


# Wide Stromal Mapping Using an Anterior Segment Optical Coherence Tomography

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**Purpose:** To quantify and assess the reproducibility of the corneal stromal thickness profiles captured by the SD-OCT. Secondly, we correlated the zonal thicknesses to the age, gender and axial length.

**Methods:** We included 227 normal eyes of 227 patients with a maximum hypermetropia of +5 and myopia of -6 diopters (D). Subjects with an intraocular pressure exceeding 22 mm Hg, evidence of cataract formation, history of ophthalmic surgery or disease were excluded. Lastly, reproducibility was evaluated in a subset of 50 participants by means of an identical scan protocol repeated by 2 different OCT operators.

**Results:** Stromal values were consistently thicker in the peripheral cornea ( $p < 0.001$ ). Age was negatively correlated with approximately every sector of the stroma with notable exceptions of the center ( $r = 0.117$ ,  $p = 0.088$ ) and the superior inner ( $r = 0.057$ ,  $0.409$ ), middle ( $r = 0.086$ ,  $p = 0.209$ ) and outer locations ( $r = 0.120$ ,  $p = 0.079$ ). There was no statistical significance in most sectors when looking at the axial length, gender and K1/K2. This method was highly reproducible in terms of both the ICC and COV.

**Conclusion:** Corneal stromal mapping is highly reproducible and shows a negative correlation to age. Additionally, the periphery of the stroma is consistently thicker to the center. Other variables like gender and axial length show no relationship to the corneal stroma.

**Keywords:** cornea, corneal stroma, OCT, AS-OCT, tomography, topography

## Introduction

The anterior segment optical coherence tomography (AS-OCT) came into existence for the first time in 1994 based on the time-domain OCT (TD-OCT).<sup>1</sup> This technology had a resolution of 30  $\mu\text{m}$  and therefore it could generate corneal pachymetry maps; however, it was not optimal to delineate individual layers, like the corneal epithelium. In 2002, the spectral-domain OCT (SD-OCT) drastically increased the scan speed and resolution; this led to the introduction of automated algorithms to map the epithelium and the stroma.<sup>2</sup>

Previous research has focused on the very high-frequency digital ultrasound to carry out stromal mapping of the cornea.<sup>3</sup> A newly released and commercially available algorithm for the Avanti RTVue XR (Optovue, Inc., Fremont, CA, USA) has allowed clinicians to map the central 9 mm of the corneal stroma using the SD-OCT. This is a potential non-contact alternative tool to map the cornea.

To our knowledge, there are a lack of studies on the corneal stromal thickness and its association with demographic variables. Furthermore, a dearth of information exists on the reproducibility of commercially available SD-OCT stromal mapping, especially in the periphery of the cornea. Therefore, we aimed to create these maps in healthy eyes and correlate them with age, gender and axial length. Also, we

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observed interobserver reproducibility of each zonal measurement using 9 mm maps.

## Methods

### Study Design and Ethical Statement

This cross-sectional study was performed in patients presenting to the Hashmanis Hospital, Karachi, Pakistan for routine eye checkup. All patients were administered a written informed consent. In addition, the study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital. This study is an extension of our previous study measuring the corneal epithelium<sup>4</sup> and therefore some of the analysis presented here is similar.

### Inclusion/Exclusion Criteria

We included one eye from self-reported ophthalmologically healthy patients between 20 and 75 years of age. If both eyes met the inclusion criteria, one was randomly selected. The following tests were administered to each patient before inclusion: auto refractometer (Topcon KR-800, Tokyo, Japan), air-puff tonometer (Reichert 7CR, Reichert, Inc., Depew, NY, USA), visual acuity via a Snellen chart, slit lamp exam with a full-dilated fundus, optical biometer (WaveLight OB-820, WaveLight, Erlangen, Germany), corneal tomography (Pentacam HR; Oculus, Wetzlar, Germany), and the commercially available SD-OCT (Optovue, Inc) for both posterior and anterior segment imaging. All eyes were scanned during the hours of 8:00 AM to 1:00 PM to account for diurnal variation in corneal thickness.

Patients with the following characteristics were included. A maximum hypermetropia of +5 diopters (D) and myopia of -6 D, a minimum best-corrected visual acuity (BCVA) of 0.8, a maximum IOP of 22 mmHg, no previous ophthalmic surgery or evidence of cataract formation.

Patients with evidence of vitreoretinal or corneal diseases like keratoconus or form fruste keratoconus, dystrophies or dry eyes (Schirmer's test 2 value below 5 mm) were excluded. In addition, those with an indication of visual field loss (clinical examination), glaucoma, amblyopia, systemic disease, pregnancy or lactation were excluded as well. No patient was on any systemic or topical medications.

### Optical Coherence Tomography Scan Protocol

All eyes were scanned using an anterior segment SD-OCT with a commercial software that can cover the central 9 mm of the cornea. The stroma includes the bowman's layer down

to the endothelium. The specifications of this OCT machine are as follows: a 5  $\mu$ m axial resolution with a 22  $\mu$ m beam width and a light source with a median wavelength of 840 nm. [Figure 1](#) demonstrates a sample image of the stromal map. The zones were divided into the central (2 mm), inner (2–5 mm), middle (5–7 mm) and outer (7–9 mm).

Each eye was dilated with 1% tropicamide for a posterior segment examination and then, each eye was permitted to return to its original non-dilated state for an anterior segment OCT scan. All scans were checked for the quality of their scans and only those without obvious artifacts were included. Additionally, any scan with a net-like pattern indicating signal blockage was excluded.

### Reproducibility

The interobserver reproducibility was measured using identical scan protocols on one eye by two OCT technicians.

### Statistical Analysis

Sample size was calculated according to the following formula  $(Z_{(1-\alpha/2)}^2 SD^2) / d^2$ , with an assumption of a 95% confidence interval ( $Z_{(1-\alpha/2)} = 1.96$ ), a margin of error of 5  $\mu$ m and a standard deviation of 32.5. The minimal sample came out to be 162.

The Statistical Package for the Social Sciences (SPSS v23; SPSS, Inc., Chicago, IL, USA) was used for all data analysis. Descriptive statistics (mean and standard deviations) were calculated for each zonal measurement and the various demographic factors. The following statistical tests were used one-way ANOVA, Pearson product moment correlation coefficient, partial correlation, student's *t*-test and a multiple regression analysis. For measuring reproducibility, the following were employed: coefficient of variation (CV) and the intraclass correlation coefficient (ICC). Statistical significance was considered when p-value was < 0.05.

## Results

### Patients

We included 227 patients with 118 males and 109 females. The mean age for this population was 40.0 years (20–75 years). The general characteristics are shown in [Table 1](#).

### Corneal Stromal Thickness

The data presented as mean and standard deviations of the various stromal sectors have been presented in [Table 2](#). Stromal values were consistently thicker in the peripheral cornea ( $p < 0.001$ ). The outermost section of the cornea was



**Table 2** Thickness by Section

Section	Inner	Middle	Outer	P-value
Central	467.0 ± 31.1			
Superior	506.3 ± 33.4	553.5 ± 35.1	600.0 ± 39.9	<0.001
Superior nasal	506.2 ± 34.9	547.0 ± 34.3	588.5 ± 40.0	<0.001
Nasal	498.1 ± 34.0	533.8 ± 35.5	574.4 ± 35.4	<0.001
Inferior nasal	489.7 ± 33.1	522.9 ± 33.6	563.3 ± 35.4	<0.001
Inferior	482.4 ± 33.0	514.0 ± 34.0	550.6 ± 35.1	<0.001
Inferior temporal	479.5 ± 37.5	504.4 ± 34.8	536.1 ± 36.4	<0.001
Temporal	479.2 ± 33.1	506.0 ± 34.2	540.2 ± 37.3	<0.001
Superior temporal	493.4 ± 34.1	530.9 ± 35.7	572.8 ± 39.0	<0.001

**Table 3** Gender Differences

Section	Male (n = 118)	Female (n = 109)	P-value
Central	467.4 ± 32.3	466.7 ± 30.1	0.877
Superior			
Inner	508.8 ± 34.9	503.7 ± 31.8	0.270
Middle	556.6 ± 36.9	550.4 ± 33.1	0.202
Outer	601.5 ± 44.5	598.5 ± 34.7	0.591
Superior Nasal			
Inner	506.7 ± 35.3	505.9 ± 34.7	0.872
Middle	547.7 ± 36.4	546.4 ± 32.1	0.788
Outer	588.1 ± 36.6	588.1 ± 36.6	0.874
Nasal			
Inner	499.7 ± 37.6	496.5 ± 29.9	0.493
Middle	533.8 ± 38.1	533.8 ± 32.9	0.995
Outer	574.3 ± 38.2	574.5 ± 32.5	0.973
Inferior Nasal			
Inner	488.5 ± 34.4	491.0 ± 31.8	0.571
Middle	521.9 ± 36.4	524.1 ± 30.6	0.633
Outer	562.4 ± 37.0	564.3 ± 33.7	0.690
Inferior			
Inner	481.3 ± 34.7	483.6 ± 31.5	0.599
Middle	513.2 ± 36.3	514.8 ± 31.6	0.727
Outer	550.2 ± 35.9	551.1 ± 34.6	0.851
Inferior Temporal			
Inner	478.5 ± 36.6	480.6 ± 38.7	0.677
Middle	504.4 ± 36.8	504.5 ± 32.9	0.971
Outer	535.2 ± 37.7	537.1 ± 35.2	0.713
Temporal			
Inner	479.1 ± 32.5	479.5 ± 34.0	0.924
Middle	506.5 ± 34.5	505.7 ± 34.1	0.860
Outer	541.6 ± 37.8	538.9 ± 37.1	0.587
Superior Temporal			
Inner	496.7 ± 36.5	490.0 ± 31.3	0.150
Middle	533.6 ± 37.7	528.2 ± 33.6	0.273
Outer	577.5 ± 40.1	567.8 ± 37.6	0.069

**Abbreviation:** n, number.

middle ( $r=0.086$ ,  $p=0.209$ ) and outer locations ( $r=0.120$ ,  $p=0.079$ ). Interestingly, the strength of the correlation was seen to increase from the center towards the periphery (inner location < middle location < outer location) for each relevant quadrant. The correlations of age and stromal thickness are displayed in [Table 4](#).

## Axial Length

Statistical significance was found in the following sectors: central ( $r=0.160$ ,  $p=0.019$ ), outer inferior temporal ( $r=0.143$ ,  $p<0.001$ ), inner ( $r=0.166$ ,  $p=0.015$ ), middle ( $r=0.150$ ,  $p=0.028$ ) and outer temporal ( $r=0.141$ ,  $p=0.039$ ), and inner superior temporal ( $r=0.168$ ,  $p=0.014$ ). The following zones fell out of statistical significance when adjusting for age: outer inferior temporal ( $r=0.143$ ,  $p<0.001$ ) and middle ( $p=0.052$ ) and outer temporal ( $p=0.090$ ). This data is represented in [Table 5](#).

## Reproducibility

For the 50 patients included in this analysis (25 males and 25 females), the mean age was  $39.0 \pm 15.0$  years. Excellent results were found throughout with a minimum CV of 0.002 and a maximum of 0.019. Similarly, there was a minimum ICC of 0.868 and maximum of 0.999. This is presented in [Table 6](#).

## Discussion

Corneal thickness as a unified measurement has been analyzed extensively in the literature for the purpose of delineating ocular pathologies; however, the individual layers have been explored less thoroughly. The clinical significance of stromal thickness has been recognized in its correlation with disease processes such as keratoconus<sup>5</sup> and myopia,<sup>6</sup> as well as its variation with surgical interventions.<sup>7</sup> The present study aimed to independently evaluate stromal thickness in different meridians of the eye in normal individuals.

The literature includes a range of values for central stromal thickness, obtained through various techniques. Similar values were obtained through the VHF digital ultrasound<sup>3</sup> and confocal microscopy.<sup>8–10</sup> Another SD-OCT study reported a slightly higher central value of  $482 \mu\text{m}$ .<sup>7</sup> It may be safe to assume, therefore, that inter-study variations are likely not attributable to differences in measurement techniques. Therefore, geographical, genetic or simply patient variation may account for these differences.

With respect to the pattern of peripheral stromal thickening, previous studies with whole-corneal stromal

**Table 4** Age Correlations

Section	Regression Equation	R-Value	P-value	Adjusted P
Central	478.4-0.29×age	0.117	0.088	0.137
Superior				
Inner	512.2-0.15×age	0.057	0.409	0.522
Middle	563.0-0.24×age	0.086	0.209	0.263
Outer	615.0-0.38×age	0.120	0.079	0.102
Superior Nasal				
Inner	517.7-0.29×age	0.106	0.124	0.173
Middle	516.8-0.37×age	0.138	<b>0.043</b>	0.053
Outer	607.7-0.49×age	0.154	<b>0.024</b>	<b>0.026</b>
Nasal				
Inner	509.5-0.29×age	0.107	0.118	0.163
Middle	557.2-0.59×age	0.211	<b>0.002</b>	<b>0.003</b>
Outer	603.9-0.75×age	0.267	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Inferior Nasal				
Inner	504.9-0.39×age	0.147	<b>0.031</b>	<b>0.042</b>
Middle	543.7-0.52×age	0.198	<b>&lt;0.001</b>	<b>0.005</b>
Outer	593.3-0.76×age	0.272	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Inferior				
Inner	497.1-0.37×age	0.142	<b>0.038</b>	0.050
Middle	532.8-0.48×age	0.178	<b>0.009</b>	<b>0.012</b>
Outer	574.2-0.60×age	0.215	<b>0.002</b>	<b>0.002</b>
Inferior Temporal				
Inner	449.6-0.51×age	0.171	<b>0.012</b>	<b>0.017</b>
Middle	524.3-0.50×age	0.183	<b>0.007</b>	<b>0.011</b>
Outer	566.5-0.77×age	0.268	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Temporal				
Inner	493.4-0.36×age	0.137	<b>0.045</b>	0.075
Middle	526.4-0.52×age	0.191	<b>0.005</b>	<b>0.009</b>
Outer	573.7-0.85×age	0.287	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Superior Temporal				
Inner	500.6-0.18×age	0.068	0.323	0.456
Middle	540.9-0.25×age	0.090	0.190	0.248
Outer	589.5-0.42×age	0.137	<b>0.045</b>	0.059

**Note:** Values in bold represent statistical significance.

mapping concur with our findings.<sup>3</sup> Multiple mapping studies have attributed this to the structural layout of collagen fibrils in the stroma, highlighting a pattern of increasing collagen lamellae towards the periphery.<sup>11-13</sup> It may also be interesting to note the study using the VHF digital ultrasound has reported maximum stromal thickness in the superior quadrant but with a considerably higher value (640 µm).<sup>3</sup>

Interestingly, we found the thinnest zone in our epithelial thickness profiles to be the superior zone; these were thicker as they approached the center.<sup>4</sup> This is the reversal of what we find in the stroma. All previous theories trying to

**Table 5** Axial Length Correlations

Section	Regression Equation	R Value	P value	Adjusted P value
Central	352.2+4.88×axial length	0.160	<b>0.019</b>	<b>0.029</b>
Superior				
Inner	408.3+4.16×axial length	0.127	0.064	0.076
Middle	476.4+3.28×axial length	0.095	0.165	0.206
Outer	520.6+3.37×axial length	0.086	0.210	0.280
Superior Nasal				
Inner	405.1+4.30×axial length	0.126	0.067	0.092
Middle	499.0+2.04×axial length	0.061	0.376	0.494
Outer	570.9+0.74×axial length	0.019	0.781	0.964
Nasal				
Inner	405.4+3.94×axial length	0.117	0.084	0.115
Middle	469.8+2.72×axial length	0.078	0.256	0.403
Outer	544.0+1.29×axial length	0.037	0.589	0.888
Inferior Nasal				
Inner	423.7+2.80×axial length	0.086	0.209	0.295
Middle	475.0+2.03×axial length	0.062	0.369	0.541
Outer	520.8+1.80×axial length	0.052	0.449	0.720
Inferior				
Inner	415.5+2.84×axial length	0.088	0.202	0.283
Middle	454.0+2.55×axial length	0.076	0.265	0.390
Outer	475.2+3.20×axial length	0.093	0.175	0.291
Inferior Temporal				
Inner	406.7+3.09×axial length	0.084	0.220	0.326
Middle	422.8+3.47×axial length	0.102	0.138	0.220
Outer	416.0+5.10×axial length	0.143	<b>&lt;0.001</b>	0.080
Temporal				
Inner	352.2+5.04×axial length	0.166	<b>0.015</b>	<b>0.024</b>
Middle	387.8+5.03×axial length	0.150	<b>0.028</b>	0.052
Outer	418.8+5.16×axial length	0.141	<b>0.039</b>	0.090
Superior Temporal				
Inner	361.0+5.63×axial length	0.168	<b>0.014</b>	<b>0.018</b>
Middle	442.0+3.78×axial length	0.108	0.116	0.149
Outer	495.7+3.27×axial length	0.086	0.213	0.294

**Note:** Values in bold represent statistical significance.

explain the regional differences in epithelial thickness profiles focused on the outside environment or the measurement method.<sup>4</sup> Perhaps the epithelium is simply reacting to the stromal profiles even in the normal cornea in an attempt to smoothen out the surface of the cornea. This may be one of the reasons as evidenced by the change in epithelial thickness profiles post refractive surgery, as well.<sup>8</sup>

Our analysis noted no significant difference in stromal thickness between male and female participants, which is



**Table 6** Reproducibility

Section	Coefficient of Variation				Intraclass Correlation Coefficient			
	Inner	Middle	Outer	Whole	Inner	Middle	Outer	Whole
Central				0.002				0.999
Superior	0.006	0.008	0.019	0.011	0.994	0.987	0.868	0.969
Superior nasal	0.005	0.007	0.009	0.007	0.996	0.992	0.983	0.994
Nasal	0.010	0.010	0.014	0.011	0.943	0.975	0.943	0.972
Inferior nasal	0.010	0.009	0.016	0.011	0.935	0.970	0.918	0.965
Inferior	0.004	0.006	0.011	0.007	0.997	0.99	0.974	0.993
Inferior temporal	0.003	0.006	0.017	0.009	0.994	0.986	0.908	0.978
Temporal	0.004	0.009	0.018	0.010	0.997	0.983	0.920	0.979
Superior temporal	0.005	0.008	0.016	0.009	0.996	0.989	0.937	0.984

in agreement with other past studies.<sup>6,14</sup> In another study, we found males to have thicker epithelial thickness profiles.<sup>4</sup> Interestingly, we found a thicker central cornea in females using the Oculus Pentacam in a large cohort of patients.<sup>15</sup> Baghdasaryan et al, on the other hand, found the corneal thickness to be significantly increased in male patients while epithelial thickness showed no difference.<sup>16</sup> The dynamic nature of the cornea makes these measurements difficult as a large array of factors can affect the corneal thickness. This can account for the large discrepancy in not only the literature but our own datasets.

In addition, older age was found to be correlated with a significant decrease in stromal thickness in select peripheral sectors of the cornea. In part, this may be explained by a decline in stromal keratocyte density with age.<sup>9,14</sup> Conversely, collagen deposition and collagen fibril diameter have been shown to increase with age, suggesting that age-related biomechanical adaptations are likely to be complex and multi-layered. In terms of corneal thickness (epithelium plus stroma), available literature has described a negative<sup>17,18</sup> as well as positive<sup>19</sup> correlation between central corneal thickness and age. Nonetheless, it is difficult to ascertain whether these changes are due to the epithelium, stroma, or a combination of both.

Studies reporting isolated impact on stromal thickness are scarce, although Kim et al have denied any significant association between age and stromal thickness in any region of the cornea.<sup>14</sup> As opposed to this, our findings not only show a significant negative correlation in the nasal, temporal and inferior quadrants, but also an increase in the strength of this correlation from inner to outer (peripheral) segments. This further cements the idea that epidemiological or age-related changes affect the cornea heterogeneously and may preferentially act on the periphery as compared to the center.

In conclusion, while epithelial thickness has been mapped extensively, stromal thickness represents an understudied domain with respect to our understanding of corneal mechanisms of adaptation. In view of how the stroma appears to respond independently to patient-related characteristics, these results encourage wide stromal mapping in larger patient populations in order to evaluate its clinical significance in the diagnosis and prognostication of ocular disease.

## Limitations

There are several limitations to this study. Firstly, this study was conducted at a single center in Karachi, Pakistan and therefore only a specific subset of patients was seen. Secondly, the Optovue OCT cannot exclude the Descemet's and the endothelium from the analysis; the stromal thicknesses, therefore, were likely overestimated due to the inclusion of these 2 layers.

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## Disclosure

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