Measurement of Crystalline Lens Volume During Accommodation in a Lens Stretcher

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PURPOSE. To determine if the lens volume changes during accommodation.

METHODS. The study used data acquired on 36 cynomolgus monkey lenses that were stretched in a stepwise fashion to simulate disaccommodation. At each step, stretching force and dioptric power were measured and a cross-sectional image of the lens was acquired using an optical coherence tomography system. Images were corrected for refractive distortions and lens volume was calculated assuming rotational symmetry. The average change in lens volume was calculated and the relation between volume change and power change, and between volume change and stretching force, were quantified. Linear regressions of volume-power and volume-force plots were calculated.

RESULTS. The mean ($\pm$ SD) volume in the unstretched (accommodated) state was $97 \pm 8 \text{ mm}^3$. On average, there was a small but statistically significant ($P = 0.002$) increase in measured lens volume with stretching. The mean change in lens volume was $+0.8 \pm 1.3 \text{ mm}^3$. The mean volume-power and volume-load slopes were $-0.018 \pm 0.058 \text{ mm}^3/\text{D}$ and $+0.16 \pm 0.40 \text{ mm}^3/\text{g}$.

CONCLUSIONS. Lens volume remains effectively constant during accommodation, with changes that are less than 1% on average. This result supports a hypothesis that the change in lens shape with accommodation is accompanied by a redistribution of tissue within the capsular bag without significant compression of the lens contents or fluid exchange through the capsule.

Keywords: crystalline lens, volume, optical coherence tomography, accommodation

The great majority of recent studies on the mechanism of accommodation provide overwhelming evidence in support of the general principles of the Helmholtz theory of accommodation.^{1–4} Contraction of the ciliary muscle decreases the tension on the zonule, which relaxes the forces applied on the lens. As a result, lens diameter decreases, lens thickness increases, and the anterior and posterior surfaces of the lens become steeper. The change in lens shape with accommodation is the product of an intricate mechanical process that has been studied using experimental techniques and computational models.

Computational models generally start with the assumption that the lens is incompressible, or near-incompressible.^{5–9} This assumption is consistent with the results of in vivo biometric studies using Scheimpflug and magnetic resonance imaging (MRI) that found that the volume of the lens and lens nucleus is constant with accommodation.^{10,11} On the other hand, there are also studies suggesting that the lens volume increases with accommodation.^{12–15} Two hypotheses have been proposed to explain how the lens volume could change during accommodation. The first hypothesis is that the lens substance is slightly compressible.^{12,15} The change in lens volume with accommodation is then due to changes in the compressive force exerted by the capsule on the lens substance. The second hypothesis is that

there is fluid exchange between the lens and its surroundings through the lens capsule during accommodation.^{13,14,16}

Changes in lens volume have been reported to range from an average of 2.3% measured in vivo in human lenses¹⁵ to an average of 5.9% measured in vitro in bovine lenses.^{13,14} However, Wendt et al.¹⁷ showed that these changes are near or below the measurement uncertainty. In addition, the volume change reported in vivo was highly variable and irregular.¹⁵ On average, volume was found to decrease in response to a 4-diopter (D) stimulus, but to increase in response to an 8-D stimulus. A study on a larger sample size or one with lower measurement uncertainty is needed to establish with greater confidence if the lens volume changes with accommodation.

The purpose of the present study was to estimate the change in lens volume in nonhuman primate lenses during accommodation in a lens-stretching system using high-resolution optical coherence tomography (OCT) images.

MATERIALS AND METHODS

Dataset

Data were acquired from 36 cynomolgus monkey eyes (*Macaca fascicularis*; age 1.4–14.3 years, average = 6.4 ± 2.8 years) that

had been subjected to lens-stretching experiments in our second-generation custom-built lens stretcher (EVAS II).^{18,19} All eyes were from different animals (i.e., there were no paired eyes). The eyes were selected by excluding tissues with any detectable rotational asymmetry in stretching, any lateral shift of the lens, or insufficient stretching force. The selection criteria helped ensure that the lenses underwent rotationally symmetric stretching with no systematic decentration. Asymmetry and decentration were assessed independently by two observers by examination of top views of the lens recorded at each step during stretching.

Tissue Preparation

All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and to the University of Miami institutional animal care and use guidelines. The eyes were obtained immediately after enucleation from the Division of Veterinary Resources at the University of Miami as part of a tissue-sharing protocol. The globes were placed in sealed jars on a bed of gauze moistened with saline, and stored at 2 to 6°C until they were used in the experiment. All experiments were performed within 48 hours post mortem (average = 15 ± 12 h).

The dissection and mounting procedure has been described in detail elsewhere.^{18–21} In summary, eight attachments (shoes) fitting the scleral curvature of the test eye are bonded to the globe. The posterior pole, cornea, and iris are then removed, and full-thickness incisions are made in the sclera between the shoes to produce eight segments for stretching. The prepared tissue sample, consisting of the intact crystalline lens, zonular fibers, ciliary body, and the segmented sclera, is mounted in the tissue chamber of EVAS II. The tissue chamber is filled with Dulbecco's modified Eagle's medium (DMEM) until the tissue is completely immersed. The lens-stretching system simulates disaccommodation/accommodation by applying and releasing a radial force on the eight scleral shoes.

Lens Stretching

All experiments started with the lens in the unstretched state, corresponding to maximal accommodation (zonular tension released). The lens stretcher was programmed to move the stretching arms outward in 0.25-mm steps, up to 2.0 or 2.5 mm of stretching, to simulate disaccommodation.^{18–22} The lens stretcher provides measurements of the stretching force at each step. Lens power was measured by finding the focus of a ring-shaped beam with a ray height of 1.5 mm from the lens center using an optical system based on the Scheiner principle.^{19–21} The lens shape was obtained from cross-sectional images acquired with a custom-built time-domain OCT system. A detailed description of the system and imaging protocol have been published previously.^{19,21,23} Images were recorded with 5000 points per A-line at a rate of 20 A-lines per second, with 500 A-lines per B-scan and a total lateral scan length of 10 mm. The axial resolution of the system, defined as the full-width half-maximum of the measured axial point-spread function was 12 μm in air (corresponding to 8 μm in tissue). The lateral resolution, defined as the calculated 1/e² beam diameter in the lens plane, was 60 μm. The lens shape and lens power recorded at each step were used for the present study.

Extraction of the Lens Contour

The raw cross-sectional images were processed using a program developed in MATLAB (MathWorks, Natick, MA, USA). A semiautomatic edge-detection algorithm is first used

to extract the contour of the lens from the OCT images^{19,21} (Fig. 1). The contour provides the position of the anterior and posterior lens boundaries measured along each A-line, in optical path length units. The contour is scaled in the axial direction to convert optical distances to geometrical distances using the measured group refractive index of DMEM ($n = 1.345$ at 825 nm²⁴) and an estimate of the average group refractive index of nonhuman primate lenses ($n = 1.414$ at 825 nm²¹).

Determination of the Axis of Symmetry and Equatorial Axis

The calculations of volume assume that the lens is rotationally symmetric. The axis of symmetry was determined by fitting the contour expressed in polar coordinates with a 10th-order cosine series with decentration and tilt terms using the general method described in Urs et al.²⁵ Lens tilt was corrected using the value of the angular tilt obtained from the fit and the curve fit was repeated for the corrected lens contour (see details in Urs et al.²⁵). The axis of symmetry of this second fit was taken as the position of the axis of symmetry of the crystalline lens. The z -position of the equatorial axis was taken as the average of the z -coordinates of the two points of the curve fit with the most positive and most negative y -coordinates. The center of the coordinate system was placed at the intersection of the lens equatorial axis and the axis of symmetry (Fig. 1).

Distortion Correction

The posterior contour of the lens is distorted due to refraction of the OCT beam at the anterior lens surface and within the lens.^{19,21,24,26} Refractive distortions were corrected using a computational ray-trace that calculates the correct position of the posterior lens surface along each A-line. The lens is assumed to be a homogeneous medium (no gradient index), with a refractive index equal to the average group refractive index.²⁴ Potential sources of error resulting from this assumption are discussed below, in the Discussion section.

The direction of the refracted ray was calculated by applying Snell's law to a curve fit of the anterior lens contour. The lens contour was fit in polar coordinates with a rotationally symmetric Fourier model using the method described by Urs et al.,²⁵ with a total of 21 Fourier terms (Fig. 2):

$$\rho(\theta) = \sum_{k=0}^{20} b_k \cdot \cos(k\theta), \quad (1)$$

where b_k is the Fourier series coefficient of order k and θ is the angle with respect to the y -axis. The Fourier model closely fit the lens shape (Fig. 2) with a root mean square (RMS) fit error that ranged from 10 to 48 μm (mean = 18 ± 6 μm).

Calculation of Lens Volume

The lens volume was estimated from the distortion-corrected lens contour in the Cartesian coordinate system (y, z) using the following discrete integration formula (rectangle method, see Fig. 3):

$$V = \pi \sum_{i=1}^{N-1} y_k^2 \cdot (z_{k+1} - z_k), \quad (2)$$

where z_k is the axial position along the axis of symmetry of the k_{th} sample, y_k is the radial distance from the axis of symmetry to the lens contour, and N is the number of contour points. For this calculation, the contour was sampled with a period of approximately 5 μm in the y -direction (i.e., $y_{k+1} - y_k = 5 \mu\text{m}$) starting with the point closest to the axis of symmetry (i.e., $y_1 =$

FIGURE 1. Scaled OCT image of a cynomolgus monkey lens (*top*) and segmented lens contour centered at the intersection of the axis of symmetry and lens equator (*bottom*). The figures on the *left* show the unstretched lens. The figures on the *right* show the lens at the final stretching step. The gap between the anterior and posterior lens contours corresponds to contour points that have the same *y*-coordinates.

0). Comparison of lens volume calculated using the rectangle method of Equation 2 and the trapezoidal rule shows that the difference between the two methods is less than 0.2 mm^3 for the lens volume and less than 0.01 mm^3 for the change in lens volume.

Evaluation of Repeatability

The repeatability of the measurement was estimated by performing five successive stretching runs on one lens. The OCT beam was taken out of position and realigned to center between successive stretching runs. The precision of repeated measurements of volume (95% confidence interval) was $\pm 1.1 \text{ mm}^3$ ($\pm 1.1\%$ of the lens volume). The worst-case expected uncertainty in the difference between stretched and unstretched lens volume is therefore $\pm 2.2\%$.

Data Analysis

For each lens, slopes of linear regressions of the diameter-power, thickness-power, volume-power, diameter-load, thickness-load, and volume-load graphs were calculated. A two-sample paired *t*-test was used to determine if the difference between unstretched and stretched lens volume is statistically significant at the 0.05 level. One-sample *t*-tests were performed to determine if the volume-power and volume-load slopes were significantly different from zero. Data acquired during the first 0.75 mm of radial displacement of the translation stages were excluded from these analyses because these initial stretching steps compensate for changes in geometry due to dissection and for postmortem tissue slackness. These steps place the tissue under tension without producing significant changes in lens shape or power. In all results shown below, the unstretched state of the lens is the state of the lens when the radial displacement of the EVAS II shoes is 1 mm.

RESULTS

General Findings

Thirty-six cynomolgus monkey eyes were subjected to stepwise stretching and the thickness, diameter, power, and volume of the lens were measured at each step. In accordance with the Helmholtz theory, the stretching was accompanied by a decrease in lens power. Typical responses to stretching, obtained for a 5.8-year-old monkey, are shown in Figure 4.

The unstretched lens power decreased with age, from 66.2 diopters (D) at 1.4 years to an average of 43.6 D for the three 13- to 14-year-old lenses (Table 1). The decrease in lens power with stretching ranged from 13.8 D to 28.8 D ($21.7 \pm 3.7 \text{ D}$; Table 2). Lens thickness decreased and diameter increased in a linear fashion as a function of power as the stretching force increased. The average thickness-power slope, obtained from plots such as shown in Figure 4A, was $0.035 \pm 0.004 \text{ mm/D}$. Similarly, the average diameter-power slope was $-0.029 \pm 0.005 \text{ mm/D}$. The finding of a linear relation between lens power, lens thickness, and lens diameter is consistent with the results of previous *in vivo* biometric studies of accommodation in rhesus monkey lenses.^{27,28} The diameter-load and thickness-load graphs were found to be nonlinear (Fig. 4B).

Lens Volume

The unstretched lens volume (average = $97 \pm 8 \text{ mm}^3$) increased with age, with values ranging from 77 mm^3 at 1.4 years to an average of 109 mm^3 for the three lenses from 13- to 14-year-old monkeys. To determine if lens volume is affected by stretching, volume-power (mm^3/D), and volume-load (mm^3/g), slopes were obtained from linear regressions for each lens. Both positive and negative slopes are observed (Fig. 5). The mean value is $-0.018 \pm 0.058 \text{ mm}^3/\text{D}$ for the volume-power slope and $0.16 \pm 0.40 \text{ mm}^3/\text{g}$ for the volume-load slope. On average, these values

FIGURE 2. Example of a Fourier fit of a lens contour (*top*) and of the distortion-corrected posterior lens contour (*bottom*). The original posterior contour is not a true representation of the posterior lens shape, due to the presence of optical distortions.

correspond to a small increase in lens volume with stretching (volume-power slope: $P = 0.09$; volume-load slope: $P = 0.03$).

The 95% confidence interval of the slopes ranged from $-0.038 \text{ mm}^3/\text{D}$ to $0.002 \text{ mm}^3/\text{D}$ for the volume-power slopes and from $0.02 \text{ mm}^3/\text{g}$ to $0.31 \text{ mm}^3/\text{g}$ for the volume-load slope. An estimate of the total volume change was obtained by multiplying the slopes by the mean change in power (-22 D) or stretching force (3.3 g). Based on this approach, we found that the 95% confidence interval for the change in volume with stretching ranges from -0.04 mm^3 to $+0.8 \text{ mm}^3$ when calculated using the volume-power slope and from $+0.07 \text{ mm}^3$ to $+1.0 \text{ mm}^3$ when calculated using the volume-load slope.

In previous studies,^{13,14} the effect of stretching on lens volume was evaluated by comparing the volume measured in the unstretched and stretched states. In our study, the mean difference between stretched and unstretched lens volumes is $0.8 \pm 1.3 \text{ mm}^3$, corresponding to a slight increase in volume with stretching that is statistically significant (two-sample paired t -test, 0.05 level, $P = 0.002$). The 95% confidence interval for the mean difference between stretched and unstretched lens volume is 0.3 to 1.2 mm^3 . These values obtained from the endpoints are consistent with the estimate obtained from the slopes.

Further information can be gained by examining the incremental increases in volume and power produced by each step in all of the stretching experiments. The data are presented in Figure 6. The increments in power range from -10.8 D to 0.3 D . The increments in volume range from -1.9 to $+2.8 \text{ mm}^3$, with an

FIGURE 3. Calculation of lens volume. The method assumes rotational symmetry and calculates the sum of discrete elementary volumes of the distortion-corrected lens contour (V_k). For this calculation, the contour was sampled with a period of approximately $5 \mu\text{m}$ in the y -direction (i.e., $y_{k+1} - y_k = 5 \mu\text{m}$) starting with the point located closest to the axis of symmetry (i.e., $y_1 = 0$). Note that the thickness of the elementary volume is exaggerated in the figure to illustrate the methodology.

average of $0.15 \pm 0.78 \text{ mm}^3$. This range is comparable with the estimated measurement variability ($\pm 2.2 \text{ mm}^3$). Regression analysis indicates that there is no relationship to the power change over a large range of powers ($R = -0.025$, $P = 0.73$). This analysis suggests that the measured volume change reflects the experimental variability rather than a true change in lens volume.

DISCUSSION

Our measurements on a total of 36 cynomolgus monkey lenses show that there are no major changes in lens volume with accommodation. On average, the volume change observed was only 0.8 mm^3 (0.8%). Overall, our results provide support for the assumption that the lens is nearly incompressible. They are in agreement with observations that the lens volume is approximately constant with accommodation,^{10,11} or that the changes are within the uncertainty of the measurement techniques.¹⁷ Given the small magnitude of its effect, it is probable that a change in lens volume is not a biologically relevant component of the physiology of accommodation.

Several previous studies have measured or predicted an increase in lens volume with accommodation. Unlike these previous studies, we find that, on average, lens volume appears to decrease with accommodation. From published values of the curvature and thickness of the human lenses and a spherical model of the lens surfaces, Gerometta et al.¹³ estimated that the human lens volume increases with accommodation by 2.6% for a 20-year-old lens and 1.7% for a 40-year-old lens. There are several sources of error that limit the accuracy of this estimate. In particular, the lens has an aspheric shape with an asphericity that changes with accommodation. A spherical model cannot produce reliable estimates of the volume or its changes. In the same study, measurements on 13 bovine lenses produced an average decrease in lens volume of 5.8% with stretching, with

FIGURE 4. Typical response to stretching. The graphs show thickness, diameter, and volume versus power (A) and load (B). The results are for a 5.8-year-old cynomolgus monkey lens. The values given on the plots are the slopes of the linear regression ($\pm$ SE of the slope).

values ranging from 1.7% to 12.5%. The changes in thickness and diameter with stretching were minimal, on average -2.8% and $+2.2\%$, respectively, consistent with the expectation that bovine lenses do not undergo significant accommodation. These small changes in lens shape measured by Gerometta et al.¹³ are inconsistent with the large (6%) decrease in lens volume that they measured on the same lenses, as discussed below.

If we assume that the crystalline lens is a solid of revolution, the volume V will be of the general form $V = A \times d^2 \times t$, where d is the lens diameter, t is the lens thickness, and A is a

parameter that depends on the shape of the lens. For instance $A = \pi/6 = 0.524$ for a sphere or ellipsoid and $A = \pi/4 = 0.785$ for a cylinder. For the monkey lenses in our study, the coefficient A increased from an average of 0.443 ± 0.010 in the unstretched state to an average of 0.478 ± 0.012 in the stretched state, corresponding to a 7.3% increase with stretching. This change is consistent with the pronounced change in shape of the monkey lens with stretching, as can be seen in Figure 1. Because there were no significant changes in the shape of the bovine lenses with stretching in the study of Gerometta et al.,¹³

TABLE 1. Cynomolgus Monkey Lens Parameters in the Unstretched State

Eye No.	Age, y	Load at Maximum Stretch, g	Unstretched Thickness, mm	Unstretched Diameter, mm	Unstretched Power, D	Unstretched Volume, mm ³
1	5.8	4.3	3.62	7.51	49.2	93.1
2	7.2	1.7	3.79	7.67	50.2	99.4
3	6.0	2.2	3.54	8.08	41.3	104.7
4	8.3	4.7	3.74	7.86	44.7	105.4
5	4.1	2.8	3.88	7.38	50.9	94.1
6	1.4	1.7	4.13	6.49	66.2	76.6
7	5.4	1.6	3.80	7.65	49.9	97.3
8	6.4	3.7	3.86	7.28	*	93.0
9	7.3	4.2	3.82	7.69	46.1	102.0
10	6.8	3.7	3.76	7.87	51.2	102.7
11	10.4	3.9	3.40	7.71	46.9	87.2
12	7.3	3.2	3.78	7.51	50.1	94.1
13	4.8	5.7	3.89	7.35	45.4	93.2
14	14.1	2.3	3.77	*	46.2	102.4
15	4.8	4.6	3.83	7.08	50.0	87.5
16	6.2	2.7	4.04	7.71	53.0	105.1
17	8.3	3.2	3.91	7.97	46.6	109.8
18	5.4	2.4	3.79	7.39	54.0	90.0
19	4.8	4.7	3.86	7.65	49.4	98.8
20	5.3	3.6	3.74	7.67	53.3	98.6
21	3.8	4.0	4.10	7.30	50.9	99.6
22	5.8	4.2	3.59	7.17	51.8	81.0
23	5.0	2.5	3.96	7.12	52.6	87.4
24	4.5	2.1	3.97	7.58	60.3	101.6
25	6.6	2.3	3.66	7.94	49.5	94.9
26	5.9	2.4	3.92	7.40	59.0	95.5
27	4.8	3.3	3.88	7.17	57.1	89.1
28	4.9	4.1	3.94	7.28	53.8	92.4
29	5.4	5.8	3.95	7.68	55.8	102.3
30	5.3	2.8	4.03	7.42	57.4	94.4
31	4.9	3.8	3.88	7.35	56.7	93.4
32	5.5	2.9	3.68	7.98	51.0	101.1
33	4.8	2.4	3.84	7.28	56.8	88.2
34	5.2	2.5	3.74	7.60	47.3	96.2
35	14.3	2.2	3.73	8.47	40.7	120.5
36	13.6	†	3.78	7.85	43.8	104.7
Mean	6.4	3.3	3.82	7.55	51.1	96.7
SD	2.8	1.1	0.15	0.36	5.4	8.4

Mean and SD are provided for reference only, because the thickness, diameter, power, and volume are age-dependent.

* Parameter was not measured.

† Problem with load measurement.

the coefficient A of these lenses should remain approximately constant with stretching. With a constant coefficient A , the -2.8% thickness change and $+2.2\%$ diameter change of the bovine lenses with stretching corresponds to a 1.5% increase in volume, as opposed to the 5.8% decrease measured by Gerometta et al.¹³ Alternatively, the coefficient A would have to decrease by 7.3% with stretching to reconcile the volume change of the bovine lenses with their thickness and diameter change. In other words, the bovine lens would have to undergo pronounced changes in lens shape, similar to those observed in our study. This finding is inconsistent with the observation that there were no significant changes in the shape of the bovine lenses.

Sheppard et al.¹⁵ used a 3 Tesla three-dimensional MRI to measure lens volume in 19 subjects at three different accommodative states. They found a non-statistically significant decrease of lens volume in response to a 4D stimulus, but a statistically significant increase in response to an 8D stimulus. The results were highly variable, with a mean volume increase of $2.4\% \pm 5.9\%$ between relaxed accommodation and the 8D

stimulus. If the lens is compressible, one would expect an increase in lens volume at all accommodative levels, not a decrease (or no change) at 4 D and an increase at 8 D. It is therefore not possible to conclude with confidence from these data that the lens volume changes with accommodation.

The two hypotheses that have been offered to explain a change in lens volume are that the lens is compressible^{12,15} and that there is fluid exchange through the lens capsule.^{13,14,16} In either case, the change in lens volume is expected to be directly correlated with the force of accommodation. In our study, contrary to these hypotheses, the volume did not decrease on stretching.

We quantified lens volume by analyzing cross-sectional images of the lens acquired using OCT, with the assumption that the lens is rotationally symmetric. Previous studies have used MRI¹⁵ shadow-photogrammetry^{25,29} or photography^{13,14} to quantify lens volume. Optical coherence tomography provides measurements with much higher resolution (typically $<10 \mu\text{m}$) than MRI ($>100 \mu\text{m}$), but unlike MRI, OCT images suffer from distortions that must be corrected to produce

TABLE 2. Difference Between Stretched and Unstretched Measurements

Eye No.	Thickness Change, mm	Diameter Change, mm	Power Change, D	Volume Change, mm ³	Thickness-Power Slope, mm/D	Diameter-Power Slope, mm/D	Volume-Power Slope, mm ³ /D	Volume-Load Slope, mm ³ /g
1	-0.62	0.46	-19.7	0.5	0.032	-0.023	-0.025	0.09
2	-0.61	0.64	-16.8	2.4	0.036	-0.038	-0.141	1.09
3	-0.55	0.38	-14.2	0.9	0.039	-0.028	-0.062	0.40
4	-0.82	0.61	-18.5	0.0	0.043	-0.034	-0.038	0.18
5	-0.60	0.52	-16.7	1.3	0.035	-0.032	-0.071	0.35
6	-0.66	0.47	-22.1	0.3	0.031	-0.021	0.005	-0.09
7	-0.76	0.64	-21.2	0.7	0.036	-0.030	-0.011	0.16
8	-0.72	0.63	*	3.4	*	*	*	†
9	-0.84	0.75	-19.7	1.9	0.042	-0.035	-0.043	0.20
10	-0.83	0.69	-23.4	-0.4	0.035	-0.031	0.003	-0.08
11	-0.70	0.60	-22.2	1.6	0.032	-0.026	-0.063	0.39
12	-0.82	0.67	-22.7	-0.5	0.035	-0.029	0.016	-0.04
13	-0.92	0.84	-21.0	1.1	0.044	-0.039	-0.052	0.20
14	-0.53	*	-16.6	-1.6	0.031	*	0.080	-0.63
15	-0.88	0.72	-21.1	-1.4	0.041	-0.033	0.083	-0.19
16	-0.83	0.53	-22.6	-0.8	0.036	-0.023	0.031	-0.27
17	-0.78	0.54	-19.2	0.3	0.039	-0.028	-0.017	0.12
18	-0.83	0.78	-23.3	2.8	0.036	-0.034	†	†
19	-0.97	0.81	-23.6	1.5	0.039	-0.033	-0.050	0.35
20	-0.92	0.78	-24.1	1.6	0.038	-0.033	-0.077	0.42
21	-0.93	0.81	-20.9	-0.7	0.044	-0.037	0.025	-0.17
22	-0.88	0.80	-25.2	1.6	0.034	-0.031	-0.044	0.32
23	-0.81	0.63	-20.3	0.5	0.039	-0.030	0.022	0.39
24	-0.79	0.60	-25.4	0.7	0.031	-0.023	-0.008	0.05
25	-0.69	0.48	-20.1	0.8	0.034	-0.024	-0.018	0.25
26	-0.62	0.65	-22.3	2.1	0.028	-0.029	†	†
27	-0.95	0.76	-27.5	1.0	0.033	-0.028	-0.033	0.24
28	-0.90	0.72	-25.6	1.7	0.035	-0.028	-0.074	0.65
29	-0.97	0.96	-28.7	3.0	0.033	-0.033	-0.115	0.61
30	-0.83	0.54	-26.2	0.2	0.030	-0.020	0.007	-0.01
31	-0.88	0.79	-28.8	1.9	0.029	-0.025	-0.064	0.65
32	-0.75	0.68	-24.0	2.3	0.032	-0.027	-0.077	0.67
33	-0.76	0.58	-24.6	0.7	0.031	-0.023	-0.027	0.40
34	-0.69	0.37	-18.4	-1.6	0.038	-0.019	0.082	-0.65
35	-0.46	0.25	-13.8	-1.6	0.035	-0.018	0.115	-0.82
36	-0.69	0.51	-20.2	-0.9	0.033	-0.025	0.052	‡
Mean	-0.77	0.63	-21.7	0.8	0.035	-0.029	-0.018	0.16
SD	0.13	0.15	3.7	1.3	0.004	0.005	0.058	0.40

Mean and SD are provided for reference only, because some parameters are age-dependent.

* Parameter was not measured.

† No slope value (response was nonlinear).

‡ Problem with load measurement.

accurate lens biometry. Optical coherence tomography images must be scaled to convert optical path length into physical distance, and they must be corrected for distortions due to refraction of light rays. In our study, refractive distortions had a minimal effect on the calculated change in lens volume. We find that the change in volume is slightly underestimated if the refractive distortions are not corrected. Without distortion correction, the average change in lens volume is 0.2 ± 1.3 mm³ for the 36 monkey lenses, instead of 0.8 ± 1.2 mm³ obtained from the distortion-corrected images.

To facilitate the ray-trace for the distortion correction, the lens contour was fit with the Fourier model of Equation 1. The Fourier curve fit provides a model of the anterior lens shape suitable for optical ray-tracing over the entire anterior boundary, including the equatorial region. The Fourier model closely fit the segmented lens contour, with a mean RMS fit error of 18 ± 6 μ m, but it may introduce some error in the calculation of the change in lens volume. To estimate this error, for all lenses, we calculated the change in lens volume obtained

directly from the segmented contour and compared it with the change in lens volume obtained with the Fourier fit before distortion correction. On average, the change in lens volume obtained directly from the segmented contour was 0.6 ± 0.5 mm³ less than the value obtained with the uncorrected Fourier fit. This analysis suggests that using the Fourier fit produces a slight overestimation of the change in lens volume.

Another potential source of error in the calculation of lens volume from OCT images is that the accuracy of the correction is limited by uncertainties in the value of the refractive index. We used an average group refractive index ($n = 1.414$) based on published data acquired on human and monkey lenses.^{21,23,30} To quantify the effect of uncertainties in the refractive index on the estimation of lens volume change, we processed one lens with different values of the refractive index, ranging from 1.400 to 1.420, in 0.05 steps. This analysis shows that an uncertainty of ± 0.01 in the refractive index produces an uncertainty on the order of $\pm 1\%$ in the lens volume. An increase in the refractive index produces a

FIGURE 5. Plots versus age (left) and histograms (right) of volume-power slope, volume-load slopes, and volume change.

comparable decrease in both the stretched and unstretched lens volumes, and therefore the uncertainty in the refractive index has a negligible effect on the estimated change in lens volume.

The presence of a refractive index gradient is an additional source of error in the distortion correction. Because the choice of refractive index and the use or omission of refractive distortion have negligible effects on the estimated change in lens volume, it is reasonable to anticipate that ignoring the gradient in the distortion correction introduces a negligible error.

Our repeatability experiments demonstrate that the precision of the measurements of change in lens volume obtained from cross-sectional OCT images is approximately $\pm 2.2\%$. In other words, even if there is absolutely no change in lens volume with accommodation, we expect that measurement uncertainties will produce an apparent change in lens volume

in individual lenses, in the range of $\pm 2.2\%$. Our experimental results are consistent with this estimate of variability (Fig. 6). There are two important sources of error that contribute to the measurement variability: the error in the determination of the position of the axis of symmetry, and potential lateral displacement of the lens during accommodation (Enten A, et al. *IOVS* 2011;52:ARVO E-Abstract 816). These sources of error can be eliminated only by using three-dimensional imaging.^{31,32} To increase the sensitivity of our analysis to detect changes in lens volume, we considered the volume-power and volume-load slopes. The slope analysis produces conclusions that are in good agreement with the analysis of the volumes at the two endpoints of stretching. However, the slope metrics are more robust because they use data from all stretching steps, as opposed to the alternative comparison that considers only the beginning and ending volume.

FIGURE 6. Plot showing the volume increment as a function of the power increment, for all stretching steps. There is no correlation between volume increment and power increment.

To the best of our knowledge, there are no other published data on cynomolgus monkey lens volume that can be used to validate our method to calculate lens volume from OCT images. However, we were able to compare volumes of unstretched human lenses that we measured and analyzed using exactly the same protocol as for the monkey lenses with the isolated human lens volumes obtained by Priestley Smith³³ using a fluid displacement method. The mean volumes obtained with the OCT method and those reported by Priestley Smith³³ were, respectively (in mm³): 184 ($n = 4$) and 177 ($n = 22$) for ages 30 to 39; 187 ($n = 5$) and 188 ($n = 23$) for ages 40 to 49, 202 ($n = 18$) and 203 ($n = 21$) for ages 50 to 59, and 220 ($n = 23$) and 223 ($n = 23$) for ages 60 to 69. This comparison demonstrates that the OCT method produces reliable measurements of lens volume.

In conclusion, our results show that lens volume remains effectively constant during accommodation, with changes that are less than 1% on average. The lack of significant changes in lens volume supports hypotheses that assert that the change in shape of the crystalline lens with accommodation is accompanied by a redistribution of tissue within the capsular bag, as opposed to alternative theories that invoke compressibility of the lens or fluid exchange through the capsule.

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References

- Helmholtz H. Ueber die Accommodation des Auges. *Archiv fuer Ophthalmologie*. 1855;2:1-74.
- Glasser A, Kaufman PL. Accommodation and presbyopia. In: Kaufman PL, Alm A, eds. *Adler's Physiology of the Eye*. 10th ed. St. Louis, MO: Mosby; 2002:197-233.
- Strenk SA, Strenk LM, Koretz JE. The mechanism of presbyopia. *Prog Retin Eye Res*. 2005;24:379-393.
- Charman WN. The eye in focus: accommodation and presbyopia. *Clin Exp Optom*. 2008;91:207-225.
- Fisher RF. The significance of the shape of the lens and capsular energy changes accommodation. *J Physiol*. 1969;201:21-47.
- Koretz JE, Handelsman GH. Model of the accommodative mechanism in the human eye. *Vision Res*. 1982;22:917-927.
- Koretz JE, Handelsman GH. A model for accommodation in the young human eye: the effects of lens elastic anisotropy on the mechanism. *Vision Res*. 1983;23:1679-1686.
- Burd HJ, Judge SJ, Cross JA. Numerical modelling of the accommodating lens. *Vision Res*. 2002;42:2235-2251.
- Weeber HA, van der Heijde RGL. On the relationship between lens stiffness and accommodative amplitude. *Exp Eye Res*. 2007;85:602-607.
- Hermans E, Dubbelman M, van der Heijde R, Heethaar R. The shape of the human lens nucleus with accommodation. *J Vis*. 2007;7(16):1-10.
- Hermans EA, Pouwels PJW, Dubbelman M, Kuijer JPA, van der Heijde RGL, Heethaar RM. Constant volume of the human lens and decrease in surface area of the capsular bag during accommodation: an MRI and Scheimpflug study. *Invest Ophthalmol Vis Sci*. 2009;50:281-289.
- Strenk SA, Strenk LM, Semmlow JL, DeMarco JK. Magnetic resonance imaging study of the effects of age and accommodation on the human lens cross-sectional area. *Invest Ophthalmol Vis Sci*. 2004;45:539-545.
- Gerometta R, Zamudio AC, Escobar DP, Candia OA. Volume change of the ocular lens during accommodation. *Am J Physiol Cell Physiol*. 2007;293:797-804.
- Zamudio AC, Candia OA, Kong CW, Wu B, Gerometta R. Surface change of the mammalian lens during accommodation. *Am J Physiol Cell Physiol*. 2007;294:1430-1435.
- Sheppard AL, Evans CJ, Singh KD, Wolffsohn JS, Dunne MCM, Davies LN. Three-dimensional magnetic resonance imaging of the phakic crystalline lens during accommodation. *Invest Ophthalmol Vis Sci*. 2011;52:3689-3697.
- Candia OA, Alvarez LJ. Fluid transport phenomenon in ocular epithelia. *Prog Retin Eye Res*. 2008;27:197-212.
- Wendt M, Bockhorst K, He L, Glasser A. Accuracy and resolution of in vitro imaging based porcine lens volumetric measurements. *Exp Eye Res*. 2011;93:741-752.
- Ehrmann K, Ho A, Parel J-M. Biomechanical analysis of the accommodative apparatus in primates. *Clin Exp Optom*. 2008;91:302-312.
- Nankivil D, Maceo Heilman B, Durkee H, et al. The zonules selectively alter the shape of the lens during accommodation based on the location of their anchorage points. *Invest Ophthalmol Vis Sci*. 2015;56:1751-1760.
- Nankivil D, Manns F, Arrieta-Quintero E, et al. Effect of anterior zonule transection on the change in lens diameter and power in cynomolgus monkeys during simulated accommodation. *Invest Ophthalmol Vis Sci*. 2009;50:4017-4021.
- Maceo B, Manns F, Borja D, et al. Contribution of the crystalline lens gradient refractive index to the accommodation amplitude in non-human primates: in-vitro studies. *J Vis*. 2011;11(23):1-13.

22. Manns F, Parel JM, Denham D, et al. Optomechanical response of human and monkey lenses in a lens stretcher. *Invest Ophthalmol Vis Sci.* 2007;48:3260–3268.
23. Uhlhorn S, Borja D, Manns F, Parel J-M. Refractive index measurement of the isolated crystalline lens using optical coherence tomography. *Vision Res.* 2008;48:2732–2738.
24. Borja D, Siedlecki D, de Castro A, et al. Distortions of the posterior surface in optical coherence tomography images of the isolated crystalline lens: effect of the lens index gradient. *Biomed Opt Express.* 2010;1:1331–1340.
25. Urs R, Ho A, Manns F, Parel J-M. Age-dependent Fourier model of the shape of the isolated ex-vivo human crystalline lens. *Vision Res.* 2010;50:1041–1047.
26. Siedlecki D, de Castro A, Gamba E, et al. Distortion correction of OCT images of the crystalline lens: GRIN approach. *Optom Vis Sci.* 2012;89:E709–E718.
27. Vilupuru AS, Glasser A. The relationship between refractive and biometric changes during Edinger-Westphal stimulated accommodation in rhesus monkeys. *Exp Eye Res.* 2005;80:349–360.
28. Glasser A, Wendt M, Ostrin L. Accommodative changes in lens diameter in rhesus monkeys. *Invest Ophthalmol Vis Sci.* 2006;47:278–286.
29. Rosen AM, Denham DB, Fernandez V, et al. In vitro dimensions and curvatures of human lenses. *Vision Res.* 2006;46:1002–1009.
30. de Castro A, Birkenfeld J, Maceo B, et al. Influence of shape and gradient refractive index in the accommodative changes of spherical aberration in nonhuman primate crystalline lenses. *Invest Ophthalmol Vis Sci.* 2013;54:6197–6207.
31. De Castro A, Ortiz S, Gamba E, Siedlecki D, Marcos S. Three-dimensional reconstruction of the crystalline lens gradient index distribution from OCT imaging. *Opt Express.* 2010;18:21905–21917.
32. Sun M, Birkenfeld J, de Castro A, Ortiz S, Marcos S. OCT 3-D surface topography of isolated human crystalline lenses. *Biomed Opt Express.* 2014;5:3547–3561.
33. Priestley Smith J. On the growth of the crystalline lens. *Trans Ophthalmol Soc U K.* 1883;3:79–99.