Photorefraction estimates of refractive power varies with the ethnic origin of human eyes

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Eccentric infrared photorefraction is an attractive tool for measuring refractive errors of young children and uncooperative subjects, for it allows quick and non-invasive acquisition of data from both eyes simultaneously over a reasonably large dioptric range. Accuracy of refraction in this technique depends on calibration of luminance slope formed across the pupil into diopters (defocus calibration factor). Commercial photorefactors, like the PowerRef 3TM used in this study, employ an universal defocus calibration factor from one population (Caucasian) to convert raw data of all populations. This study reports significantly larger defocus calibration factors of PowerRef 3TM in 132 East Asian, African and Indian eyes, relative to the machine’s default calibration (p < 0.001). The calibration slope of 50 Indian eyes was over-estimated by 64 ± 11% (mean ± 95%CI), vis-à-vis, retinoscopy (p < 0.001). The error reduced to ~6–7% upon rescaling the data using a calibration factor specific for Indian eyes or to that individual (p > 0.9, relative to no over-estimation). Our results therefore strongly suggest the use of an ethnicity- or individual-specific defocus calibration factor for accurate estimation of refraction using photorefraction. Inaccurate refraction estimates due to calibration errors will otherwise severely undermine the advantages of this technique.

Several techniques are currently employed to quantify the eye’s uncorrected refractive error (e.g. retinoscopy, autorefraction and photorefraction). Of these, the technique of slope-based eccentric infrared (IR) photorefraction is especially attractive for young children and uncooperative subjects, for it allows simultaneous estimation of both eye’s refractive power in a quick (e.g. 50 Hz for the PlusOptix PowerRef 3TM photorefactor) and non-invasive fashion over a reasonably large dioptric range (e.g. +5D to −7D for the PowerRef 3TM). The ability to synchronously measure binocular gaze position and pupil size also makes this technique attractive for studying the near triad of accommodation, vergence and pupil size in many laboratory-based experiments. The technique of photorefraction involves projecting light into the eye from an array of IR LED’s positioned eccentrically from the camera aperture and calibrating the slope of reflected light formed across the pupil to indicate the polarity (myopic or hyperopic) and magnitude of the eye’s defocus. This calibration of luminance slope formed across the pupil into units of diopters – referred herein as the defocus calibration factor – is critical for obtaining accurate refractive power estimates using photorefraction. Commercially available photorefactors, like the PlusOptix PowerRef 3TM used in this study, typically use a universal defocus calibration factor for converting raw luminance slope values into diopters. While this calibration factor cannot be directly accessed due to its proprietary nature, it can certainly be validated by inducing known amounts of refractive error before the eye using trial lenses and measuring the instrument’s output for each of these lens powers. The defocus calibration factor depends on several parameters, including the contrast and luminance response curve of the photorefraction video system (i.e. camera, video digitizer and LED light source), the eye’s pupil size, lower- and higher-order wavefront aberrations, etc [see Blade and Candy and Bharadwaj et al for the complete list]. Recently, Bharadwaj et al made a preliminary observation that the defocus calibration factor might also depend on the ethnic origin of the population tested. Using a custom-designed photorefactor, they observed the defocus calibration factor of Indian eyes to be statistically significantly higher than those of North American eyes. That is to say, unit change in the eye’s dioptric power resulted in a larger change in the photorefraction luminance slope in the former group than in the latter. Qualitative differences in the photorefraction luminance profiles for visible light across one Asian and one Caucasian volunteer have also been reported by Chen et al.
These results, if held true, have significant implications for the accuracy of refractive power estimated using photorefraction across people of different ethnic origin. The universal defocus calibration factor used in commercial photorefractors is typically obtained from one population (typically, Caucasian) and it is applied to the raw data of all populations indiscriminately. Systematic errors may therefore arise in the refractive power estimates of different populations – refractive power will be over-estimated for populations with calibration factor greater than the population average value while it will be under-estimated for those with calibration factor lesser than the population average value. The inaccuracy in refractive error estimates will also scale with the magnitude of refractive error due to the multiplicative nature of the defocus calibration factor.

Several issues related to the ethnic dependency of this defocus calibration factor remain unanswered from the preliminary observations of Bharadwaj et al. First, the data on the two populations in their study were obtained in different locations by different set of investigators and using two different IR cameras. The observed ethnic difference in calibration factors could therefore, at least in part, be due to subtle unintended variability in the experimental protocol (e.g. variations in equipment design, vertex distance of trial lenses used). Second, defocus calibration factor of other ethnicities were not investigated. Third, the study was performed using a custom-designed photorefractor and the possibility of commercial photorefractors exhibiting similar trends was not explored. Experiment I of the current study addressed all these issues by determining the defocus calibration factor of the PlusOptix PowerRef 3™ photorefractor in subjects of European and North American Caucasian (n = 31; 19 to 52 yrs; mean ± 1SD age: 31.8 ± 9.3 yrs), East Asian (n = 30; 19 to 35 yrs; 22.0 ± 4.3 yrs), African (n = 30; 18 to 50 yrs; 29.7 ± 6.1 yrs) and Indian (n = 41; 19 to 27 yrs; 23.2 ± 2.4 yrs) origin in a single location with a single set of investigators. Experiment II determined the impact of such an ethnicity-dependent difference in defocus calibration factor on the accuracy of myopia recorded by this photorefractor in a separate group of Indian eyes (n = 50; 20 to 34 yrs; 25.2 ± 2.8 yrs), vis-à-vis, gold-standard retinoscopy.

Defocus calibration factor obtained in this study is all relative to the default proprietary calibration of the PowerRef 3™ photorefractor. It is therefore a unitless quantity. A value of unity indicates that the calibration factor estimated here equaled the default calibration of the instrument – i.e. there was no over- or under-estimation of the subject’s refractive error. Calibration values greater than or lesser than unity indicate an under-estimation or over-estimation of the subject’s refractive error, respectively. Based on the preliminary findings of Bharadwaj et al., the defocus calibration factor obtained in Experiment I was hypothesized to be close to unity for Caucasian eyes (i.e. eyes for which the PowerRef 3™ is inherently calibrated) and greater than unity for Indian eyes. Such hypotheses could not be generated for African and East-Asian eyes due to lack of preliminary data.

**Results**

All statistical analyses were performed using Matlab®, Microsoft Excel® and SPSS®. Figure 1 shows the raw data of the defocus calibration function obtained from one representative Indian subject in Experiment I. The data showed a linear change in the left eye’s refraction (i.e. eye before which the trial lenses were placed) and no systematic change in the right eye’s refraction over the entire range of induced trial lens powers (Figure 1). The anisometropia (right eye refraction – left eye refraction) also therefore changed linearly over the range of induced lens powers (Figure 1). The defocus calibration factor (see Methods for details) was greater than unity for this subject (1.45), indicating that the PowerRef 3™ over-estimated the calibration slope by ~45% (Figure 1). This was generally the trend for most subjects of Indian, African and East-Asian origin, as discussed below in detail.

![Figure 1](link)

Figure 1 | Raw data of the defocus calibration protocol obtained from a representative Indian subject. Abscissa shows the range of trial lenses used to induce anisometropia while the ordinate shows the refractive error recorded by the PowerRef 3™ for each induced lens power. The diagonal black line indicates the ideal line with unity slope. The solid blue line indicates the best-fit linear regression line to the data of output anisometropia versus induced lens power. Error bars show ±95% confidence intervals.

Defocus calibration factors of the four ethnic groups were not normally distributed according to the Kolmogorov-Smirnov test. Median [25th–75th inter-quartile range (IQR)] defocus calibration factor of Caucasian, East Asian, African and Indian eyes were 1.13 (IQR: 1.05–1.31), 1.40 (IQR: 1.20–1.70), 1.43 (IQR: 1.25–1.68) and 1.55 (IQR: 1.41–1.73), respectively (Figure 2). Data of 3 caucasian subjects were outliers while there were not outliers in other ethnic groups. Non-parametric Kruskal-Wallis test indicated that there was a statistically significant difference in the defocus calibration factor across different ethnic groups [χ²(3) = 26.92, p < 0.0001]. Mann-Whitney U test (with appropriate Bonferroni correction) confirmed that the calibration factors of Indian (U = 195, z = -4.92, p < 0.0001), African (U = 203, z = 3.77, p = 0.0002) and East Asian (U = 680.5, z = -3.1, p = 0.0019) eyes were statistically significantly larger than those of Caucasian eyes while the data from the three groups were not statistically significantly different from each other (U ≥ 453, z ≤ 1.16, p ≥ 0.08 for all). The median calibration factor however showed an overall increase in the following order: Caucasian, East Asian, African and Indian eyes (Figure 2). There was also significant inter-subject variability in the defocus calibration factor within each ethnic group, as has been observed previously for the MCS PowerRefractor™ and a custom-designed photorefractor (Figure 2).

The slope of photorefraction luminance profile formed across the pupil tends to saturate with an increase in the refractive state of the eye. This implies that subjects with steeper defocus calibration slopes (i.e. those in whom the luminance profile slope changes more rapidly per diopter of induced refractive error) may reach saturation sooner than their counterparts with more flatter calibration slopes. The effective linear operating range of the photorefractor [+5D of hyperopia to −7D of myopia (12D operating range) for the PowerRef 3™, as per the manufacturer’s recommendation] may therefore decrease with an increase in the value of the defocus calibration factor. In other words, the defocus calibration function may saturate at smaller values of induced lens power with an increase in the slope of the calibration function (Figure 1). Data of the 132...
subjects who participated in Experiment I was further analyzed to determine if this hypothesis was true for the range of trial lenses induced in this study (1.5D to 2.5D; 10D operating range). The defocus calibration function of all these subjects was independently visually inspected by the three authors of this study after the subjects were masked for their ethnic origin and defocus calibration factor value. Each examiner indicated if the calibration function showed a saturation and, if so, at what dioptric value did this begin in hyperopic and myopic direction. The results of all three examiners were then pooled together and only subjects where there was a majority consensus (i.e. 2 out of 3 examiners indicated the same result) on the presence/absence of saturation and the dioptric value of the saturation was considered for this analysis. Of the 132 subjects examined, data from 110 subjects met the consensus criterion. Twenty-one out of 110 subjects did not show any saturation (i.e. they had the entire 10D operating range) while 33, 33, 18 and 5 out of 110 subjects had 9D, 8D, 7D and 6D operating range, respectively. The median linear operating range of the PowerRef 3TM reduced from 10D to 7D across the entire calibration slope range (0.63 to 2.13) of this cohort and the operating range was moderately but statistically significantly negatively correlated with the defocus calibration slope of this cohort (Spearman’s rank correlation; \( r = -0.37; p < 0.001 \)).

Figure 3 shows the impact of an error in the defocus calibration factor on the accuracy of refraction estimated by the PowerRef 3TM in Experiment II. Figure 3A shows a scatter diagram of the magnitude of myopia estimated by PowerRef 3TM using its default proprietary defocus calibration factor plotted against myopia estimated using retinoscopy in a cohort of Indian eyes. Orthogonal linear regression fit to this data indicated a slope of 1.64 (±0.07) and y-intercepts were −0.05D (±0.89D) and −0.06D (±0.10D) for panels B and C, respectively. Similar ANOVA analysis with post hoc test indicated that the slopes of the re-scaled data were not significantly different from unity (\( p > 0.9 \) for both), indicating that the over-estimation of myopia was reduced to a statistically insignificant level when the PowerRef 3TM recordings were re-scaled using the Indian eye’s average defocus calibration factor and the individual’s own defocus calibration factor, both obtained from the first experiment, respectively. Orthogonal linear regression slopes were 1.06 (±0.07) and 1.07 (±0.09) and y-intercepts were −0.00D (±0.08D) and −0.00D (±0.10D) for panels B and C, respectively. Similar ANOVA analysis with post hoc test indicated that the slopes of the re-scaled data were not significantly different from unity (\( p > 0.9 \) for both), indicating that the over-estimation of myopia was reduced to a statistically insignificant level when the PowerRef 3TM recordings were re-scaled using the Indian eye’s average defocus calibration factor and the individual’s own defocus calibration factor. The constant offset error was also nearly eliminated following data re-scaling.

The data obtained in the second experiment were also analyzed using Bland-Altman analyses to systematically understand the relation between the mean and the difference in refractive errors estimated by the two techniques (Figure 3D–F). As expected, the results
showed a linear relation between the mean and difference in refractive error estimates (y = -0.99x + 0.52; r² = 0.64; p < 0.001) when using the generic calibration factor of the PowerRef 3™ (Figure 3D). This linear relation was eliminated when the PowerRef 3™ data were rescaled using the Indian eye's population-average and individual's own calibration factor (y = -0.04x + 0.06; r² = 0.02; p = 0.83) (Figure 3E) or using the individual defocus calibration factor (y = -0.04x + 0.09; r² = 0.03; p = 0.84) (Figure 3F). The mean difference ±95% limits of agreement for panels E (0.17 ± 1.31D) and F (0.22 ± 1.51D) were also very similar to each other, indicating that rescaling of the PowerRef 3™ values using the Indian eye's population-average and individual's own calibration factor, respectively. The solid red line in (panel D) show the best-fit orthogonal linear regression line plotted to the data. The solid and dashed red lines in (panels E and F) show the mean difference and ±95% limits of agreement, respectively.

Figure 3 | Myopia of Indian eyes estimated using PowerRef 3™ plotted against those obtained using retinoscopy. (Panel A) shows data obtained using the instrument’s default calibration factor while (panels B and C) show data obtained by rescaling the PowerRef 3™ values using the Indian eye’s population-average and individual’s own calibration factor, respectively. The diagonal black line in each panel indicates equal refractive error estimates recorded by both instruments. The solid red line in each panel shows the best-fit orthogonal linear regression line plotted to the data while the dashed curves around the best-fit line show the ±95% confidence interval of the linear regression fit. (Panels D–F) show Bland-Altman type plots of the difference in refractive error estimates obtained from PowerRef 3™ and retinoscopy plotted as a function of the mean refractive error estimates obtained from the two techniques. (Panels D) shows data obtained using the instrument’s default calibration factor while (panels E and F) show data obtained by rescaling the PowerRef 3™ values using the Indian eye’s population-average and individual’s own calibration factor, respectively. The solid red line in (panel D) show the best-fit orthogonal linear regression line plotted to the data. The solid and dashed red lines in (panels E and F) show the mean difference and ±95% limits of agreement, respectively.

Discussion
This study documented variations in the defocus calibration factor of photorefraction across individuals of four different ethnicities and determined the impact of such a difference on the accuracy of myopia recorded using this technique. This is the first systematic study of its kind, producing results that have important implications for the utility of photorefraction as a technique for measuring refractive errors in the population. The present study confirms the preliminary findings of Bharadwaj et al who found mean defocus calibration factor of Indian and Caucasian eyes to be 0.65 Ls/D (luminance slope per diopter) and 0.40 Ls/D, respectively, using their custom-
Indian eyes were over-estimated by study (Figure 3). Relative to retinoscopy, the calibration slope of SCIENTIFIC REPORTS designed photorefractor14. If their photorefractor were to be calibrated for Caucasian eyes, then a given change in luminance slope across the pupil would be interpreted as a larger dioptric value in Indian than in Caucasian eyes. This would lead to an over-estimation of the calibration slope by ~62.5% in Indian eyes, relative to Caucasian eyes (0.65 Ls/D ÷ 0.4 Ls/D = 1.625 or 62.5%) in the previous study. The over-estimation of slope in Experiment I of the current study will be ~37.2%, with the median defocus calibration factor being larger in Indian eyes (1.55) than in Caucasian eyes (1.13) (1.55 ÷ 1.13 = 1.372 or 37.2%) (Figure 2). The smaller magnitude of over-estimation in the present study could be partly related to differences in the subject pool in the two studies or because of the larger than unity median defocus calibration factor of Caucasian eyes (1.13), relative to the default calibration of the PowerRef 3™ (Figure 2). If the difference of the Caucasian values from unity were to be ignored, then the over-estimation of Indian eyes slope by the PowerRef 3™, relative to its default calibration, becomes ~55% (1.55 ÷ 1.0 = 1.55 or 55%; closer to what was observed in the previous study14). Alternatively, the defocus calibration value of 1.13 obtained in Caucasian eyes may reflect an inherent over-estimation bias induced by the calibration protocol used in the study. True over-estimation of slope in Indian eyes by the PowerRef 3™ may therefore only be ~37%, as indicated by the earlier analysis. Over-estimation of slope in East-Asian and African eyes is expected to be ~23.8% (1.40 ÷ 1.13 = 1.238 or 23.8%) and ~26.5% (1.43 ÷ 1.13 = 1.265 or 26.5%), respectively, following the same aforementioned analysis. Overall, these results indicate that ethnicity-dependent difference in defocus calibration factor is not unique to a specific type/brand of photorefractor but it may be inherent to the technique of photorefraction itself.

The implications of these results for refraction estimated using the PowerRef 3™ were evident from the results of Experiment II of this study (Figure 3). Relative to retinoscopy, the calibration slope of Indian eyes were over-estimated by ~64% by the PowerRef 3™ when using its in-built defocus calibration factor (Figure 3A). This value was very close to the ~62.5% over-estimation of refractive error predicted from the previous study by Bharadwaj et al14. This over-estimation of refractive error by PowerRef 3™ refers strictly to only that induced by the slope of the linear regression line – it ignores any effect of the non-zero y-intercept. The total over-estimation of refractive error in dipters would, however, be a combination of errors induced because of the slope and y-intercept of the linear regression line. The regression line shown in Figure 3A had a slope of 1.64 and a hyperopic y-intercept of +0.73D, indicating that the over-estimation of refractive error by the PowerRef 3™ was somewhat lesser than what was predicted by the slope value alone (e.g. over-estimation using the combination of slope and y-intercept will be 0.6D and 1.8D for 2D and 4D of myopia, respectively, while it will be 1.3D and 2.6D for the same two refractive errors using the slope value alone). Bland-Altman analysis confirmed this over-estimation by revealing a systematic linear relation between the mean and the difference in refractive errors estimated by the two techniques (Figure 3D). Although not tested, similar over-estimation of refractive errors may exist for hyperopic refractive errors and for other ethnic groups that participated in Experiment I. Over-estimation of hyperopia using the protocol employed here can be tricky due to fluctuations in the natural accommodative state of the eye. The eye may need to be cyclopeged if such an endeavour is to be pursued in the future. The fundamental advantages of photorefraction in rapidly estimating the eye’s refractive error from a remote distance is therefore severely undermined because of such systematic errors induced by the defocus calibration factor. Caution must be exercised in interpreting the refraction values recorded by this technique in populations for which the instrument is not calibrated. Photorefractors that are currently available in the market are typically calibrated for Caucasian eyes and they will need to be recalibrated if they are to be used successfully in other ethnic groups.

Over-estimation of refractive error was nearly eliminated when the data were re-scaled using the Indian eye’s average calibration factor or using the individual’s own calibration factor, as indicated by the near-unity slope and near-zero y-intercept of the linear regression lines in Figure 3B & C. The small percentage of over-estimation seen following the re-scaling process (6% and 7% for the two strategies, respectively) was not significantly different from unity (i.e. no over-estimation) and they may have arisen from the inherent inter- and intra-subject variability of retinoscopy and photorefraction techniques13,14,16. Bland-Altman analyses further indicated that the difference in refractive error estimates did not vary significantly with the mean refractive error following the two re-scaling strategies and that the mean ± 95% limits of agreement were also similar in the two

Figure 4 | Scatter plot of the defocus calibration factor of each subject plotted against their respective pupil diameter (panel A) and grayscale iris intensity (panel B). Data from different ethnic groups are indicated in different colors.
strategies (Figure 3E & F). These results indicate that the accuracy of refractive error estimates obtained using PowerRef 3™ in Indian eyes can be restored by recalibrating the data using one of the two aforementioned strategies. Interestingly, both strategies resulted in similar levels of accuracy restoration, suggesting that the easier of the two may be followed when collecting data with this instrument (Figure 3B & C). Re-scaling of data using the second strategy may not be practical as it is not possible to determine the defocus calibration factor of each individual due to time constraints and subject cooperation41. The population-specific average defocus calibration factor may be used instead to re-scale the data using the PowerRef 3™. Similar results may be expected for other ethnic groups as well, although it was not directly tested here. The average defocus calibration factors for different ethnic groups may therefore be built into the instrument’s algorithm and can be selected by the user depending on the ethnic origin of the subject being tested. The results of both rescaling strategies being similar is somewhat surprising as we intuitively expected the second rescaling strategy (i.e. using the individual’s own defocus calibration factor) to produce more accurate refraction estimates than the first rescaling strategy (i.e. rescaling data using the Indian eye’s average defocus calibration factor). This intuitions was because the second strategy accounted for the inter-subject variability in the defocus calibration factor while the first strategy did not (Figure 2). The defocus calibration routine employed here is however limited in its repeatability (i.e. the technique shows intra-subject variability in addition to inter-subject variability41,42, which may prevent the first strategy from achieving greater levels of accuracy in refraction relative to the second strategy. Subjects fixated relatively stably and with minimal fluctuation of refractive state during the data collection process of the second experiment, suggesting that instability of gaze position and accommodation added minimal variability to data recorded in this study.

A second implication of the results of Experiment I is with regards to the linear operating range of PowerRef 3™, as was observed from the second analysis of this data. The linear operating range of PowerRef 3™ tended to decrease with an increase in the defocus calibration slope of the subject, indicating that, like refractive accuracy, the effective operating range of the instrument may also be dependent on the defocus calibration factor of the subject. By extension, it may also be expected that the linear operating range of PowerRef 3™ may overall be smaller for Indian and African eyes with steeper defocus calibration slopes than for their Caucasian counterparts. Such an ethnicity-dependent difference in the linear operating range was however not observed in the current data, perhaps due to the large inter-subject variability in the data within a given ethnic group.

Four factors that might account for the observed inter-ethnic differences in defocus calibration factor of photorefractor could be eliminated. The individual’s pupil diameter and IR iris reflectance (as a surrogate for IR reflectance of the choroid) appear to play only a limited role in determining the defocus calibration factor of photorefractor as inferred from the poor correlation between these factors and the corresponding defocus calibration factor (Figure 3). Poor correlation between the defocus calibration factor and pupil diameter is in line with the previous observation of Bharadwaj et al in a custom-designed photorefractor41. Poor correlation between the defocus calibration factor and iris reflectance is also somewhat unexpected from the results of Delori and Pillbings26 and Elser et al43 who noted similar levels of fundal reflectance of IR light in people with a wide range of eye colors using a scanning laser ophthalmoscope—that is quite different in technology from eccentric infrared photorefraction. Minimal role of IR fundal reflectance in determining the defocus calibration factor is also indirectly supported by the accuracy of IR-light based autorefractors and automated optometers in determining the eye’s refractive power across various ethnicities, vis-à-vis, retinoscopy22,23. These devices typically use reflected IR light in a Scheiner-disk principle or using a grating focus principle to determine the eye’s refractive power, unlike the photorefractor that utilizes the luminance profile of IR light formed across the entire pupil for this purpose1. The current study was limited in that the fundal reflex was not directly estimated but it was only indirectly inferred from the reflectance of the iris. It is therefore possible that a direct measure of fundal reflectance is indeed correlated with the subject’s defocus calibration factor. This remains to be tested in future studies. Schaeffel et al also observed a large inter-subject variability in the defocus calibration factor, which was significantly correlated with the brightness of the IR fundal reflex and pupil size of the subject27. Accordingly, they suggested normalizing the photorefractor luminance profile to the average brightness of the fundal reflex before deriving the defocus calibration factor27. Such a normalization factor is already built into the software algorithm of PowerRef 3™ (as confirmed by the manufacturer) and, therefore, as suggested by Schaeffel et al27, any inter-subject difference in the defocus calibration factor may arise from factors other than fundal reflectivity. The four ethnic cohorts that participated in this study were also roughly age-matched and hence the subject’s age could not account for the observed ethnic difference in the defocus calibration factor average. The defocus calibration factor of photorefractor has anyhow been shown to poorly correlate with the subject’s age in previous studies27. Finally, any difference in the biometry of the human eye across different ethnicities (e.g. differences in curvature and refractive index of the cornea and lens, anterior chamber depth, retinal thickness) would only induce a constant dioptric offset and not a multiplicative error in refractive power estimates as observed in the present results24,25. Other factors that might play a role in the observed results include ethnic differences in the higher-order monochromatic and polychromatic wavefront aberrations and scattering properties of the eye. Suryakumar et al19 have previously observed local non-linearities in the luminance profile slope due to monochromatic wavefront aberrations of the eye. Perhaps these local non-linearities influence the way in which the luminance slope across the pupil is calculated, thereby having an impact on the defocus calibration slope. No such obvious non-linearities in the luminance profile was observed in this study while visually inspecting the pupils during data collection. However, the PowerRef 3™ does not provide access to the raw data and hence the role of any subtle ethnicity-specific non-linearities in the luminance slope cannot be ruled out. Recently, Nischler et al26 and Teel et al27 have observed differences in ocular scattering with eye color, and therefore with ethnicities. How this might relate to differences in defocus calibration slopes observed across different ethnicities in this study needs further investigation. Overall, the reason for differences in defocus calibration factor across different ethnicities remains elusive even though some of the relatively straightforward factors were eliminated in this study. Further experiments therefore appear necessary to fully understand this phenomenon.

Methods

Volunteers of Caucasian, African and Indian origin were recruited for the study from the staff, students and visitors of the L V Prasad Eye Institute (LVPEI), Hyderabad. Volunteers of East-Asian origin were recruited from Manipal College of Allied Health Sciences (MCOAHS), Manipal University, Karnataka. Different set of subjects participated in the two experiments of this study. All subjects in the first experiment were emmetropic or were corrected to emmetropia using soft contact lenses. All subjects in the second experiment were restricted to within range in order to remain within the linear operating zone of the PowerRef 3™ photorefractor (+5.0D to –7.0D)38. Uncorrected astigmatism and uncorrected anisometropia were ±0.5D in all subjects in both experiments. None of the subjects reported to have any ocular or systemic abnormality and their best-corrected visual acuity was 20/20 or better in both eyes. Subjects with history of inter-ethnicity marriage of parents or grandparents (based on self-report), strabismus, <3.5 mm pupil diameter (minimum diameter required for the PowerRef 3™ to collect data) and excessive blinking were excluded from the study. A sample size calculation, based on the preliminary data obtained from Bharadwaj et al (2013)44, with a study power of 80% and Type-I error of 5% indicated that a minimum of 25
volunteers were required to show a statistically significant difference in the defocus calibration factor between Indian and Caucasian eyes. Similar sample size calculation could not be performed for African and East-Asian eyes due to lack of preliminary data. Accordingly, 30–41 volunteers were recruited in each ethnic group for this study (see introduction section for details of subject recruitment). In total, 189 volunteers were screened for both experiments and 182 volunteers were inducted into the study. All procedures were performed in accordance with the tenets of the declaration of Helsinki. All subjects signed an informed consent form that was duly approved by the Institutional Review Board of LVPEI and the Institutional Research Committee of MOCAHS.

The overall design and functioning of the PowerRef 3™ (PlusOptix, Germany) is very similar to its predecessors (Multichannel Systems PowerRefractor™ and PlusOptixPowerRefPower III)13,14,15. Details of the MCS PowerRefractor™ can be found in previous publications13,14. Briefly, the PowerRef 3™ consists of nine IR LED’s (850 nm) arranged in a trapezoidal fashion, ~4 mm above the camera aperture for measuring refractive power of the eye along the vertical meridian; unlike its predecessors, the PowerRef 3™ does not measure refractive error in other meridians)14,15. Refractive power is recorded simultaneously from both eyes at 50 Hz (20 ms per acquisition). The automatic gain control function of the phoroe折射or camera was switched off and the gain was manually set to 50% for all participants during data collection, independent of their ethnic origin. Left and right gaze positions and pupil diameters of both eyes are also recorded simultaneously at the same sampling rate by tracking the 1st Purkinje image and by using standard grayscale-intensity based image processing algorithms, respectively16,17.

In Experiment I, subjects fixated on the PowerRef 3™ camera positioned in between the two eyes at 1 m viewing distance with their right eye while the left eye was occluded using an IR transmitting filter (Opticat filter Edmund Optics™, NT43-954) in an otherwise dark adapted environment. Trial lenses (from +3D to −5D) were placed before the occluded left eye in 1D steps at 10–14 mm vertex distance for ~5 s each. No lens was placed before the right eye. 2 s of stable data within this epoch was averaged in both eyes and the right eye refractions were subtracted from the left eye refractions to obtain the induced anisometropia. This anisometropia was plotted against the induced lens power and a linear regression line was fit to the linear portion of the data using custom-written Matlab® software, the slope of which gave the defocus calibration factor (Figure 1). The effective power of the trial lenses for the aforementioned vertex distance was not incorporated into the analysis as the difference between the actual power and the effective power of the lenses would be small for the range of lens powers used and, even if it was incorporated, it would not induce any differential effect in the results obtained across different ethnicities. Overall, this technique minimized the impact of ocular accommodation on the calibration measurements and permitted calibration without cycloplegia and mydriasis. Even if accommodation changed in one eye, there would be a compensational change in the fellow eye that would help in maintaining the interocular difference in refraction22,23. In reality, refraction of the uncovered eye varied very little during the experiment indicating relatively stable accommodation.

The y-intercept of the aforementioned linear regression line gives the baseline anisometropia experienced by the subject without any induced lenses. The y-intercept was close to zero diopters in all subjects, confirming that the subjects had any baseline uncorrected anisometropia in the first experiment. The slope of a similar linear regression line to the left eye data (i.e. eye before which trial lenses are placed) will estimate the change in output refraction recorded by the PowerRef 3™ per diopter change in induced lens power while the y-intercept of this line will estimate the differences in output refraction of the left eye with respect to the photorefractor camera. The slope of the left eye’s linear regression fit is expected to match the slope values obtained from the anisometropia plot (i.e. Figure 1) as the fellow right eye’s refraction remains constant, whereas the y-intercept of this regression line was also close to zero diopters in all four ethnic groups [Caucasian: +0.08D (−0.11D to 0.27D); East Asian: +0.07D (+0.34D to +0.74D); African: +0.27D (−0.07D to 0.76D); Indian: −0.01D (−0.37D to 0.35D)], indicating that the subject’s eye was more or less optically conjugate to the plane of the photorefractor camera. Since the main focus of this study was to report changes in defocus calibration slopes across ethnicities, no further analysis of the y-intercept values was performed. Even if the y-intercept values varied across subjects or ethnicities, they should only create a constant offset in the data and not have any bearing on the defocus calibration slopes reported in this study.

In Experiment II, refractive error was measured simultaneously using the PowerRef 3™ and streak retinoscopy (Heine Beta 200™) while subject’s binocularly fixated on a 2/00-sized target at 3 m viewing distance. Retinoscopy was performed by one experienced optometrist in the vertical meridian at a constant working distance of 1 m on the right eye of all subjects. Neutralizing lenses were briefly placed on a trial frame worn by the subject to maintain constant vertex distance. Refractive error was noted as the lens power for which the retinoscopy reflex changed from ‘with’ to ‘against’ movement or vice versa. The optometrist was masked to the results of the PowerRef 3™ at all times during the experiment. Data were obtained using the PowerRef 3™ for a total of 20 s and 10 s worth of data free of blink and other artifacts was averaged from this recording to obtain the refractive error of the subject. Gaze positions were obtained from the PowerRef 3™-worn camera (optometrist). Comparison of the techniques of videorefraction and static retinoscopy in the measurement of refractive error in infants. Ophthalmic Physiol Opt 14, 20–24 (1994).
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**Acknowledgments**

The authors would like to thank all subjects who participated in the study. The authors would also like to thank Dr. Ramesh Venkatassubbu and Mr. Avinash Prabhu from Manipal College of Allied Health Sciences, Manipal University, Karnataka for support with data collection and Dr. Ashiq Mohammed for help with statistical analyses. This research was supported by a Fast-Track Grant for Young Scientists scheme, Science and Engineering Research Board, Department of Science and Technology, Govt. of India to SB.

**Author contributions**

G.S. and V.K.N. conducted the experiment and analyzed the data. S.B. analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

**Additional information**

Competing financial interests: The authors declare no competing financial interests.

**How to cite this article:** Sravani, N.G., Nilagiri, V.K. & Bharadwaj, S.R. Photorefraction estimates of refractive power varies with the ethnic origin of human eyes. *Sci. Rep.* **5**, 7976; DOI:10.1038/srep07976 (2015).

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