The Precision of Wavefront Refraction Compared to Subjective Refraction and Autorefraction

KONRAD PESUDOVS, BScOptom, PhD, FAAO, KATRINA E. PARKER, OD, FAAO, HAN CHENG, OD, PhD, and RAYMOND A. APPLEGATE, OD, PhD, FAAO

Department of Ophthalmology, NH and MRC Centre of Clinical Eye Research, Flinders University and Flinders Medical Centre, Bedford Park, South Australia, Australia (KP), and Visual Optics Institute, College of Optometry, University of Houston, Houston, Texas (KP, KEP, HC, RAA)

ABSTRACT

Purpose. To determine the precision (repeatability) of several methods of calculating refraction from higher-order wavefront aberration data and to compare these wavefront refractions with lower-order (LO) wavefront refraction, subjective refraction, and autorefraction.

Methods. Four clinicians refracted 16 normal participants aged 23.6 \pm 1.2 years, 69% female with an average spherical equivalent refractive error of -3.03 ± 2.55 D, median sphere -2.50 D (minimum -7.50, maximum +4.75), and median cylinder -0.50 D (minimum -3.00, maximum 0). Participants were cyclopleged and underwent subjective refraction, autorefraction on two machines (Nidek AR-800, Topcon KR-8000), and wavefront sensing using the Wavefront Sciences Complete Ophthalmic Analysis System. Wavefront error was used to calculate: LO refraction, refractions that incorporated higher-order spherical and astigmatism terms from up to the 4th, 6th, and 10th orders (PCM₄, PCM₆, and PCM₁₀), and a method based on optimizing image quality metrics [wavefront analysis technology (WAT) refraction]. Within and between examiner agreements for total dioptric difference were determined using Bland–Altman limits of agreement (LOA).

Results. The interexaminer LOA for individual measurements for M, J0, J45 were: Topcon (±0.18, ±0.10, ±0.06), Nidek (±0.28, ±0.16, ±0.09), LO (±0.17, ±0.10, ±0.06), PCM₄ (±0.26, ±0.09, ±0.06), PCM₆ (±0.37, ±0.17, ±0.34), PCM₁₀ (±0.54, ±0.32, ±0.40), WAT (±0.28, ±0.20, ±0.15), and subjective refraction (±0.48, ±0.20, ±0.13) and averaging across three measures LOA: Topcon (±0.15, ±0.08, ±0.05), Nidek (±0.21, ±0.13, ±0.07), LO (±0.12, ±0.06, ±0.04), PCM₄ (±0.16, ±0.05, ±0.04), PCM₆ (±0.23, ±0.09, ±0.19), PCM₁₀ (±0.29, ±0.19, ±0.24), and WAT (±0.18, ±0.12, ±0.10). The within-examiner LOA for M, J0, J45 were: Topcon (±0.08, ±0.04, ±0.02), Nidek (±0.13, ±0.07, ±0.05 D), LO (±0.11, ±0.07, ±0.04), PCM₄ (±0.17, ±0.07, ±0.04 D), PCM₆ (±0.28, ±0.12, ±0.24 D), PCM₁₀ (±0.42, ±0.24, ±0.32 D), and WAT (±0.19, ±0.14, ±0.09 D).

Conclusions. All objective refractions except for PCM_{10} were more repeatable across clinicians than subjective refraction. The precision of all refractions were improved by an expected amount through averaging over multiple measurements. Wavefront refractions were not as precise as standard autorefractions, although not clinically significantly worse. (Optom Vis Sci 2007;84:387–392)

Key Words: aberration, autorefraction, optics, refraction, refractive errors, vector analysis, wavefront

The aim of this study was to examine the precision (repeatability) of methods for calculating refraction from higherorder wavefront aberrations and to compare this precision with that of traditional refraction methods. In recent years, wavefront sensing has become a widely available clinical test of the wavefront errors (WFE) of the eye.¹ The WFE can be used in several ways to determine a sphero-cylindrical correction.² A common method is to fit the wavefront error with a normalized Zernike expansion as detailed by the ANSI Z-80 standard and use specific coefficients to calculate a sphero-cylindrical correction. In

the simplest version, just the lower-order (LO) terms of the Zernike expansion (sphere and cylinder) are used. Higher-order sphere and cylinder terms (e.g., spherical aberration and secondary astigmatism, etc.) can also be incorporated to calculate refraction in a method known as paraxial curve matching.³ An alternative strategy is to use the WFE to calculate a single-value metric of retinal image quality and iterate all possible combinations of sphere, cylinder, and axis to find which combination produces the optimum retinal image quality as defined by the metric.³ Thibos et al. described and determined the accuracy and precision of 33 objective metrics of optical quality in determining a sphero-cylindrical refraction.³ They found that most of these metrics had precision of 0.50 to 1.00 D for predicting subjective refraction (-0.50 to +0.25 D for sphere and 1/8 D for astigmatism).

Although subjective refraction has long been the de facto gold standard for refraction, the precision of a subjective refraction between or within clinicians is relatively poor with 95% limits of interexaminer agreement of the spherical equivalent being 0.62 to 0.75 D.^{4-6} This is twice the limits of agreement (LOA) found for autorefraction^{7,8} or wavefront-guided refractions.^{3,9,10} We would argue that high precision is the first essential feature of a clinical test, or indeed a potential clinical gold standard should one consider it were possible for another method of refraction to usurp subjective refraction. Therefore, in this experiment, we attempt to establish the precision of a range of methods for determining refraction from higher-order wavefront aberrations. To place these results in the context of currently used methods, we compare them with the precision of subjective refraction and autorefraction, both within and between clinicians.

METHODS Patients

Sixteen healthy people free of ocular or systemic disease were recruited from the University of Houston, College of Optometry Class of 2007 to participate in this study. The average age was 23.7 \pm 1.2 years, average refractive error in dioptric vector space $(M, J_0, J_{45}) - 3.03 \pm 2.55 \text{ D}, 0.05 \pm 0.39 \text{ D}, 0.04 \pm 0.30 \text{ D} \text{ or}$ median sphere -2.50 D (minimum -7.50, maximum +4.75) and median cylinder -0.50 D (minimum -3.00, maximum 0), 11 were female and 5 were male. Approval from the University of Houston Institutional review board (IRB) was obtained, each participant signed an informed consent at enrollment and the study protocol was undertaken in compliance with the Declaration of Helsinki. The right eye of each participant served as the test eye. Accommodation was paralyzed with 1% cyclopentolate before clinical testing. To ensure minimal residual accommodation, a push-up test to first blur was performed 15 minutes after instillation and repeated every 5 minutes. If the participant could read clearly at 50 cm 30 minutes after instillation, an additional drop of 1% cyclopentolate was instilled. All participants reported blur at 50 cm before commencing the examination.

Clinical Assessment

Participants underwent: (1) subjective refraction using a phoropter, (2) autorefraction using Nidek AR-800 autorefractor and Topcon KR-8000 autorefractor, and (3) WFE measurement using Wavefront Sciences Complete Ophthalmic Analysis System (COAS) wavefront sensor.

Subjective refraction was performed by four clinicians—RAA, KP, HC, KEP. Each clinician used the endpoint criterion of maximum plus to best visual acuity and recorded their result to the nearest 0.25 D.

Both autorefractors work according to Scheiner's double pinhole principle. Although the machines are designed slightly differently, in both cases a photodetector observes the degree of coincidence between the two images and proprietary software calculates the sphero-cylindrical correction.¹¹ Each clinician was trained using the standard operating procedure as defined in the operating manual before formal data collection. Three individual measurements were taken and averaged. The Nidek AR autorefractor reported the refraction to the nearest 0.25 D (measurement range from -18.0 to +23.0 D in sphere and up to ± 8.0 D in cylinder) and the Topcon KR-8000 reported to the nearest 0.125 D (measurement range from -25.0 to +22.0 D in sphere and up to ± 8.0 D in cylinder) for both sphere and cylinder. We did not correct for differences in step size thus using the instruments as they are used clinically.

The Complete Ophthalmic Analysis System Model G200, by WaveFront Sciences was used to measure ocular aberrations. Each investigator made three measurements on each participant. The COAS is a Shack Hartmann aberrometer that measures the monochromatic aberrations of the eye and fits the resulting error with a Zernike expansion. We use the Zernike output to determine five different sphero-cylindrical refractive corrections. Four of these simply incorporate Zernike sphere and cylinder terms, but the fifth follows a completely different philosophical approach, dependent on retinal image quality. The use of different combinations of Zernike terms to report sphero-cylindrical refractive error recognizes that a complete description of wavefront requires recognition of all terms used in the expansion. The sphero-cylindrical refractive error is not simply described by the second-order sphere and cylinder terms, but also is influenced by the spherical aberration and cylinder terms in the higher orders. For the sake of simplicity the number of orders in the expansion may be truncated especially given that diminishing returns will occur through incorporating higher and higher orders.

- 1. Lower order refraction uses the second-order aberration terms C_2^{-2} , C_2^0 , C_2^0 and is consistent with the Non-Seidel output terms from COAS¹² (see equations 1 to 3 below).
- 2. Paraxial curve matching refraction to fourth radial order (PCM₄) uses the second order and spherical aberration C_4^0 and astigmatism terms C_4^{-2} , C_4^2 from the fourth radial order. This is consistent with the COAS Seidel output terms¹² (see equations 4-6 below).
- 3. Paraxial curve matching refraction to sixth radial order (PCM₆) uses the PCM₄ refraction plus the addition of spherical aberration C_6^0 and astigmatism terms C_6^{-2} , C_6^2 from the sixth radial order (see equations 7–9 below).
- 4. Paraxial curve matching refraction to 10th radial order (PCM₁₀) uses the PCM₆ refraction plus the addition of spherical aberration C_8^0 , C_{10}^0 and astigmatism terms C_8^{-2} , C_8^2 , C_{10}^{-2} , C_{10}^2 from the 8th and 10th radial order (see equations 10–12).

Equations

Lower Order Refraction

$$M = \frac{-c_2^0 4\sqrt{3}}{r^2}$$
(1)

$$J_0 = \frac{-c_2^2 2\sqrt{6}}{r^2}$$
(2)

$$J_{45} = \frac{-c_2^{-2} 2\sqrt{6}}{r^2}$$
(3)

PCM₄ Refraction

$$M = \frac{-c_2^0 4\sqrt{3} + c_4^0 12\sqrt{5}}{r^2}$$
(4)

$$J_0 = \frac{-c_2^2 2\sqrt{6} + c_4^2 6\sqrt{10}}{r^2}$$
(5)

$$J_{45} = \frac{-c_2^{-2} 2\sqrt{6} + c_4^{-2} 6\sqrt{10}}{r^2} \tag{6}$$

PCM₆ Refraction

$$M = \frac{-c_2^0 4\sqrt{3} + c_4^0 12\sqrt{5} - c_6^0 24\sqrt{7}}{r^2}$$
(7)

$$J_0 = \frac{-c_2^2 2\sqrt{6} + c_4^2 6\sqrt{10} - c_6^2 12\sqrt{14}}{r^2}$$
(8)

$$J_{45} = \frac{-c_2^{-2} 2\sqrt{6} + c_4^{-2} 6\sqrt{10} - c_6^{-2} 12\sqrt{14}}{r^2}$$
(9)

PCM₁₀ Refraction

$$M = \frac{-c_2^0 4\sqrt{3} + c_4^0 12\sqrt{5} - c_6^0 24\sqrt{7} + c_8^0 40\sqrt{9} - c_{10}^0 60\sqrt{11}}{r^2}$$

(11)

$$J_0 = \frac{-c_2^2 2\sqrt{6} + c_4^2 6\sqrt{10} - c_6^2 12\sqrt{14} + c_8^2 20\sqrt{18} - c_{10}^2 30\sqrt{22}}{r^2}$$

$$-e^{-2}$$
 12 $\sqrt{14} + e^{-2}$ 20 $\sqrt{18} - e^{-2}$ 20

$$J_{45} = \frac{-c_2^{-2} 2\sqrt{6} + c_4^{-2} 6\sqrt{10} - c_6^{-2} 12\sqrt{14} + c_8^{-2} 20\sqrt{18} - c_{10}^{-2} 30\sqrt{22}}{r^2}$$
(12)

where r = pupil radius.

5. The wavefront analysis technology (WAT) refraction uses WFE to calculate a single-value metric of retinal image quality. Then the effect of varying sphere, cylinder, and axis is iterated through all possible combinations to find which produces the optimum retinal image quality as defined by the metric. In this study the retinal image quality metric used was Visual Strehl calculated by the optical transfer function method.³ Visual Optics Laboratory software (VOL-version 6.33, Sarver and Associates, Carbondale, IL, http://www.sarverassociates.com) generates the WAT refraction using the second through 10th radial order Zernike coefficients from each measured wavefront to systematically investigate the combination of sphere, cylinder, and axis that optimizes the Visual Strehl metric.

Sphere and cylindrical components of the correction were calculated to the nearest 100 of a diopter.

Analysis

When statistically analyzing the repeatability of refraction, the individual terms of sphere, cylinder, and axis values cannot be directly compared across refractions because these terms are not independent (orthogonal).⁴ Therefore, each refraction was transformed from the conventional sphere, cylinder, and axis format into three-dimensional dioptric vector space (M, J_0 , J_{45}) where the three components are orthogonal.⁴

The *M* parameter is the spherical equivalent (or mean power), J_0 and J_{45} parameters are Jackson cross-ed cylinder components with the power at axis 180 and axis 45.^{13,14} [see equations 13–15 detailing the calculation of *M*, J_0 , and J_{45} given sphere power in diopters (*S*), cylinder power in diopters (*C*) and axis in degrees (α)].

$$M = S + \frac{C}{2} \tag{13}$$

$$J_0 = \left(-\frac{C}{2}\right)\cos(2\alpha) \tag{14}$$

$$J_{45} = \left(-\frac{C}{2}\right)\sin(2\alpha) \tag{15}$$

In addition, the results are displayed as an astigmatism vector (equation 16) which is the total difference in astigmatism (two-dimensional astigmatic plane difference vector), and also as the total dioptric difference, TDD (equation 17), which is the total dioptric change (threedimensional dioptric space difference vector).⁴

Astigmatism Vector = $\sqrt{(\Delta f_0)^2 + (\Delta f_{45})^2}$ (16)

$$TDD = \sqrt{(\Delta M)^2 + (\Delta f_0)^2 + (\Delta f_{45})^2}$$
(17)

Within and between examiner agreements were determined using Bland-Altman 95% LOA for each method of refraction; a parametric approach for comparing methods. Bland-Altman LOA are estimated by the mean difference ± 1.96 standard deviation of the differences and provide an interval within which 95% of differences between measurements by the two methods are expected to fall. Poor repeatability of one method causes poor repeatability between two methods for individuals.¹⁵ For example if the repeatability for subjective refraction is poor, then the agreement between subjective refraction and any other method of refraction will also be poor. For the astigmatism vector and the TDD, the difference between measures is reported as median and 95th percentile as surrogates for Bland-Altman analysis because the data for these two vectors are not normally distributed. The lower the number value, the better the agreement is between the two methods.⁴ All statistical analyses were performed in Microsoft Excel 2003 (Microsoft Inc., Redmond, WA).

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RESULTS

The agreement between examiners for each refraction measure, in terms of M, J_0 , J_{45} , astigmatism vector and TDD, is shown in Table 1. When comparing overall precision, the TDD value is used because it reflects the dioptric difference between all of the refractive parameters: M, J_0 , and J_{45} . The interexaminer agreement (Table 1) or the "between" examiner agreement is better for autorefraction at the 95th percentile (Topcon 0.18 D and Nidek 0.26 D) than subjective refraction (0.61 D). For the wavefront refraction methods, LO refraction

has a higher level of agreement (0.21 D) than PCM₄ (0.27 D), WAT (0.36 D), PCM₆ (0.48 D), or PCM₁₀ (0.62 D) refractions. All six Zernike-derived wavefront refractions benefit from averaging across measures more so than averaging the autorefraction measurements (due to higher initial variability) (Table 2).

The agreement within examiners for each refraction measure, in terms of M, J_0 , J_{45} , astigmatism vector and TDD, is shown in (Table 3). These are all slightly tighter than the between examiner agreement. Again the simple LO Zernike-derived refraction performs very well

TABLE 1.

The agreement between four clinicians for each method of refraction

Interobserver agreement	Bland–Altman limits of agreement			Median (95th percentile)	
	М	J _o	J ₄₅	Astigmatism vector	TDD
Topcon autorefraction	±0.180	±0.099	±0.056	0.053 (0.087)	0.101 (0.176)
Lower-order refraction	±0.166	± 0.095	± 0.059	0.044 (0.102)	0.085 (0.212)
Nidek autorefraction	±0.277	±0.156	± 0.090	0.079 (0.172)	0.184 (0.255)
PCM_4 refraction	±0.257	±0.094	± 0.059	0.043 (0.102)	0.103 (0.268)
WAT refraction	±0.276	±0.199	±0.150	0.109 (0.224)	0.151 (0.358)
PCM_{6} refraction	±0.370	±0.168	±0.336	0.120 (0.352)	0.190 (0.484)
Subjective refraction most plus	± 0.484	±0.202	±0.125	0.110 (0.345)	0.197 (0.611)
PCM_{10} refraction	±0.537	±0.320	±0.400	0.234 (0.399)	0.327 (0.623)

The units are diopters presented as Bland–Altman limits of agreement for M_{i} , J_{0} , J_{45} and as median and 95th percentile for the astigmatism vector and the total dioptric difference (TDD).

TABLE 2.

The agreement between four clinicians for each method of refraction averaged over three measurements

	Bland–A	ltman limits of a	greement	Median (95th percentile)	
Interobserver agreement averaged	М	J _o	J ₄₅	Astigmatism vector	TDD
Topcon autorefraction averaged	±0.149	±0.083	±0.049	0.050 (0.072)	0.089 (0.146)
Lower-order refraction averaged	±0.123	± 0.055	± 0.039	0.034 (0.058)	0.058 (0.167)
PCM ₄ refraction averaged	±0.159	± 0.053	± 0.039	0.033 (0.059)	0.078 (0.190)
Nidek autorefraction averaged	±0.213	±0.130	±0.072	0.074 (0.163)	0.175 (0.277)
WAT refraction averaged	±0.181	±0.123	±0.097	0.077 (0.146)	0.103 (0.280)
PCM ₆ refraction averaged	±0.229	±0.091	±0.193	0.086 (0.188)	0.133 (0.321)
PCM ₁₀ refraction averaged	±0.291	±0.193	±0.241	0.134 (0.296)	0.210 (0.421)

The units are diopters presented as Bland–Altman limits of agreement for M, J_0 , J_{45} and as median and 95th percentile for the astigmatism vector and the total dioptric difference (TDD). Because only one measure of subjective refraction was performed by each clinician this is not included in this table.

TABLE 3.

The agreement within examiners (test-retest) for each method of refraction

	Bland-Altman limits of agreement			Median (95th percentile)	
Test-retest (within examiner) agreement	М	J _o	J ₄₅	Astigmatism vector	TDD
Topcon autorefraction	±0.076	±0.035	±0.021	0.026 (0.056)	0.048 (0.115)
Lower-order refraction	±0.107	± 0.067	± 0.040	0.038 (0.104)	0.067 (0.163)
Nidek autorefraction	±0.133	±0.067	±0.046	0.043 (0.088)	0.102 (0.166)
PCM_4 refraction	±0.170	±0.067	±0.040	0.036 (0.103)	0.089 (0.248)
WAT refraction	±0.186	±0.139	± 0.094	0.096 (0.214)	0.142 (0.299)
PCM_6 refraction	±0.283	±0.123	±0.236	0.102 (0.400)	0.174 (0.484)
PCM ₁₀ refraction	±0.424	±0.240	±0.315	0.192 (0.394)	0.319 (0.602)

The units are diopters presented as Bland–Altman limits of agreement for M, J_0 , J_{45} and as median and 95th percentile for the astigmatism vector and the total dioptric difference (TDD). Because only one measure of subjective refraction was performed by each clinician this is not included in this table.

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(0.16 D) and the agreement decreases as higher-order terms are added. The PCM₄ refraction (0.25 D) has better agreement than WAT refraction (0.30 D), PCM₆ (0.48 D), and PCM₁₀ (0.60 D) refraction, but is not as good as the LO refraction. Autorefraction, particularly with the Topcon instrument performs to a high level of retest agreement (0.12). The Nidek instrument LOA (0.17) was similar to that of the LO refraction. Because of the design of this study, subjective refraction was not assessed for within examiner agreement because it was performed only once by each clinician.

DISCUSSION

The methods with consistently the best agreement were the two autorefraction methods and LO refraction whether interexaminer (Topcon 0.18 D; LO 0.21 D; Nidek 0.26), interexaminer averaged (Topcon 0.12; LO 0.16 D; Nidek 0.17 D), or within examiner (Topcon 0.12; LO 0.16 D; Nidek 0.17 D). The superiority for the Topcon autorefraction over the Nidek is accounted for by the smaller step size in reporting refractive error with the Topcon (0.125 D) when compared with the Nidek (0.25 D); rounding with the Nidek will increase the noise of the measurement. These tight agreements for autorefraction are comparable to previous studies.^{5,7,8} The consistent feature of these methods is not including higher-order terms in calculating sphere, cylinder, and axis. Notably, the difference between the two methods with the greatest precision (Topcon 0.18 D, lower order refraction 0.21 D) is small when compared with the difference between the top method and subjective refraction (Topcon 0.18 D, subjective refraction 0.61 D). This poor level of agreement for subjective refraction is consistent with previous studies (0.62 to 0.75 D).⁴⁻⁶

The reliability of wavefront-derived refractions varied from being as tight as autorefraction to being as poor as subjective refraction depending on the method. The PCM₄ refraction (which is the same as COAS Seidel refraction) repeatability is worse than autorefraction and the LO refraction (which is the same as COAS non-Seidel refraction). This is comparable to a previous study of COAS repeatability of non-Seidel refraction having better repeatability than Seidel refraction.^{9,12} Because the precision of LO refraction (0.21) is comparable to autorefraction [Topcon (0.18), Nidek (0.26)], wavefront sensors such as the COAS can also be implemented into the type of refraction studies¹⁶⁻¹⁸ and screenings^{19,20} that currently use autorefraction especially if simple LO refraction is chosen, although WAT and PCM4 refractions perform comparably especially when averaged over several measurements.^{9,12} Indeed averaging across multiple measurements improves the reliability of wavefront refraction by an expected amount, although the COAS wavefront sensor is not set up to do this as a routine clinical function. Because all wavefront refractions except PCM₁₀ are more repeatable than subjective refraction, all these methods are more appropriate outcome measures for research studies than subjective refraction. The decreasing reliability with paraxial curve matching methods with increasing number of orders included likely illustrates the decreased signal to noise ratio occurring in higher orders of the Zernike polynomial.²¹ Although these higherorder terms are small in amplitude, their normalization when included in the PCM formula amplifies the noise in their value.

This study contains a large number of results, many of which are very similar. This begs the question: do the small differences in precision across methods really matter? Currently clinicians correct refractive error in quarter diopter steps. Therefore one could argue all methods with precision of about 0.25 D or less are equivalent. If averaging is used, this would include all methods except subjective refraction and PCM₁₀. This may seem reasonable because correcting refractive error more precisely, e.g., using 1/8th or less dioptric steps would likely not benefit the visual performance (e.g., visual acuity) of an average patient. However, in this era of wavefront-guided optimization of refractive correction, it is important that highly precise methods for measurement exist to facilitate accurate correction.

Further, in wavefront-guided optimization of refractive correction, it is worth emphasizing in the context of this article that although the representation of the optical errors of the eye in either a threedimensional dioptric vector space (M, J_0, J_{45}) or in the more inclusive space of a Zernike expansion are mathematically orthogonal, they are not visually orthogonal.²²⁻²⁵ That is, each type of optical aberration has a different impact on visual performance²³ and depending how they are combined can either improve or decrease visual performance, but never better than the aberration free condition.²² Consequently, as the field moves forward it will be important to intelligently minimize the optical errors of the eye to optimize visual performance using single value metrics of optical retinal image quality³ that are predictive of visual performance.^{26,27} These single value metrics of retinal image quality can be easily derived from the coefficients of the Zernike expansion representation of the optical errors of the eye³ and used to objectively decide how best to minimize the optical errors to optimize visual performance.

In this study we do not address the "accuracy" of refraction, only the precision. The difficulty with accuracy studies is determining the gold standard. In the case of refraction, subjective refraction is used at the gold standard^{9,11,12,19,28,29} because its methodology is based on providing the clearest vision. However, it is possible that the WAT refraction system of objectively determining the best retinal image quality could be more "accurate" than subjective refraction in determining the best refractive endpoint in the majority of cases. However, if WAT refraction was compared with subjective refraction and differences were found, it would be the decision of which was the gold standard that would determine to which the treatment error was ascribed. Therefore, we chose not to investigate accuracy, nor to present the differences in refractive results between the methods. A visual performance criterion, with a method particularly sensitive to wavefront aberration like mesopic low contrast visual acuity,³⁰ would be an alternative determination of which refraction method was most accurate. Indeed, in one study comparing subjective refraction with autorefraction, VA was better with autorefraction than subjective refraction in 15% of cases.³¹ Although this was probably large due to test-retest variation in VA, it may illustrate that flawed results can occur with subjective refraction. However, this type of study design with visual performance testing was beyond the scope of this study.

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Konrad Pesudovs

Department of Ophthalmology Flinders University, Flinders Medical Centre Bedford Park SA 5042, Australia e-mail: Konrad.Pesudovs@flinders.edu.au