on trabecular cells,[5] as well as the role of the TGF-βs in modulating the production of the ECM, our findings are important for investigations regarding whether this class of growth factors is implicated in the normal aging process of the trabecular meshwork, in the pathogenesis of the glaucomatous state of the eye, and in the failure of filtration procedures for this disease.

***Key Words***

aqueous humor, betaglycan, competitive binding, extracellular matrix, heparinase, receptor types, Scatchard analysis.

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# Aging Studies on Normal Lens Using the Scheimpflug Slit-Lamp Camera

*Kayoko Kashima,* Benes L. Trus,† Michael Unser,‡ Paul A. Edwards,* and Manuel B. Datiles**

***Purpose.*** To study the changes in density and thickness in normal lenses related to aging, and to study changes in anterior chamber depth related to aging.

***Methods.*** Eighty nine normal volunteers (ages 9–80 yr) were examined and their eyes were photographed to obtain Scheimpflug photographs. The images were digitized and linear densitometry was performed, dividing the lens into five areas: posterior capsular (area 1), posterior cortical (area 2), nuclear (area 3), anterior cortical (area 4), and anterior capsular (area 5). Total lens thickness and anterior chamber depth were similarly measured for 90 normal eyes from the densitometry profiles. These were correlated with age.

***Results.*** There was a strong positive correlation between increasing age and the density in all lens areas (area 2: r = 0.805; $P < 0.0001$; area 3: r = 0.836, $P < 0.0001$; area 4: r = 0.767, $P < 0.0001$; and area 5: r = 0.319, $P < 0.0023$), except the posterior capsular area, where correlation was negative (area 1: r = −0.426; $P < 0.0001$). In addition, there was a significant correlation between age and overall lens thickness (r = 0.756; $P < 0.0001$), thickness of nucleus (r = 0.543; $P < 0.0001$), and cortex (r = 0.632; $P < 0.0001$), and a negative correlation with anterior chamber depth (r = −0.513, $P < 0.0001$).

***Conclusion.*** This report shows human lens changes in density and thickness correlated with aging using Scheimpflug photography and image analysis techniques. The results will aid future development of systems for automated detection, classification, and monitoring of human cataracts, as well as other anterior segment disorders. Invest Ophthalmol Vis Sci. 1993; 34:263–269.

With aging, the normal human lens changes in density as well as thickness. It is important to study and document these normal age-related changes to distinguish these from pathologic changes such as cataract forma-

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tion. These changes have not been documented quantitatively in a broad age range. Hockwin et al[1,2] reported age-related transparency changes using the Scheimpflug camera. They performed linear densitometry at the central axis of the lens and superimposed their results for two age groups (30–40 yr and 50–60 yr) and demonstrated by visually comparing the densitometry graph profiles that the older age generally showed higher density profiles. However, no statistical analysis was performed on any of the numerical data.

We developed a system using the Scheimpflug Topcon SL-45 camera and image processing techniques to quantitate, document, and analyze human lens density changes sensitively and objectively, using linear microdensitometry through the central axis of the lens.[3,4] Therefore, we used the same system with which we analyzed the cataractous change to statistically study the aging change on normal lenses in a broad age range.

Anterior chamber depth and lens thickness changes also are associated with aging, and important information can be obtained with the Scheimpflug camera. Good photographic results are influenced by the lens thickness, even with maximum pupillary dilation. Charles et al[5] measured lens thickness with Brown's Scheimpflug camera and showed the age-dependent increase of the lens thickness directly from the 35 mm photographs. They analyzed a wide range of age groups—from about 10–80 yr—and plotted a regression line, but did not analyze normal subjects and cataractous eyes separately. Hockwin et al[6] analyzed changes in lens thickness with linear densitometry profiles in cataractous patients aged 10–89 yr who were divided into several age groups and cataract types and compared the change in each group. Karino et al[7] measured the thickness of the lenses in normal subjects 6–79 yr old with a grid system overlaid on the Scheimpflug photographs and compared the findings from different age groups by decades.

We conducted a study wherein we analyzed the change in the thickness of the lens and anterior chamber depth in normal eyes with the new image analysis technique using densitometry profiles of Scheimpflug images. We then performed a correlation analysis with age. This data then could be used as a factor when calculating quantitative changes in cataractous lenses over time.

**MATERIALS AND METHODS.** For the lens transparency study, 89 normal volunteer lenses were photographed with the Topcon SL-45 Scheimpflug camera (Topcon America Corp., Paramus, NJ) after maximal dilation with 2.5% phenylephrine and 1% tropicamide ophthalmic solution. All eyes had 20/20 best corrected vision without pathologic findings on slit-lamp biomicroscopy and fundus examination. Informed consent was obtained from all volunteers in this study. All volunteers were part of a National Eye Institute Intramural Review Board-approved protocol. Tenets of the Declaration of Helsinki regarding human subjects were followed. Patients with any history of eye injury or systemic disorders were excluded. It was difficult getting normal volunteers older than 60 yr or younger than 10 yr of age, because most patients over 60 had opacities and those in the latter group had difficulty keeping still and fixing on the target. Eighteen photographs were taken from 90° to −90° (a 360° sweep around the central axis of the lens of the eye) at 10° intervals for each lens. Four of the images from the 18 sets of photographs (approximately equally spaced between 90° and −90°) were selected. They then were digitized as previously reported[3] using the Perkin Elmer 1010MG microdensitometer (Perkin-Elmer Corp., Garden Grove, CA) and analyzed with a VAX8350 computer (Digital Equipment Corp., Maynard, MA) using specially developed software. The four densitometry profiles from each lens were averaged and the average optical density value was calculated for each of five areas of every lens. These areas were determined based on densitometry peaks that represent posterior capsular area (area 1), posterior cortical area (area 2), nuclear area (area 3), anterior cortical area (area 4), and anterior capsular area (area 5; Fig. 1). An average optical density profile was obtained for the different age groups and plotted. Correlation analysis was done for each mean density of each of the areas.

For the lens thickness and anterior chamber depth analysis, 90 normal lenses were photographed, and one of the views for each lens was digitized. The anteroposterior thickness of the whole lens, the nucleus,

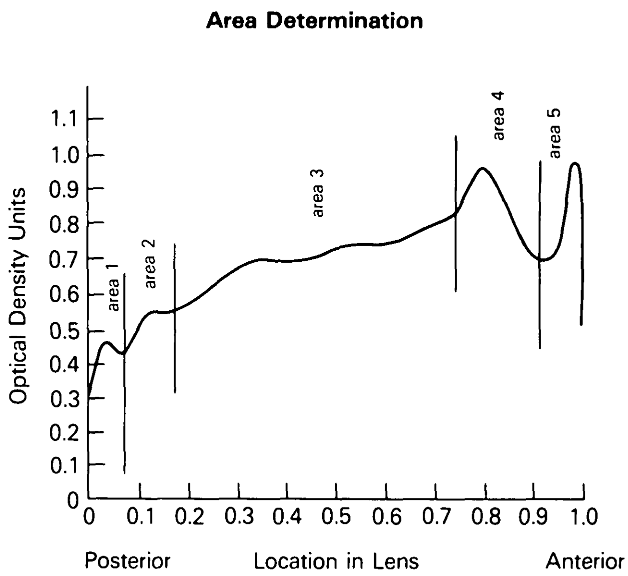


FIGURE 1. Diagram of lens profiles used for area determination in lens clarity. Area 1 = posterior capsule; area 2 = posterior cortex; area 3 = nucleus; area 4 = anterior cortex; area 5 = anterior capsule.

the anterior cortex, and the posterior cortex, and the anterior chamber depth were measured from the densitometry profile. These measurements were correlated with age. Figure 2 shows a representative image from the different age groups with densitometry profiles showing where four lines were located in the lens according to the anatomic structure. Whole lens thickness was measured from vertical line 2 to line 1. Nuclear thickness was measured from line 5 to line 6. Anterior chamber depth was measured from line 2 to line 3. Cortical thickness was calculated as whole lens thickness minus nuclear thickness.

**RESULTS.** Table 1 summarizes the age distribution of the normal volunteers. Some eyes showed diffuse high density at the supranuclear cortical area or posterior cortical area on the Scheimpflug image, although they were determined to be normal lenses by slit-lamp biomicroscopy and had 20/20 vision. These were included in this analysis. Figure 3 shows the aver-

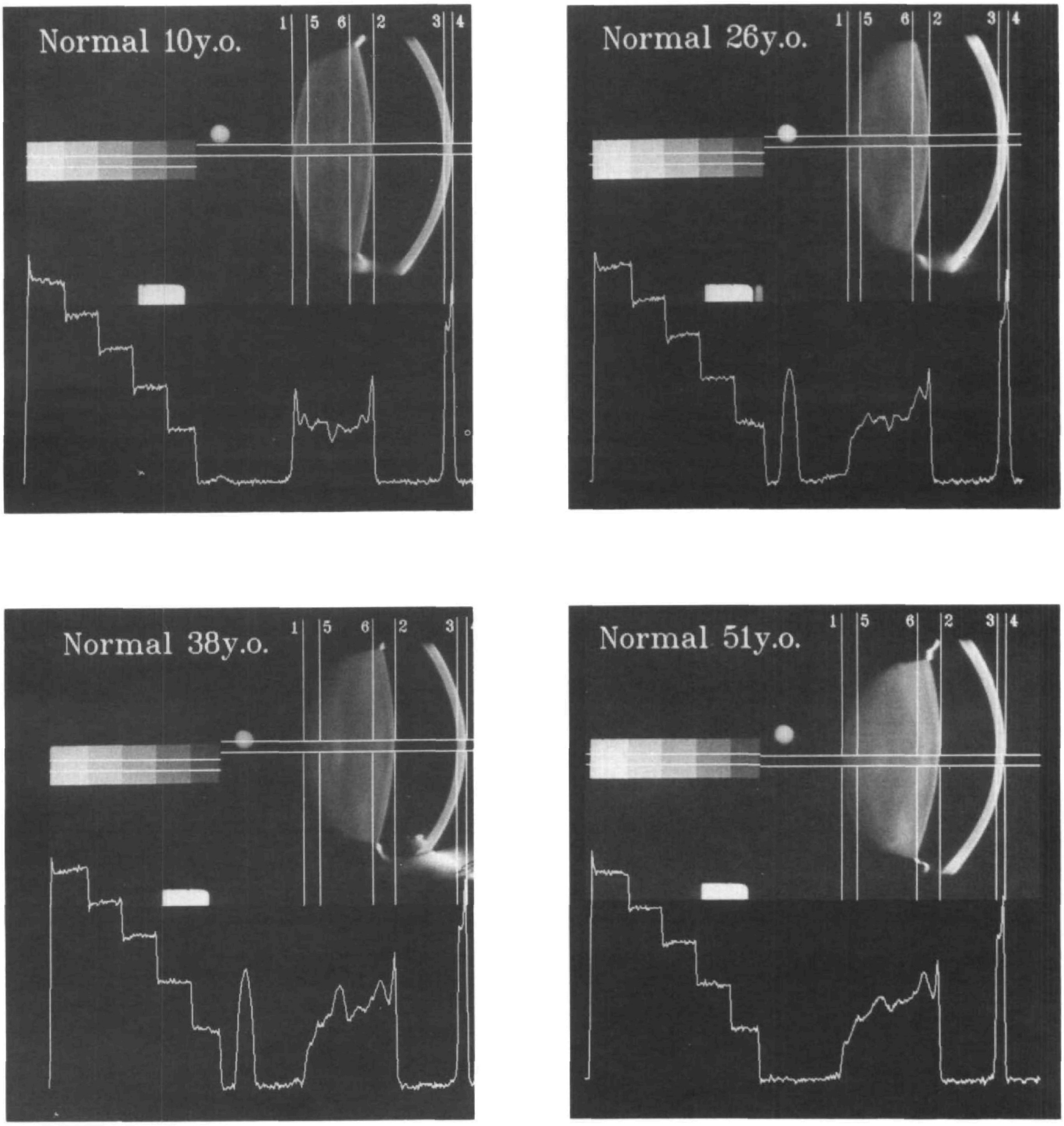


FIGURE 2. Representative Scheimpflug image of different ages with densitometry profiles. See text for detailed description.

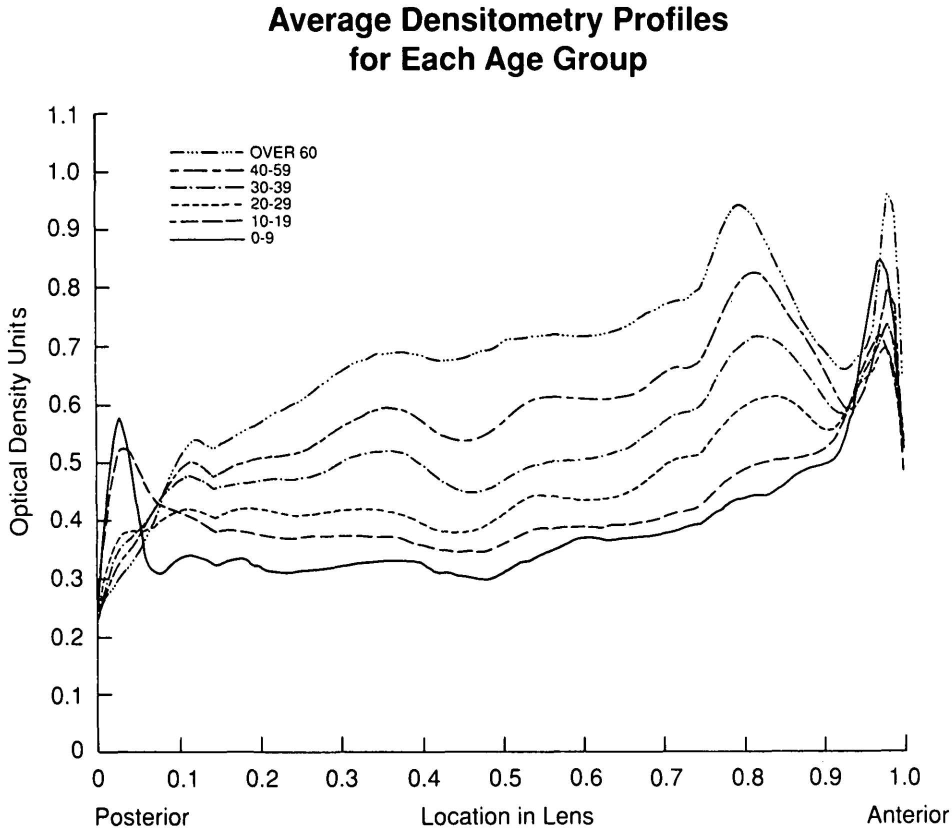


FIGURE 3. Average densitometry profiles plot for each age group.

age densitometry plot for the different age groups. This figure illustrates an increase in density regarding age for all anatomic layers of the lens, except the posterior capsule. Younger age groups (eg, 0–9 yr and 10–19 yr) showed no localized opacities, and the clinical examination revealed no abnormalities in these groups.

Table 2 shows the correlation coefficient results in the five areas. It shows that the lens density in the five areas strongly correlated with age. Areas 2, 3, and 4 had very high positive correlation; area 5 had a weaker correlation with age, but this still was significant. The correlation value of area 1 (posterior capsular area) was negative, indicating a decrease in densitometry with age. Figure 4 shows the correlation line and plot for each lens area.

Table 3 shows a summary of the correlation coefficient values for lens thickness, nuclear thickness, cortical layer thickness, and anterior chamber depth. The correlation coefficient was positive for the whole lens thickness, nucleus of the lens, and cortical thickness. It was negative for the anterior chamber depth. Figure 5 shows the correlation lines and plots for each of the above parameters.

**DISCUSSION.** This study shows the effect of aging on the density and thickness of the lens and anterior chamber depth in normal eyes. There is a strong positive correlation between density in the anterior capsular, anterior cortical, nuclear, and posterior cortical areas and age. A negative correlation was found at the posterior capsular area, in which the densitometry

**TABLE 1.** Age Distribution of Normal Volunteers for Density Study

| *Age* | *No. of Normal Volunteers for Density Study* | *No. of Normal Volunteers for Lens Thickness Study* |
|---|---|---|
| 0–9 | 2 | 2 |
| 10–19 | 14 | 13 |
| 20–29 | 21 | 25 |
| 30–39 | 16 | 15 |
| 40–49 | 12 | 18 |
| 50–59 | 19 | 13 |
| 60–69 | 3 | 3 |
| 70–79 | 1 | 2 |
| Total | 89 | 90 |

**TABLE 2.** Correlation of Density of Age

| *Area* | *Correlation/Significance* |
|---|---|
| 1 | r = −0.426, $P < 0.0001$ |
| 2 | r = 0.805, $P < 0.0001$ |
| 3 | r = 0.836, $P < 0.0001$ |
| 4 | r = 0.767, $P < 0.0001$ |
| 5 | r = 0.319, $P < 0.0023$ |

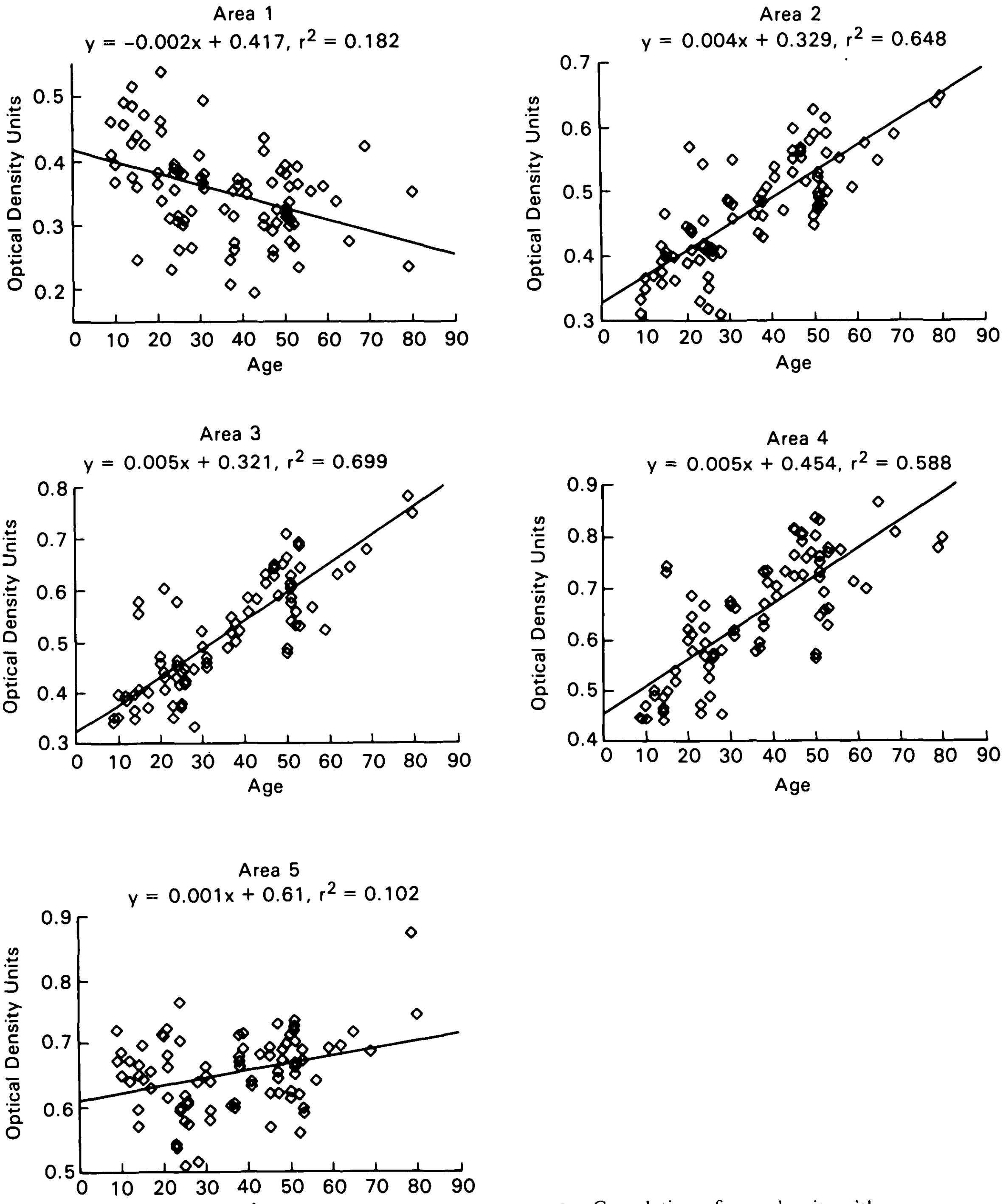


**FIGURE 4.** Correlation of area density with age.

profile may incorrectly resemble a posterior subcapsular cataract if it were presented without clinical correlation. One possible explanation for the high posterior peak in the densitometry profiles in young eyes is that these lenses are more transparent and thinner, allowing more light to reach the posterior capsule. The light then is reflected back from the posterior capsule by specular reflection. We excluded the two youngest age groups (0–9 and 10–19 yr) and again looked at the correlation coefficient in this area (posterior capsular). The correlation with age no longer was significant ($P = 0.10$), and although the coefficient still was negative ($r = -0.184$), it was less than the original overall correlation coefficient ($r = -0.426$). This suggests that specular reflection caused the peaks in the density profiles in the posterior capsular area, which was worse in the young age group and less in the older age group. This warns future researchers about the possible artifacts that may be obtained in the region of the posterior cortex and capsule. This also supports pre-

**TABLE 3.** Correlation of Lens Thickness and Anterior Chamber Depth to Age

| | |
|---|---|
| Whole lens thickness | r = 0.756, $P < 0.0001$ |
| Lens nucleus thickness | r = 0.543, $P < 0.0001$ |
| Cortical layer thickness | r = 0.632, $P < 0.0001$ |
| Anterior chamber depth | r = −0.513, $P < 0.0001$ |

vious recommendations that this technique may be best applied mainly for studying the nuclear area, rather than the other lens areas. These age-related findings should be considered in studying cataractous changes based on densitometry when cross sectional (for example, studies of cataract patients at one time frame) and longitudinal studies (for example, natural history studies or clinical trials of anti-cataract drugs) are done.

The age-related increase in lens thickness has been reported by several authors.[5–7] Our study confirms these previous reports, as well as the decrease in the anterior chamber with aging, although we used different image processing and analysis methods. In addition, we demonstrated an increase in the thickness of the cortex and nucleus with age, which can be calculated using Scheimpflug densitometry profiles.

We have confirmed that the Scheimpflug camera is a sensitive, quantitative method for measuring geometric changes in the human lens. As mentioned, we found reasons why we sometimes have difficulty acquiring good photographs in older and very young subjects. In addition, in older patients the lens becomes quite thick, sometimes making it difficult to include the posterior part of the lens using the Topcon SL-45. However, because of construction differences, this is not true with the Zeiss Scheimpflug video camera. Variabilities in lamp voltage, stability of storage media (such as film, videotape, or optical disc) and ease of handling of stored data/images also are important factors. Patient cooperation and attention and reliability in returning for appointments also are problems. We are conducting further studies to address these issues. We are determining the minimum number of views needed to characterize the lens adequately with the Scheimpflug camera, because the

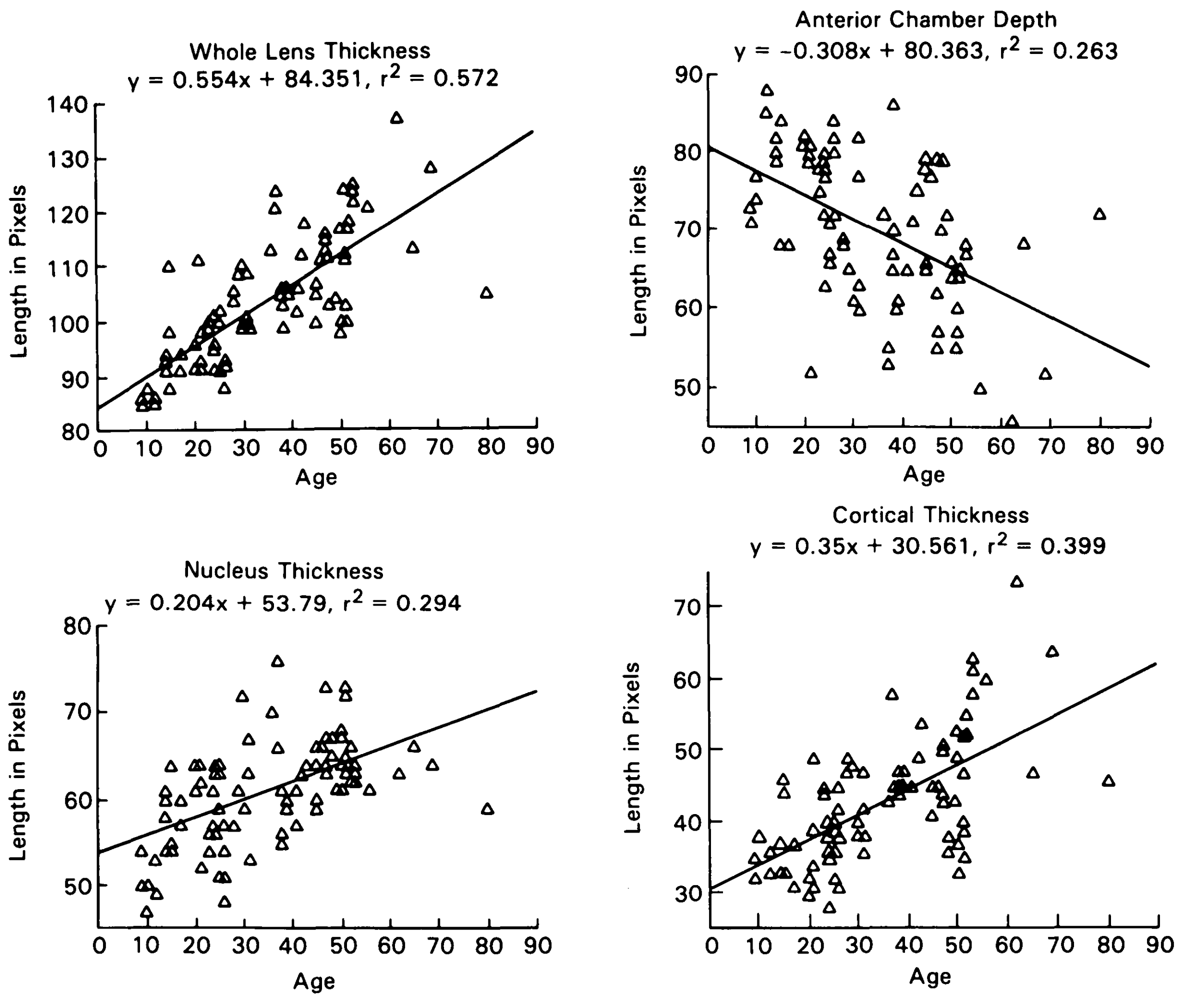


**FIGURE 5.** Correlation of lens thickness and anterior chamber with age.

current practice of using one view may be enough for normal lenses but not for cataracts. We also are developing new computer hardware and software using the MAC computer with an improved optical disc storage system to resolve image storage and recall problems. In addition, we are using a video camera on the Scheimpflug to decrease variability resulting from film batches and development and to negate the need to digitize films.

We are developing standards for "live" on-line video digitization, so one can accept or reject an image as acceptable or not while the patient is available. The video images may be calibrated to optical density units, and a method can be devised and standardized to allow precise comparisons between images obtained using various levels of illumination to solve the problem of variability in lamp voltage. This is needed, because cataracts that grow denser over time will get overexposed if the illumination is subsequently not reduced. Results of these studies will be presented in future reports.

This report presents a quantitative analysis of human lens changes in density and thickness correlated with aging using the Topcon Scheimpflug camera and image analysis techniques. The measurements were done using semi-automated computer image processing, and the numerical data were analyzed statistically. The results will assist the future development of systems for automated detection, classification, and monitoring of human cataracts, as well as other anterior segment disorders.

***Key Words***

age-related lens changes, anterior chamber depth, cataract, lens thickness, Scheimpflug camera

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# The Effects of Acetazolamide on Visual Function in Retinitis Pigmentosa

*Vivienne C. Greenstein, Karen Holopigian, Elizabeth Siderides, William Seiple, and Ronald E. Carr*

***Purpose.*** To study the effects of acetazolamide on central and peripheral visual function in patients with retinitis pigmentosa (RP) who showed no evidence of macular edema.

***Methods.*** Thirteen patients with retinitis pigmentosa participated in a preliminary study. Measures of central and peripheral visual function were obtained before and after an 8 wk period on acetazolamide. An additional 10 patients participated in a cross-over study. They were placed on a placebo for an 8 wk period, then on acetazolamide for a second 8 wk period.

***Results.*** None of the patients in the preliminary study showed significant changes in visual acuity, color vision, foveal cone pathway sensitivities, focal electroretinogram (ERG) amplitudes, or in any ERG parameter. Three patients, however, showed significant changes in visual field area and in dark-adapted thresholds. None of the patients in the cross-over study showed significant increases in visual field area.

***Conclusions.*** Given the results and the reports of side-effects, it is difficult to justify using acetazolamide to improve retinal function in RP patients who show no evidence of cystoid macular edema. Invest Ophthalmol Vis Sci. 1993;34:269–273.

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