Architecture, New Zealand; Shareware available for the Macintosh at http://www.swin.edu.au/astronomy/pbourke/software). This software tool calculates the Hausdorff–Besicovitch dimension by superimposing meshes of various sizes over the edgedetected image and counting the number of mesh boxes containing part of the boundary (see Fig. 1F).\textsuperscript{15} The fractal dimension is determined from the linear best-fit slope of a plot of $\log(N_s)$ versus $\log(1/s)$, where $s$ is box size, and $N_s$ is the number of boxes of size $s$ containing edge regions.

We compared the mean values of each parameter in the human versus the rabbit eye using an unpaired, two-tailed $t$-test. We examined the parameters for periodicities by ordering the values by plane number, centering the data in a 128-point array, and computing the spectrum using a fast Fourier transform. Lastly, we used a continuous wavelet transform (Morlet with 6 df) to analyze the data.\textsuperscript{16} This method determines the presence of periodicities in a spatially localized manner. (Wavelet software is provided in the public domain by Christopher Torrence and Gilbert Compo, University of Colorado, Boulder, and is available at http://paos.colorado.edu/research/wavelets.)

**RESULTS**

Figures 3 (top) is an example of a 3-D volume-rendered image of the scanned area in the rabbit eye. Below the 3-D rendering is a series of coronal plane cuts through the above volume that
demonstrate the radial arrangement of processes. Figure 4 provides a series of false-color 3-D renderings of the rabbit eye. With the separately color encoded cornea and sclera, iris, and ciliary body, anatomic structure is more readily appreciated. In the rabbit eye, we can see the ciliary web and its attachments to the posterior surface of the iris. Figure 5 provides a series of edge-on 3-D false-color renderings of the ciliary processes of a normal human subject (the 49-year-old man). Individual processes are apparent, as is a small occult and asymptomatic cyst. Figure 6 provides a gray-scale 3-D volume-rendered image of the scanned region in the same person, viewed from a posterior perspective.

Table 1 provides a statistical comparison of the average values of each biometric factor for human and rabbit eyes. Averaged over the scan sets, the rabbit ciliary processes have both a significantly larger periphery and area than the human ciliary processes. It was interesting to note that the rabbit’s shape factor was smaller than that of the human, whereas the rabbit fractal dimension was larger. Taken together, these indicate that although the rabbit ciliary processes were more convoluted than those of the human, the actual ratio of surface area to mass was lower in the rabbit.

If we assume a diameter of 10 mm for the ciliary band, then we get a circumference of 31.4 mm. We therefore scanned approximately one tenth of the circumference of the eye. Given the spacing of 0.04 mm between successive planes, we can compute the mean surface area of the rabbit ciliary processes per plane to be 16.80 mm $\times$ 0.04 mm = 0.67 mm$^2$, and the total surface area to be 0.67 mm$^2 \times (31.4/0.04) = 528$ mm$^2$. We can similarly calculate the total surface area of the processes for the human subject to be 222 mm$^2$. Ciliary body volumes can be calculated in the same manner. The volume of ciliary processes for the rabbit and human eye was found to be 59.6 mm$^3$ and 19.2 mm$^3$, respectively.

Figure 7 provides plots of periphery, area, shape factor, and fractal dimension as a function of scan plane position for the rabbit eye. The corresponding plots for the human eye are provided in Figure 8. In some instances, periodicity of the biometric parameters is quite evident, such as for the ciliary process area and shape factor of the rabbit. The human data seem noisier overall, which may well be due to inevitable small eye movements that occurred during scanning.

Spectral plots for each factor are provided in Figures 9 and 10 for rabbit and human eyes, respectively. The spectral plots for all parameters in the rabbit eye show a peak at a frequency of 10, indicating that 10 cycles occurred over 5.1 mm (128 $\times$ 40 $\mu$m). This suggests a spacing of ciliary processes at approximately 0.5-mm intervals. In human subjects, a 0.5-mm period-
icity was also observed for all parameters except fractal dimension, for which no prominent peak was evident.

Figure 11 provides comparative wavelet transform maps of the peripheral dimension of the rabbit and human ciliary processes. The abscissas for these maps are directly comparable to those of the plots of peripheral dimension in the upper left of Figures 7 (rabbit) and 8 (human). The ordinates indicate detected periodicities in mm. The wavelet map of the rabbit shows a dominant band extending from approximately 0.5 to 0.75 mm. For the human data, a similar but less regular pattern is seen but at somewhat higher spatial frequency, approximately 0.3 to 0.6 mm. Comparable results were obtained for the other three factors.

Fourier and wavelet findings was consistent with the spacing of processes as seen in the 3-D images shown in Figures 4 and 6. In Figure 4 (rabbit), six processes are seen over the 3.2-mm width of the scan series. The image also shows that the spacing between successive iridal processes can be somewhat irregular. In Figure 6 (human) seven to eight processes are seen over a 3.6-mm range.

**DISCUSSION**

In this study we demonstrated that not only can the ciliary processes of human and animal eyes be visualized three dimensionally, but that quantitative descriptors can be defined that might be useful for clinical studies related to ciliary body function. These descriptors relate to the surface area, volume,

<table>
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<th>Parameter</th>
<th>Rabbit</th>
<th>Human</th>
<th>T</th>
<th>P</th>
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<tr>
<td>Periphery (mm)</td>
<td>16.80 ± 1.28</td>
<td>7.06 ± 1.48</td>
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<tr>
<td>Area (mm²)</td>
<td>1.90 ± 0.13</td>
<td>0.61 ± 0.12</td>
<td>67.75</td>
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<tr>
<td>Shape factor (mm⁻¹)</td>
<td>8.88 ± 0.68</td>
<td>11.66 ± 1.75</td>
<td>-13.38</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fractal dimension</td>
<td>1.284 ± 0.024</td>
<td>1.138 ± 0.063</td>
<td>19.60</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
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Measurements were made in 80 planes in the rabbit eye and 90 planes in human eye. Data are mean ± SD.
FIGURE 7. Plots of peripheral dimension (A), area (B), shape factor (C), and fractal dimension (D) of successive scan planes in a rabbit eye.

FIGURE 8. Plots of peripheral dimension (A), area (B), shape factor (C), and fractal dimension (D) of successive scan planes in a human eye.
Figure 9. Plots of spectral amplitude of peripheral dimension (A), area (B), shape factor (C), and fractal dimension (D) for the rabbit eye. The prominent peak at a frequency of 10 seen for all factors corresponds to a spatial frequency of 0.5 mm, the expected frequency of ciliary processes.

Figure 10. Plots of spectral amplitude of peripheral dimension (A), area (B), shape factor (C), and fractal dimension (D) for the human eye. Peaks corresponding to a 0.5 mm spacing between processes appear, but less prominently than in the rabbit eye.
and degree of convolution of the ciliary processes. They differed significantly between rabbit and human eyes and also showed periodicities that corresponded to the spacing of successive processes.

The significance of differences between the values of the above quantitative descriptors between human and rabbit eyes and between scan planes encompassing and not encompassing individual processes is not that we can prove that these anatomic differences exist. This is evident by casual inspection of the 3-D images. Rather, the significance is that these descriptors can be used in experimental studies as quantitative measures of anatomic morphology.

Our goal is to develop improved systems that allow scanning at higher frame rates with consequent reduction of the time necessary to acquire high-resolution 3-D data sets. The methods described in this report will allow study of ciliary body functional anatomy in glaucoma, hypotony, aging, and other conditions in the clinical population.

**References**


