Effects of Refractive Power on Quantification Using Ultra-Widefield Retinal Imaging

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Abstract

**Background:** Ultra-widefield (UWF) retinal images include significant distortion when they are projected onto a two-dimensional surface for viewing. Therefore, many clinical studies that require quantitative analysis of fundus images have used stereographic projection algorithm, three-dimensional fundus image was mapped to a two-dimensional stereographic plane by projecting all relevant pixels onto a plane through the equator of the eye. However, even with this impressive algorithm, refractive error itself might affect the size and quality of images theoretically. The purpose of this study is to investigate the effects of refractive power on retinal area measurements (quantification) using UWF retinal imaging (Optos California; Dunfermline, Scotland, UK).

**Methods:** A prospective, interventional study comprised 50 healthy eyes. UWF images were acquired first without the use of a soft contact lens (CL) and then repeated with six CLs (+9D, +6D, +3D, -3D, -6D, and -9D). Using stereographically projected UWF images, the optic disc was outlined by 15-17 points and quantified in metric units. We divided the subjects into three groups according to axial length: Groups A (22-24mm), B (24-26mm), and C (≥26mm). The primary outcome was percentage change before and after use of the CLs. Secondary outcome was proportion of subjects with magnification effects, maximal changes >10%.

**Results:** The study population was 6, 28, and 16 eyes in each group. Overall changes for the measured area were not significantly different in the whole study population. Group C had a larger proportion of magnification effects compared to Groups A and B (50.0%, 0%, and 3.6%, P=0.020). Measured area with plus lenses was significantly higher in Group C (P<0.001).

**Conclusion:** The use of CLs might affect quantification of eyes with long axial length when using UWF images. Ophthalmologists should consider refractive error when measuring area in long eyes.

**Background**

Advances in ultra-widefield (UWF) imaging technology have allowed noninvasive, single images of the peripheral retina [1, 2]. Regardless of this advantage, UWF images include significant distortion when they are projected onto a two-dimensional surface for viewing [1, 3, 4]. Thus, stereoscopic projection algorithms have been employed to correct image distortion [1, 2, 5, 6]. A three-dimensional object (fundus image) was mapped to a two-dimensional stereographic plane by projecting all relevant pixels onto a plane through the equator of the eye [1, 3]. As this stereographic projection preserves the shape of a sphere, it enables the correct measurement of area based on calculations made on the sphere [1]. Therefore, many clinical studies that require quantitative analysis of fundus images have used this technology [1, 4, 7-10]. Moreover, a stereographic projection algorithm using axial length information can produce accurate and precise measurements relative to an intraocular ground truth standard [1, 2, 6].

However, even with these impressive advancements, refractive error itself might affect the size and quality of images theoretically [11]. In routine clinical practice, UWF images are obtained in subjects
without refractive error correction, although refractive errors are capable of causing image distortion and magnification effects. Further, several previous studies using optical coherence tomography (OCT) have indicated that refractive errors affect the analysis of retinal nerve fiber layer (RNFL) thickness, leading to lower values in myopic eyes compared to normal eyes due to the differences in axial lengths and the magnification effects [12, 13].

There are many controversies regarding the effects of refractive errors on measurement of the retina. Certain investigations using OCT report that contact lens diopter does not significantly affect measurement of RNFL [14, 15]. On the contrary, other reports have suggested that RNFL thickness decreases as refractive power becomes more negative [12]. To date, there are no studies regarding the effects of refractive error on area measurement using UWF. In this context, many studies using stereoscopic measurement have empirically employed inclusion criteria between +3 Diopters to −3 or −6 Diopters. Therefore, the purpose of this study was 1) to investigate the effects of refractive error in contact lenses (CLs) on area measurement in UWF images in healthy subjects with myopia and (2) to compare the magnification effects according to axial lengths.

Methods

Study subjects and baseline examinations

This is a single-center, prospective, interventional study conducted at Yeungnam University Medical Center between March 2018 and April 2018. The study protocol was approved by the Institutional Review Board of Yeungnam University Medical Center (IRB No. 2017-04-026). All participants provided signed informed consent, and the study adhered to the tenets of the Declaration of Helsinki.

The participants included healthy young subjects aged 18 years or older. Normal subjects were defined as those with no history of systemic or ocular disease or no presence of ocular surgery. The exclusion criteria were as follows: myopia < -6 D, astigmatism > 1.5 D, intraocular pressure (IOP) > 21 mmHg, media opacity that obscured image acquisition, pathologic myopic changes of the retina such as posterior staphyloma, Lacquer crack, tessellated fundus, or myopic foveoschisis, or changes of the optic disc such as tilted disc configuration, myopic parapillary atrophy or glaucomatous changes. Media opacity was defined to be the presence of corneal scarring, corneal edema, cataract, or vitreous haze. Each participant underwent a routine ophthalmic examination, including evaluation of past medical history, best-corrected visual acuity, refractive error without pupil dilation (Auto-refracto-keratometer, HRK-7000A; Huvitz Co., Ltd., Korea), IOP (TX-20 Full Auto Tonometers; Canon, Tokyo, Japan), and axial length (IOL Master500; Carl Zeiss Meditec, Gena, Germany).

Considering the fact that axial length and degree of baseline refractive error are capable of influencing the measurement of area in UWF images, we classified the subjects into three groups based on their corresponding axial lengths: below-average axial length group (Group A, between 22 and 24 mm), above-average axial length group (Group B, between 24 and 26 mm), and long axial length group (Group C, 26
mm or above). Further, we classified the subjects into two groups based on refractive error: mild myopia (Plano to - 3 D) and moderate myopia (- 3 D to - 6 D).

**Image acquisition, projection, and quantification**

UWF imaging was performed using an Optos UWF system (Optos California; Dunfermline, Scotland, UK). UWF images were acquired first without the use of soft CL, and then the process was repeated using CLs of six different diopters (+ 9 D, + 6 D, + 3 D, - 3 D, - 6 D, and - 9 D). Acquired images were transformed to a stereographic projection image using proprietary software from the manufacturer [1]. With stereographically projected UWF images, two masked, trained ophthalmologists manually outlined the optic disc area by 15–17 points where the disc margin meets the blood vessel using Image J V.1.49b (US National Institutes of Health, Bethesda, Maryland).

The area of the optic disc corresponding to each image was measured in square millimeters (mm$^2$) by summing the anatomically-correct sizes of all pixels that comprise the disc margin (Figure 1). Two independent, masked graders performed annotations of the optic disc twice, and the average value was used for subsequent statistical analyses. These values obtained using CLs were compared with the measurements obtained from the baseline image without CL (100.0%), and the respective percentage differences were determined. The maximal difference (%) in each case was defined as absolute value differences (% area difference) compared to those for the baseline image without CL. The magnification group comprised subjects exhibiting maximal difference > 10%.

**Statistical analysis**

Statistical analyses were conducted using SPSS software (version 19.0; IBM Corp., Armonk, NY) and MedCalc (version 15.8; MedCalc, Inc., Ostend, Belgium). The Kolmogorov-Smirnov test was used to assess sample distribution. The differences in numerical data were analyzed using repeated measure analysis of variance (ANOVA), Kruskal Wallis test, independent t-test, and Mann-Whitney test. Categorical variables were evaluated using Chi-square tests. Area under the curve, sensitivity, and specificity were calculated using the receiver operating characteristics (ROC) curve. A multiple comparison with Bonferroni correction was performed in cases that exhibited significant difference. Intra-grader and inter-grader agreement values were evaluated by intra-class correlation coefficient (ICC) values. Statistical significance was defined as P < 0.05.

**Results**

**Baseline Characteristics**

A total of 50 eyes from 25 healthy subjects (13 male and 12 female) were included. The average age of the subjects was 25.0 ± 3.5 years; the average spherical equivalent and axial length were - 2.80 ± 1.61 D and 25.31 ± 1.07 mm, respectively. Other demographic information has been detailed in Table 1.
Subgroup analysis according to axial length and magnification effect

The study population was composed of 6 eyes in Group A, 28 eyes in Group B, and 16 eyes in Group C. As depicted in Table 2, the corresponding mean axial lengths were 23.13, 25.20, and 26.33 mm, respectively (P < 0.001). In subgroup analysis, there was a significant proportion of moderate myopia in Group C, which also contained a larger proportion of patients experiencing magnification effects than Groups A and B (50.0%, 0%, and 3.6%, respectively, Chi-square, P = 0.020). Moreover, the absolute value of the corresponding maximal difference was also observed to exhibit statistical significance (P = 0.046, one-way ANOVA).

The data presented in Table 3 indicates that the magnification group contained a larger proportion of patients with long axial lengths (≥ 26 mm, 61.5% vs. 21.6%, P = 0.020) and moderate myopia (< -3 D, 35.2% vs. 84.6%, P = 0.002), as well as greater myopic refractive error (- 4.03 D vs. –2.37 D, P = 0.001).

Receiver operating curves (ROC) and area under the ROC (AUROC)

Figure 2 depicts receiver operating curves for magnification effects with corresponding cutoff values. The cutoff values are 25.44 mm (sensitivity/specificity = 100%/62.2%) for axial length and – 3.5 Diopter (sensitivity/specificity = 84.6%/73.0%) for refractive error. Although areas under the ROC (AUROC) for axial length (0.867, 95% C.I 0.741 – 0.946) were higher than those for spherical equivalent (0.793, 95% C.I 0.655 – 0.895), there was no statistical significance. Moreover, the values with specificity > 80% were 26.04 mm for axial length and – 4.125 Diopters for refractive error.

Percentage changes in measured area before and after the use of the CLs

There was no significant difference between the CLs of different diopters (- 9 D, - 6 D, - 3 D, + 3 D, + 6 D, + 9 D) among the entire study group (Figure 3A). In subgroup analysis, the measured optic disc areas with the plus lenses (+3 D, + 6 D, +9 D) were significantly higher than those with minus lenses in the moderate myopia group and Group C (Figures 3B and 3C). No adverse event associated to the application or removal of the CLs was observed.

Discussion

In the present study, measurement of area was unaffected by refractive power in the normal axial length group (A, 22–24 mm) and the mid-axial length group (B, 24–26 mm). On the contrary, the measurement of area in the long axial length group (C, 26 mm or above) was affected by changes in refractive power.

To the best of our knowledge, no previous studies have examined the use of CL to compare area measures using UWF images. The use of CLs is capable of providing refractive error correction from either astigmatism or axial length without the use of a correction formula. In this specific population with AL ≥ 26 mm, the refractive correction achieved with a CL affected the area measurement with a statistical significance and an effect size measured in maximal difference of 9.36 ± 10.37 %. These findings are important to avoid misinterpretations or false interpretations of area measurements.
Compared to measurements on UWF images using minus lenses, those using plus lenses were observed to be more affected in Group C, compared to Groups A and B.

This phenomenon is likely to be related to the myopia, with the possible hypotheses; 1) deviation from the spherical retinal model, 2) ocular magnification and 3) overestimation by grader; also a combination of these effects is possible First, we primarily consider ocular magnification effects by CL alone. A plus CL is convex, which causes light convergence. This additional lens power further focuses the scan in front of the retina, and, in particular, a positive refractive defocus error causes the scan circle to shrink in the retinal plane and become smaller in terms of the usual scan size or degrees in diameter [16, 17]. These changes could lead to an overestimation of area measurement, whereas minus CLs may similarly lead to underestimation [11]. Second, myopic eyes are elongated and may deviate from the spherical model used to perform anatomically-correct measurements.

The mean ICCs for grader 2, in particular, and between graders, were observed to be 0.942 and 0.927, respectively, suggesting good intra-grader and inter-grader reproducibility of these measurements; however, the possibility of over-estimation owing to image quality still remained [18]. With the influence of both the aforementioned effects on the estimation of the optic disc margin, ocular magnification effects are also prone to being exacerbated by the graders.

These findings are supported by previous studies using OCT. In spectral domain OCT models, signal strength and scan reliability decreased in long axial lengths [19, 20]. Similarly, increase in axial length has been confirmed to lead to ocular magnification and an underestimation of the nerve fiber layer (NFL) in SD-OCT scans without the use of a correction formula for a refractive error of less than −4 D [13, 21, 22]. However, even correction formulas including the Litmann formula or Bennett formula are known to not be able to guarantee an accurate assessment of the optic nerve head [12, 23]. These effects may increase with increasing axial length [24].

The limitations of our study are as follows. (1) Relatively small cohort and specific sample composed of young healthy adults. (2) We were unable to exclude accommodation effects. (3) No subjects with hyperopic eyes or short axial lengths < 22 mm were included in the study. (4) Myopic eyes, which are often elongated, can deviate from a spherical shape assumed for quantification. Despite these limitations, our models have certain advantages. This is the first study to analyze the effect of refractive error on the area measured after correcting the peripheral distortion by the stereographic method. In addition, the use of CL could provide a refractive error correction for either astigmatism or axial length without the use of a correction formula, such as the Littmann or the Bennett formulae.

Conclusions

To the best of our knowledge, this is the first study to investigate the effect of refractive error on area measurement in UWF images. Our study demonstrated that refractive errors might affect area measurement accuracy in subjects with long axial length. Although the majority of the subjects exhibited
no definite magnification effects, it was concluded that careful correction or consideration is required for area measurement in eyes with long axial lengths.

### Abbreviations

UWF = ultra-widefield

OCT = optical coherence tomography

RNFL = retinal nerve fiber layer

D = diopter

CL = contact lens

IOP = intraocular pressure

ROC = receiver operating characteristics

### Declarations

#### Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Institutional Review Board of Yeungnam University Medical Center (IRB No. 2017-04-026). All participants provide signed informed consent to participate.

#### Consent for publication

Written informed consents were obtained from all the participants. Copies of the written consent are available for review by the editor of this journal

#### Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Competing interests

S.H. Lim, None; S. Jeong, None; J.H. Ahn, None; and M. Sagong, None

J.v.H. is the research director at Optos, a Nikon Company (Employment) and held patents (Improvement in and relating to Imaging of the Eye) pending to Optos PLC.
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Authors' contributions
Conceptualization, S.H.L., S.J. and M.S.; methodology, S.H.L., S.J., J.H.A., J.v.H., and M.S.; writing-original draft preparation, S.H., and S.J.; writing-review and editing, S.H.L., S.J., J.v.H., and M.S.; Critical editing, M.S.; image acquisition and processing, S.H.L., S.J., J.H.A., and J.v.H.; interpretation of data, S.H.L., S.J., and M.S; funding acquisition, M.S. All the authors read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Tables

Table 1. Baseline characteristics of the study participants

| Variables                  | Study participants (50 eyes of 25 subjects) |
|----------------------------|--------------------------------------------|
| Age, years                 | 25.0 ± 3.5 (Range 20-33)                   |
| Male/Female                | 13/12                                      |
| IOP, mmHg                  | 15.1 ± 2.0 (Range 11-20)                   |
| Axial Length, mm           | 25.31 ± 1.07                               |
| Avg. Keratometry (Diopters)| 42.63 ± 1.49 D                            |
| Spherical Equivalent (Diopters) | -2.80 ± 1.61 (Range, -0.125D ~ -5.375D) |

Values are presented as mean ± SD unless indicated otherwise.

IOP intraocular pressure.

Table 2. Comparison of ocular parameters according to the axial length
| Variables                              | Group A (AL 22-24mm) N=6 eyes | Group B (AL 24-26mm) N=28 eyes | Group C (AL > 26mm) N=16 eyes | P-value  |
|---------------------------------------|-------------------------------|-------------------------------|-------------------------------|----------|
| Age, years                            | 24.7 ± 3.4                    | 25.9 ± 3.6                    | 23.8 ± 3.4                    | 0.159*   |
| IOP, mmHg                             | 14.2 ± 1.3                    | 14.9 ± 2.5                    | 15.6 ± 1.0                    | 0.298*   |
| Axial length, mm                      | 23.13 ± 0.67                  | 25.20 ± 0.50                  | 26.33 ± 0.23                  | <0.001*  |
| Average Keratometry (D)               | 43.89 ± 1.39                  | 42.79 ± 1.54                  | 41.88 ± 1.02                  | 0.011*   |
| Spherical Equivalent (D)              | -1.06 ± 0.49                  | -2.41 ± 1.54                  | -4.13 ± 0.88                  | <0.001*  |
| Proportion of Refractive Error        |                               |                               |                               | <0.001†  |
| Plano to – 3D (Mild)                  | 6 (100.0%)                    | 18 (64.3%)                    | 2 (12.5%)                     |          |
| - 3 D to – 6 D (Moderate)             | 0 (0%)                        | 10 (35.7%)                    | 14 (87.5%)                    |          |
| Max. Difference (%)                   | 3.40 ± 4.16                   | 2.65 ± 7.9                    | 9.36 ± 10.37                  | 0.046*   |
| Proportion of Subjects                |                               |                               |                               |          |
| Max. Difference > 10%                 | 0/6 (0%)                      | 5/28 (17.9%)                  | 8/16 (50.0%)                  | 0.020†   |

Values are presented as mean ± SD unless indicated otherwise.

* P value using the Kruskal Wallis test.

† P value using the χ² test.

AL axial length; IOP intraocular pressure.

**Table 3. Comparison of ocular parameters according to magnification effects**
| Variables                  | No Magnification Group | Magnification Group (Max. Diff > 10%) | P-value |
|----------------------------|------------------------|---------------------------------------|---------|
|                            | N = 37 eyes             | N = 13 eyes                           |         |
| Age, years                 | 25.4 ± 3.3              | 23.9 ± 3.8                            | 0.235*  |
| IOP, mmHg                  | 15.2 ± 2.1              | 14.7 ± 1.7                            | 0.381*  |
| Axial Length – 24 (mm)     | 1.01 ± 1.05 (25.01 ± 1.05) | 2.17 ± 0.45 (26.17 ± 0.45)          | <0.001* |
| (Mean Axial length (mm))   |                        |                                       |         |
| Axial Length (Proportion)  |                        |                                       | 0.020†  |
| 22-24 mm                   | 6 (16.2%)               | 0 (0%)                                |         |
| 24-26mm                    | 23 (62.2%)              | 5 (38.5%)                             |         |
| 26mm or above              | 8 (21.6%)               | 8 (61.5%)                             |         |
| Avg. K value (D)           | 42.87 ± 1.51            | 41.94 ± 1.21                         | 0.034*  |
| Spherical Equivalent       | - 2.37 ± 1.47           | - 4.03 ± 1.38                        | 0.001*  |
| Refractive Error           |                        |                                       | 0.002†  |
| Plano to – 3D (Mild)       | 24 (64.8%)              | 2 (15.4%)                             |         |
| - 3 D to – 6 D (Moderate)  | 13 (35.2%)              | 11 (84.6%)                            |         |

Values are presented as mean ± SD unless indicated otherwise.

* P value using the Mann-Whitney test.

† P value using the χ² test.

IOP intraocular pressure.