# Three-Dimensional Morphometric Analysis of the Iris by Swept-Source Anterior Segment Optical Coherence Tomography in a Caucasian Population

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**Purpose.** We analyzed by swept-source anterior segment optical coherence tomography (SS-ASOCT) the three-dimensional iris morphology in a Caucasian population, and correlated the findings with iris color, iris sectors, subject age, and sex.

**Methods.** One eye each from consecutive healthy emmetropic (refractive spherical equivalent ±3 diopters) volunteers were selected for the study. The enrolled eye underwent standardized anterior segment photography to assess iris color. Iris images were assessed by SS-ASOCT for volume, thickness, width, and pupil size. Sectoral variations of morphometric data among the superior, nasal, inferior, and temporal sectors were recorded.

**Results.** A total of 135 eyes from 57 males and 78 females, age 49 ± 17 years, fulfilled the inclusion criteria. All iris morphometric parameters varied significantly among the different sectors (all $P < 0.0001$). Iris total volume and thickness were significantly correlated with increasingly darker pigmentation ($P < 0.0001$, $P = 0.0384$, respectively). Neither width nor pupil diameter was influenced by iris color. Age did not affect iris volume or thickness; iris width increased and pupil diameter decreased with age ($r_s = 0.52$, $r_s = -0.58$, respectively). There was no effect of sex on iris volume, thickness, or pupil diameter; iris width was significantly greater in males ($P = 0.007$).

**Conclusions.** Morphology of the iris varied by iris sector, and iris color was associated with differences in iris volume and thickness. Morphological parameter variations associated with iris color, sector, age, and sex can be used to identify pathological changes in suspect eyes. To be effective in clinical settings, construction of iris morphological databases for different ethnic and racial populations is essential.

Keywords: iris, morphometry, optical coherence tomography, iris color, morphology

The iris has several roles in anterior chamber physiology, including accommodative and light-adaptive changes in pupil size, aqueous flow modifications, and immune system balance in response to anterior chamber inflammatory conditions.[1] Moreover, iris morphology and dynamics are associated with angle-closure pathogenesis, and changes in iris thickness occur during acute or chronic inflammatory diseases, such as herpetic anterior uveitis[2] and Fuchs' iridocyclitis.[3]

Relative to the importance of the iris, there are few in vivo morphological studies of either healthy or pathological eyes, because older imaging techniques did not allow for precise and repeatable measurements. With the introduction of anterior segment (AS) time-domain optical coherence tomography (TD-OCT) and then spectral-domain optical coherence tomography (SD-OCT), iris measurements became easier to obtain, and an increasing number of studies have focused on this topic. Time-domain OCT has demonstrated iris thinning in Fuchs' uveitis,[4] whereas patients affected by angle closure glaucoma have thickened irises.[5] Iris thickness varies in healthy eyes of different ethnic groups,[6] and in a Chinese population, iris surface characteristics, such as color and number of crypts, were correlated with iris thickness.[7] A recent accurate SD-OCT–based morphological analysis of the iris in healthy eyes demonstrated sectoral patterns of change in iris thickness.[8] All of these results suggest the need for a more detailed volumetric study that considers regional physiological differences that exist among the iris quadrants.

The relative slow scan speed of older OCT instruments and the absence of automatic segmentation algorithms limited the accuracy of iris structural measurements. Thus, complete morphological analysis of the iris volume was almost impossible. With the introduction of swept-source anterior segment optical coherence tomography (SS-ASOCT), which is characterized by improved scan speeds and axial resolution, and by dedicated software for automatic anterior chamber measurements, in vivo 360° imaging of the iris became affordable and reliable.[9] This improved imaging technique could help in the identification of pathological variations in iris morphometry, thus helping clinicians in the diagnosis and follow-up of several diseases. However, as a prerequisite for use in disease diagnosis, it is essential to establish a detailed, baseline dataset for iris morphology in healthy eyes. Such a dataset should include normal sectoral variations and correlations with other influencing factors, such as age and iris color. Therefore, we used SS-ASOCT to analyze in vivo the three-dimensional morphology of normal Caucasian irises. We measured iris thickness, width, volume, and pupil size, and determined if variations in these parameters were associated with iris color, iris sectors, and age or sex of the subjects.

## Materials and Methods

### Study Population

Consecutive healthy volunteers were recruited for the present study during routine ocular examinations in June 2014 at the Eye Unit, Luigi Sacco Hospital, Milan, Italy. The study was performed in accordance with the tenets of the Declaration of Helsinki and the protocol was approved by the local ethic committee. Informed consent was obtained by participants. Subject inclusion criteria were age over 18 years, Caucasian ethnicity, refractive error spherical equivalent between −3 and +3 diopters, no history of eye surgery, no history of ocular inflammatory conditions or angle closure glaucoma, no ocular topical medications at the time of enrollment or during the previous 3 months, and no ongoing systemic conditions/treatments known to affect pupillary diameter.

### Procedures

All of the subjects underwent a complete ocular examination to determine refractive error and the eligibility of the selected eye according to the above listed criteria. In case both eyes fulfilled the inclusion criteria, the studied eye was chosen randomly. The enrolled eye underwent standardized anterior segment photography obtained by the Righton RS-1000 Digital Imaging Slit Lamp (S4OPTIK, Tonawanda, NY, USA). Color pictures were made without flash in a darkened room (20 lux) at ×10 magnification. The iris was illuminated at an angle of 45° temporally, with the light beam at full width and height, and the brightness maintained at 30% of the maximum brightness.

We also obtained SS-ASOCT images of the enrolled eye in a darkened room (20 lux) by the Casia SS-1000 OCT instrument (Tomey Corporation, Nagoya, Japan). Patients were asked to fixate on an internal target and to keep the eye open for the duration of the scan (≈2.4 seconds). In case of frequent blinking, the eyelids were kept open manually without compressing on the bulb. Imaging was achieved with the preset volume scan protocol comprising 128 radial scans, each 16 mm in length and 6 mm in depth.

### Image Analysis

Anterior segment color photographs were reviewed by two independent operators to grade each eye according to a nine-category grading system for iris color classification.[10] In case of disagreement between the operators, a third observer was asked to grade the eye.

Volumes for the superior, nasal, inferior, and temporal iris sectors, and for the total volume were automatically generated by the OCT software after image acquisition. Pupil diameter was automatically generated by the software as well. In case of failure to automatically determine the pupil diameter, pupil size was calculated as the mean of the vertical and horizontal diameters manually measured on the corresponding OCT scans.

On horizontal (0°–180°) and vertical (90°–270°) scans passing through nasal/temporal and superior/inferior iris sectors, respectively, the width of the iris from the pupillary margin to the iris root was assessed manually along the posterior surface of the iris by a linear caliper integrated into the OCT software. Sectoral width values were recorded. The same tool was used to manually measure the iris thickness at the thickest point of the scan in each sector (Fig. 1). Sectoral thickness values were recorded. The overall mean width and thickness of each iris was calculated as the average of the values from each of the four sectors. Manual measurements were repeated twice by two independent operators. The intraclass correlation coefficient for agreement between the two operators in manually measuring iris width and thickness was 0.82 and 0.95, respectively.

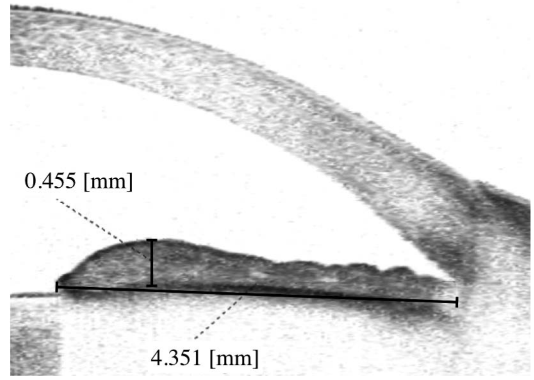


Figure 1. Manual measurements of iris width and thickness on an OCT scan. The measurements were performed with a linear caliper tool available in the OCT software. Iris thickness was measured at the thickest point. Iris width was measured from the pupillary margin to the iris root. All measurements were repeated twice by two independent operators with high agreement between the observers.

### Statistical Analysis

Statistical calculations were done with open-source software R (R version 2.14.0; The R Foundation for Statistical Computing, Vienna, Austria). For continuous variables, the mean and standard deviations were determined. The influence of iris color on the morphometric parameters of volume, thickness, width, and pupil diameter was assessed by ANOVA. The influence between iris sector and iris volume, thickness, and width was assessed by ANOVA. Pairwise comparisons with Bonferroni adjustment were used to assess differences among the four sectors.

Relationships between age of the subject and iris volume, thickness, width, and pupil diameter were assessed by Spearman correlation coefficient. Relationships between sex and the same morphometric parameters were assessed by the Wilcoxon Mann-Whitney test. Intraclass correlation coefficient (ICC) was used to assess the agreement between the two operators for iris thickness and width manual measurements.

## Results

One eye each from 135 subjects (57 males and 78 females) fulfilled the inclusion criteria and was enrolled in the study. The mean age of the subjects was 49 ± 17 years (range, 22–82 years). The color of each iris was assigned to one of the nine categories described by Mackey et al.[10] For each iris category, mean and standard deviation of the morphometric measurements of total volume, mean thickness among the sectors, mean width among the sectors, and pupil diameter are reported in the Table.

The total iris volume significantly increased with color variation from light blue (Category 1) to dark brown (Category

**Table.** Morphometric Values of Iris Total Volume, Mean Thickness Among the Sectors, Mean Width Among the Sectors, and Pupil Diameter in the Nine Color Categories

| Iris Category* (*n*) | Iris Description | Total Volume, μm³ | Thickness,† μm | Width,‡ μm | Pupil Diameter, mm |
|---|---|---|---|---|---|
| 1 (7) | Light blue | 27,336 ± 3,750 | 463.0 ± 58.3 | 3,961 ± 419 | 4.16 ± 1.30 |
| 2 (7) | Dark blue | 20,846 ± 5,237 | 491.5 ± 12.3 | 3,606 ± 456 | 5.20 ± 0.80 |
| 3 (16) | Brown | 28,591 ± 4,932 | 505.5 ± 35.4 | 3,962 ± 536 | 4.56 ± 0.96 |
| 4 (4) | Green | 27,755 ± 3,629 | 524.1 ± 33.3 | 3,841 ± 442 | 4.34 ± 1.42 |
| 5 (10) | Green with brown iris ring | 30,148 ± 3,852 | 501.4 ± 44.9 | 3,946 ± 288 | 4.38 ± 0.75 |
| 6 (9) | Central brown with peripheral green | 30,063 ± 3,231 | 513.8 ± 33.4 | 3,900 ± 542 | 4.44 ± 1.08 |
| 7 (24) | Brown with some peripheral green | 32,107 ± 4,453 | 536.8 ± 52.3 | 3,687 ± 514 | 4.85 ± 1.11 |
| 8 (39) | Light brown | 33,498 ± 5,076 | 528.6 ± 69.2 | 3,791 ± 430 | 4.73 ± 1.10 |
| 9 (19) | Dark brown | 35,657 ± 4,240 | 532.7 ± 49.3 | 3,506 ± 551 | 5.01 ± 0.88 |
| *P* value | | <0.0001 | 0.0384 | 0.19 | 0.453 |

*P* values represent the statistical significance of iris color influence on morphometric values. Total volume, thickness, and width are reported as mean ± SD of the values found in the iris included into the corresponding category.

* Described by Mackey et al.[10]

† Iris thickness was calculated as the mean of four thickness measurements assessed at the thickest point of each iris sector.

‡ Iris width was calculated as the mean of four width measurements assessed as the distance from the pupillary border to the iris root in the middle of each iris sector.

9, $P < 0.0001$, Table, Fig. 2A). The iris thickness increased in a similar fashion ($P = 0.0384$, Table, Fig. 2B). There was no significant influence of iris color on iris width or pupil diameter (Table; Figs. 2C, 2D).

Iris sector significantly influenced iris volume, thickness, and width (all $P < 0.0001$, Fig. 3). The volumes of the inferior and temporal sectors were similar to each other, but were greater than the volumes of the nasal and superior sectors (Fig. 3A). The thickness of the iris also varied by sector, but followed a different pattern compared to the volume. The superior sector was the thickest and temporal sector the thinnest (Fig. 3B). The width of the iris followed a pattern similar to that of the volume. Thus, the inferior and temporal sectors were similar to each other, but were wider than the nasal and superior sectors (Fig. 3C). Based upon these data, a graphic representation of volume, thickness, and width variations among the different iris sectors was generated (Figs. 3D, 3E).

Age had no apparent effect on iris volume or thickness (Figs. 4A, 4B); however, there was a slight, but significant increase ($r_s = 0.52$) in iris width with aging (Fig. 4C). In contrast, pupil diameter decreased significantly ($r_s = -0.58$) with the subjects' age (Fig. 4D). There were no significant correlations between sex and iris volume or thickness (Figs. 4E, 4F); however, the iris width was significantly greater in males ($P = 0.007$, Fig. 4G). There were no differences in pupil diameter between male and female subjects (Fig. 4H).

## Discussion

The in vivo 360° morphometric analysis of normal irises in a Caucasian population revealed significant data that must be taken into consideration while analyzing iris morphology in a clinical setting. In the irises of normal eyes, each of the four sectors has a unique combination of volume, thickness, and width.

To our knowledge, this is the first population-based study of iris volume, and we found that the volumes of the temporal and inferior sectors were significantly greater than the nasal and superior sectors. For iris thickness, differences among the sectors already have been established.[8] Our analysis confirms these findings, with the superior sector being the thickest and the temporal sector being the thinnest. The apparently discordant results between volume and thickness sector variations probably are associated with variations in the width of the iris. The extent of the iris from the pupil edge to the iris root was different among the sectors, and it followed the same pattern of change found for the volume; that is, temporal ≃ inferior > nasal ≃ superior. The greater influence the iris width had on the volume compared to the iris thickness probably is due to the fact that the width extent of each iris quadrant is approximately 10 times the thickness.

The disparity in the width of the different iris sectors indirectly causes the pupillary foramen to be eccentrically positioned superonasally instead of in the center of the iris disk. This finding is consistent with a previous report in which

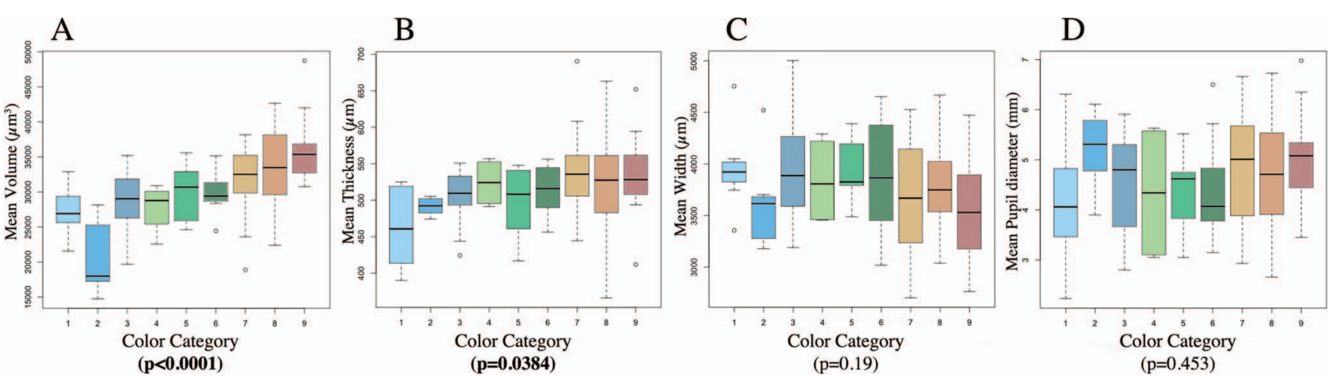


**Figure 2.** Distribution of iris total volume, thickness, width, and pupil diameter among the nine iris color categories. Volume (**A**) and thickness (**B**) increased from *light blue* (Category 1) to *dark brown* (Category 9). There was no significant correlation of iris color with pupil width (**C**) or diameter (**D**). *P* values represent the statistical significance of iris color influence on morphometric values.

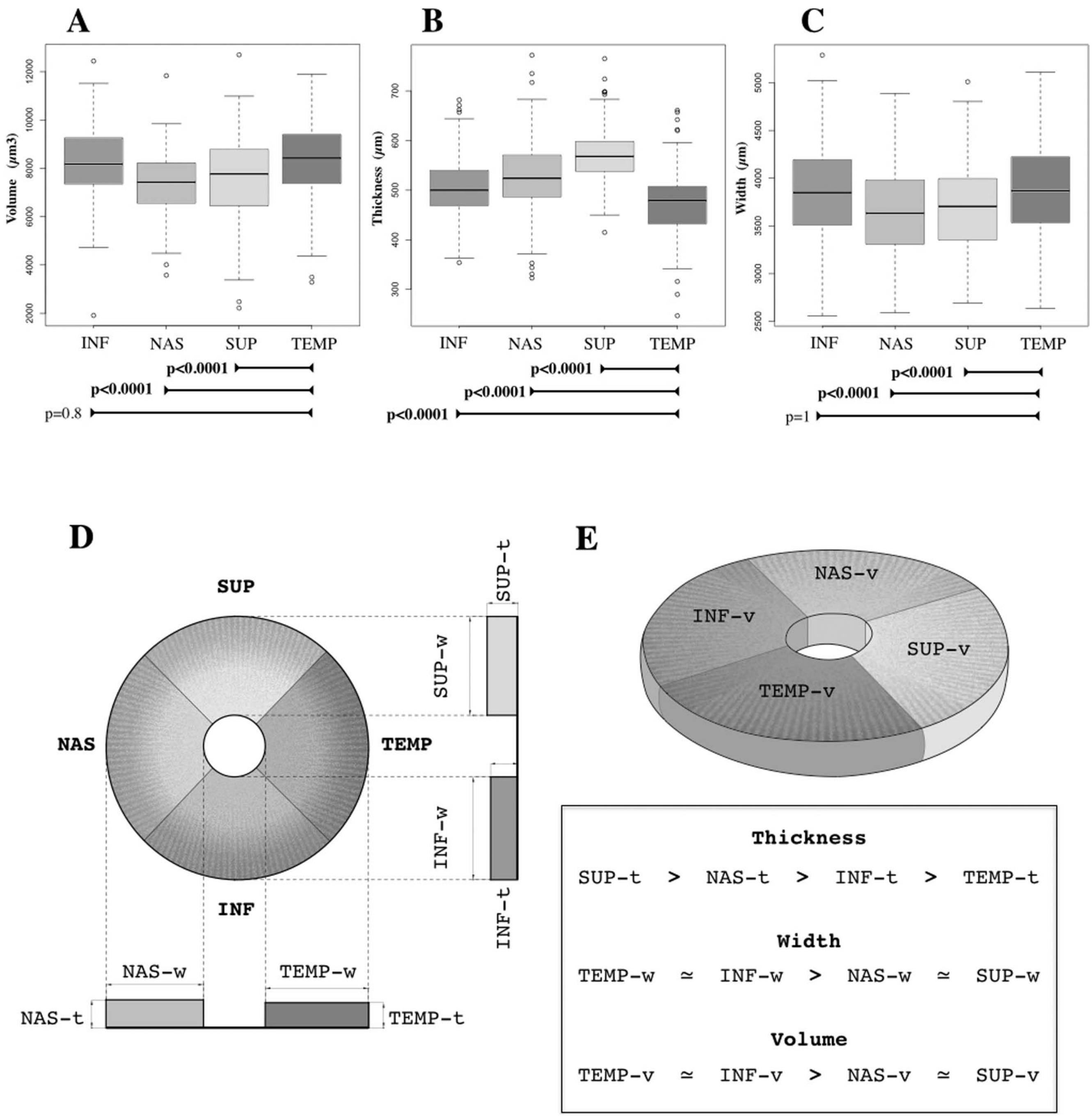


FIGURE 3. Iris volume, thickness, and width in the four iris sectors. (**A**) The volumes of the inferior and temporal sectors were similar to each other and significantly greater than nasal and superior sectors. (**B**) The thickness of the temporal sector was significantly smaller than those of the inferior, nasal, and superior sectors. (**C**) The widths of the inferior and temporal sectors were similar to each other, but significantly greater than nasal and superior sectors. *P* values are for pairwise multiple comparison *t*-tests in relation to the temporal sector. Each iris sector had a unique combination of thickness and width (**D**) and volume (**E**). The patterns of change for the morphometric parameters among the sectors also are reported at the *bottom right* corner of the image. INF, inferior sector; NAS, nasal sector; SUP, superior sector; TEMP, temporal sector. Box plots in the (**A**–**C**) and corresponding sectors in the graphic representations (**D**, **E**) are colored by the same shade of *gray.*

the pupil location was described relative to the center of the cornea.[11] According to our analysis, the pupil is significantly shifted in the superonasal direction, but only by 5% of the iris width. This explains the difficulty in detecting this uncentered position during standard examinations. Moreover, the temporal iridocorneal angle is physiologically narrower than the nasal one, hiding the iris root by the wider temporal limbus overlying it.[12,13]

Sector variations in iris volume, thickness, and width characterize healthy eyes of our Caucasian study population independently from age, sex, or iris color. The presence of consistent morphometric patterns in the four iris sectors has two main implications in clinical practice. First, the patterns can serve as guidelines or benchmarks against which a suspect eye can be compared for the diagnosis of an underlying pathological condition. Second, when used for diagnostic

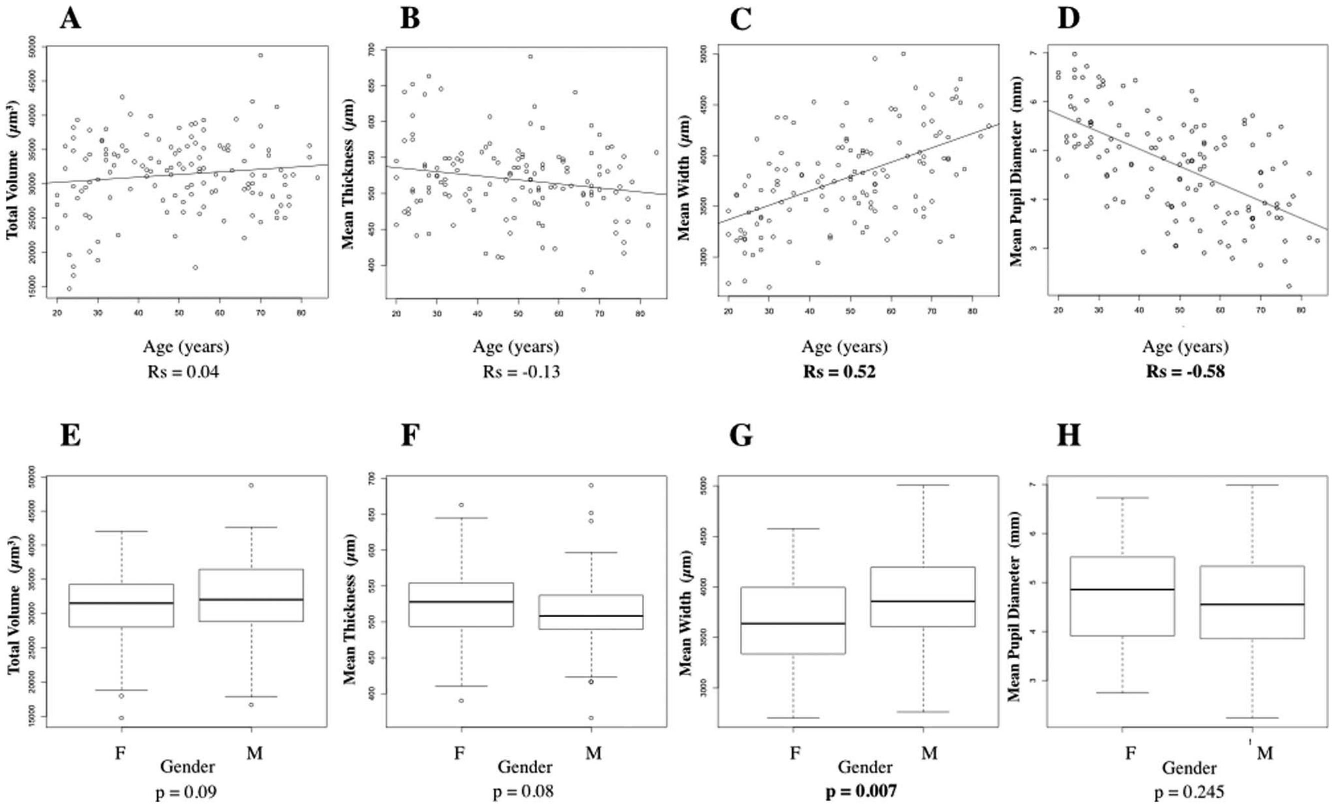


**FIGURE 4.** Correlation of iris total volume, thickness, width, and pupil diameter with subject age and sex. There were no significant changes in iris volume (**A**) or thickness (**B**) with age. However the iris width (**C**) increased and the pupil diameter (**D**) decreased significantly with age. There were no significant differences in iris volume (**E**), thickness (**F**) between males and females. Iris width (**G**) was significantly wider for males and for females. However, pupil diameter (**H**) did not differ among the sexes.

purposes, comparisons must be made between the same sectors of the benchmark database eyes and the suspect eye.

In a Chinese population with brown eyes, the thickness of the iris was correlated with darkness of the pigmentation.[7] We also found that in our Caucasian study population, the darker brown irises were thicker. Further, the relationship between darker shades and iris thickness was true also for blue and green eyes as well. Based on the nine-category color scale proposed by Mackey et al.,[10] we found a trend of increasing thickness and volume starting from light blue irises (Category 1) up to dark brown (Category 9). Dark blue irises (Category 2) appeared to be an exception to this trend because the volumes of these irises were smaller than expected. This deviation could have been due to the small sample size for this category or to automated segmentation errors.

The correlation of categorical iris colors with iris volume was stronger than it was for iris thickness. The stronger correlation with volume probably is due to the higher accuracy of the automatic volumetric analysis as compared to the manual thickness measurement of the iris sections. In fact, the volumetric measurements were based on the complete automatic segmentation of the OCT scan of the iris surface, including crypts and evaginations. In contrast, the manual thickness measurements were performed on only a single vertical plane at the thickest point of the scan.

There was no correlation between iris color and width or pupil diameter. Since the number of stromal melanocytes does not vary significantly in irises of different colors,[14] a possible explanation for the correlations between color and morphology can be found in the higher content of pigment granules within the melanocytes of darker irises.[15] The increased number of pigment granules likely increases the thickness and consequently the volume of the iris stroma, but does not affect the width of the structure.

Age did not influence iris volume or thickness, whereas iris width did increase with aging. Because the iris is a part of the vascular tunic of the eye, it might be expected that aging could reduce iris thickness and volume as described for the choroid.[16,17] It is possible that the high water content of the iris stroma, due to the presence of aqueous humor that fills the spaces between stromal cells,[18] prevents the reduction in thickness and volume. Thus, we speculate that although the stromal cells and/or the iris vascular components can decrease with age, the residual space generated by this loss could be filled by aqueous humor, resulting in a global maintenance of iris morphology and volume. Histological analysis could support this hypothesis. The increase in iris width with aging could be related to mechanical factors. Our measurements were made from the pupillary margin to the iris root, and, thus, were influenced by pupil size. Because the pupil diameter significantly decreased with aging, a phenomenon known as senile miosis,[19] our finding can be attributed to the reduction in pupil size rather than a real increase in iris width.

Sex did not influence iris thickness or volume; however, the width was slightly, but significantly, greater for males. Contrary to long-held beliefs, but consistently with data previously reported by others,[19,20] neither sex nor iris color influenced pupil size. Thus, we speculate that the difference in iris width between males and females is related to sex-determined anatomical variations that occur during growth of the face. This hypothesis must be confirmed.

Our study has some limitations. First the consecutive strategy applied for enrollment resulted in an unbalanced distribution among the nine color categories in favor of brown irises. This reflects the normal prevalence of iris color in the Italian population. The uneven inclusion of iris colors could have partially affected the results even though the correlations between color and morphology were significant. Second, despite the consecutive enrollment, the sex distribution also was partially unbalanced (males/females ratio = 0.73) possibly affecting the results. Third, while volume calculation was automatically generated by the instrument software, other parameters were measured manually. Manual procedures could have decreased the accuracy of measurements although they were repeated twice and had high agreement between the observers. Finally other characteristics that we did not consider, such as subject ethnicity, axial length, and pupil dynamics, could influence iris morphometry.[9] We tried to reduce the possible bias generated by these variables by enrolling only Caucasian emmetropic eyes and by capturing OCT images under standardized illumination conditions.

In conclusion, SS-ASOCT opens a new era in the in vivo analysis of iris morphology. Automatic measurements can be performed easily in a clinical setting and can offer a wide set of adjunctive information that is potentially useful during the diagnostic process and follow-up of several pathologies. With the present study, we found morphology of the iris to be more complex than expected, and yet it provided quantitative benchmark values that can be applied to identify pathological variations in single subjects. To reach a high degree of reliability, a large normative database of iris morphology that takes into account the multiple influences identified so far, such as iris color, sectoral variations, and many more that still are unknown, should be created. To achieve this purpose, further studies are needed to confirm our findings and to elucidate other variables that affect iris morphology.

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