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Reproducibility of Macular Pigment Optical Density Measurement by Two-wave Length Auto-fluorescence in a Clinical Setting

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Abstract

Purpose—Macular pigment, composed of lutein, zeaxanthin, and meso-zeaxanthin, is postulated to protect against age-related macular degeneration (AMD), likely due to filtering blue light and its antioxidant properties. Macular pigment optical density (MPOD) is reported to be associated with macular function evaluated by visual acuity and multifocal electroretinogram. Given the importance of macular pigment, reliable and accurate measurement methods are important. The main purpose of current study is to determine the reproducibility of MPOD measurement by two-wave length auto-fluorescence method using scanning laser ophthalmoscopy.

Methods—Sixty eight eyes of 39 persons were enrolled in the study, including 11 normal eyes, 16 eyes with wet AMD, 16 eyes with dry AMD, 11 eyes with macular edema due to diabetic mellitus, branch retinal vein occlusion or macular telangiectasia and 14 eyes with tractional maculopathy including vitreomacular traction, epiretinal membrane or macular hole. MPOD was measured with a two-wavelength (488 and 514 nm) auto-fluorescence method with the Spectralis HRA+OCT after pupil dilation. The measurement was repeated for each eye 10 minutes later. The Analysis of variance (ANOVA) and Bland-Altman plot were used to assess the reproducibility between the two measurements.

Results—The mean MPOD at eccentricities of 1° and 2° was 0.36±0.17 (range: 0.04–0.69) and 0.15±0.08(range: −0.03, 0.35) for the first measurement and 0.35±0.17 (range: 0.02, 0.68) and 0.15±0.08 (range: −0.01, 0.33) for the second measurement respectively. The difference between the two measurements was not statistically significant, and the Bland-Altman plot showed 7.4% and 5.9% points outside the 95% limits of agreement, indicating an overall excellent reproducibility. Similarly, there is no significant difference between the first and second measurements of MPOD volume within eccentricities of 1°, 2° and 6° radius, and the Bland-Altman plot showed 8.8%, 2.9% and 4.4% points outside the 95% limits of agreement respectively. The data for the reproducibility did not differ significantly among the various disease and normal eyes.

Conclusion—Under routine examination conditions with pupil dilation, MPOD measurement by two-wave length auto-fluorescence method showed a high reproducibility.

Keywords

Macular pigment optical density; Age-related macular degeneration; auto fluorescence; vitamins supplementation

Macular pigment, composed of lutein, zeaxanthin, and meso-zeaxanthin, has been shown to enhance visual function in normal subjects, and is postulated to protect against age-related macular degeneration (AMD), which is the leading cause of blindness in economically developed countries, likely through filtering blue light and its antioxidant properties[1–5]. Several reports show that the macular pigment level is associated with macular function evaluated by visual acuity, contrast sensitivity and multifocal electroretinogram[6–8]. Given the importance of macular pigment, it is clearly needed to measure it reliably and accurately in clinical practice.

A variety of methods have been used to measure macular pigment levels in clinical or research settings, which can be classified into subjective psychophysical techniques or objective optical techniques[9–10]. The subjective psychophysical techniques available include color matching motion photometry and heterochromatic flicker photometry (HFP). Among them, HFP is well known and widely used, for which there are instruments commercially available. However, previous studies showed that commercially available heterochromatic flicker photometers for macular pigment optical density assessment in clinical settings demonstrated poor coefficients of repeatability, which limit their application in clinical practice[9]. Optical techniques currently used for measuring MP include resonance Raman spectroscopy, fundus reflectance, and fundus auto-fluorescence. Dual wavelength auto fluorescencemethod has shown a concordance with HFP[11,12]. Recently, this technique for measurement of MPOD has been incorporated into Spectralis HRA+OCT (Heideberg Engineer, Heideberg, Germany), a common clinically used instruments. There is a paucity of data on reproducibility of MPOD measurement by dual wavelength auto-fluorescence (AF) with Spectralis HRA+OCT. It is the main purpose of current study to determine the repeatability of MPOD measurement by dual wavelength auto-fluorescence with Spectralis HRA+OCT in a clinical setting.

Methods

The study was approved by the Institute Review Board of University of California San Diego and was conducted in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained. Thirty-nine consecutive patients referred to Jacobs retina center, Shiley Eye Institute, University of California San Diego during October to December were examined. Those with severe cataract or poor fixation due to severe macular lesion were excluded. Pupils of all the participants were dilated with 2.5% phenylephrine and 0.5% tropicamide to ≥6 mm in diameter. MPOD was measured with a two-wavelength (488 and 514 nm) autofluorescence method with Spectralis HRA+OCT after pupil dilation as part of clinically indicated Scanning Laser Ophthalmoscopy imaging for disease or to rule out retinal pathology. During imaging, the subject was asked to put his/her chin on chin rest and forehead against an upper bar to keep their head still, and he/she was instructed to fixate on an internal fixation target. Initial camera alignment, illumination and focus were done in

infrared (IR) mode. Once the image was evenly illuminated and in good focus, the camera mode was switched to simultaneous blue AF and green AF imaging (BAF + GAF) mode for MPOD measurement. An additional adjustment to illumination and focus was performed to ensure optimal image quality, and then a 30 second video was recorded. Subsequently, a raster optical coherence tomography (OCT) scanning centered on the foveola and covering 30°×30° posterior pole was performed for clinical purpose. The measurement of MPOD was repeated for each eye after 10 minutes.

To simulate a second day measurement, we measured the right eye first then left eye as part of the first session. The patient also had other imaging performed as clinically indicated and was allowed to rest, move and walk to another area as needed. The second measurements were then performed. Both the focus and alignment were changed between the two measurements due to the refraction difference of the right and left eye and in between sessions. The two measurements were done by the same operator.

Heidelberg Eye Explorer software (HEYEX, version 1.7.1.0) was used to align and average the images in the videos, and an MP density map is created. Due to the variation in ocular transmission between people it is not possible to calculate an absolute calibration value for MPOD. Therefore, we have used the region of the macula known to be free of macular pigment as in-image normal control. All MPOD values are relationship to this normal control value. For analysis, the plateau (equivalent to the reference point) was set to 6-degree eccentricity automatically. The average MPOD at 1 and 2 degree radii and sum of MPOD volume corresponding to the eccentricities of 1-, 2- and 6-degree radii were recorded (Fig.1). Statistical analyses were conducted using SAS software version 9.4 (SAS Institutes, Cary, North Carolina, USA). The ANOVA and Bland-Altman plot were used to assess the reproducibility between the two measurements. P-values represent results for 2-sided tests, with values less than 0.05 considered statistically significant.

Results

Sixty eight eyes of 39 persons were enrolled in the study, including 11 normal eyes, 16 eyes with wet AMD, 16 eyes with dry AMD, 11 eyes with macular edema due to diabetic mellitus, branch retinal vein occlusion or macular telangiectasia and 14 eyes with tractional maculopathy including vitreomacular traction and epiretinal membrane. Control normal eyes recruited eyes without previous disease history and with best corrected visual acuity ≥ 20/20, intraocular pressure ≥ 10 mmHg and ≤ 21 mmHg, without any abnormality found on dilated fundus examination and OCT scanning. The mean age of the participants was 71.9±13.4 years (range: 34–99 years), and 57% of them were men. The demographic characteristics of the participants stratified by clinical diagnosis were summarized in table 1.

The mean MPOD at eccentricities of 1° and 2° radius was 0.36±0.17 (range: 0.04–0.69) and 0.15±0.08(range: −0.03, 0.35)for the first measurement and 0.35±0.17 (range: 0.02, 0.68), 0.15±0.08 (range: −0.01, 0.33) for second measurement respectively. The mean difference between the two measurements was −0.01 and 0.00 for the two eccentricities respectively. The difference between the first and second measurement was not statistically significant

(P=0.81 and 0.26 respectively), and the Bland-Altman plot showed 7.4% and 5.9% points outside the 95% limits of agreement. (Table 2)

Similarly, the difference between the two measurements of sum of MPOD volume within eccentricities of 1°,2° and 6° radius was not statistically significant (P=0.50, 0.43 and 0.62 respectively), and the Bland-Altman plot showed 8.8%, 2.9% and 4.4% points outside the 95% limits of agreement, indicating an overall excellent reproducibility (Fig.2–4).

The data for the reproducibility did not differ significantly among the various disease and normal eyes. (Table 2)

Discussion

Macular pigments play an important role in protecting the retina via filtering blue light and against oxidative stress[13]. It was reported that higher levels of macular pigments were associated with lower risk of AMD[14,15]. The retina itself cannot synthesize macular pigments, and supplementation of lutein and zeaxanthin is the only method to increase the macular pigment level[16]. It has been reported that reduced intake of lutein or zeaxanthin might be associated with an increased risk of AMD[5,17,18]. The Age-related Eye Disease Study (AREDS) group reported that dietary lutein/ zeaxanthin intake was inversely associated with the incidence of neovascular AMD; the incidence was lower in the high-carotenoid intake group than in the low-carotenoid intake group.[19] Furthermore, the AREDS 2 study has discovered that micronutrient supplementation with lutein/zeaxathin together with multivitamins and zinc can suppress the rate of AMD progression[20]. Given the importance of macular pigment, in recent years, the interest in measuring MPOD has increased. To better evaluate the supplementation effect, an objective, simple, and reliable method is needed to evaluate macular pigment levels in clinical practice. Several methods have been proposed for measuring MPOD. However, to date, there is no clear gold standard method for measuring MPOD[9,10]. A key issue for the clinical applicability of any method is its repeatability. The finding reported herein showed in a clinical setting, under routine imaging examination conditions with pupil dilation, MPOD measurement by two-wave length auto-fluorescence method had a high reproducibility, both for normal eyes and for eyes with macular diseases. Although the accuracy of the measurement is yet to be determined, the high reproducibility suggested that this objective and simple method could be reliable in monitoring macular pigment levels in clinical practice. In terms accuracy, the only definite accurate way to measure macular pigment level is to be measured chemically in vitro, which is not clinically relevant.

Previous studies on young healthy subjects with normal eyes showed MPOD measurement by dual-wavelength auto-fluorescence had an overall good intra-session and inter-session repeatability[21,22]. And several studies have shown good agreement between dual-wave length auto-fluorescence technique andpsychophysical techniques such as heterochromatic flickerphotometry[12] and motion photometry[23]. Very recently the dual-wavelength module for measuring MPOD was integrated into the Heidelberg Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany), but few studies have evaluated this new device. It has been used in evaluating MPOD in early AMD[24] and macular telangiectasia type 2[25,26]. Our

study evaluating this new device confirmed previously reported good repeatability of the method. Furthermore, our study showed that high repeatability is not only true for young healthy normal eyes, but also for eyes with macular diseases such as AMD in old age patients. The high repeatability of MPOD measurement by dual-wavelength auto-fluorescence found in diseased retina is clinically much more relevant than that for young healthy eyes.

Limitations of current study should be mentioned. Firstly, we didn't validate the accuracy of the MPOD measurement by dual-wavelength auto-fluorescence. However, due to the lack of an in vivo gold standard method, any validation of accuracy of measuring MPOD by any method will be indefinite. Second, we did not perform any serum carotenoid measurements to assess the relation between serum carotenoids and macular pigment measurement. However, the purpose was not to evaluate the relation between MPOD and serum carotenoid, but rather to determine the reliability of the method. The strength of the current study is that we showed an excellent repeatability of MPOD measurement with a recently available module in a widely used device in an actual clinical setting on population of older patients with macular diseases.

In conclusion, under routine examination conditions with pupil dilation, MPOD measurement by two-wave length auto-fluorescence method showed a high reproducibility in a clinical setting.

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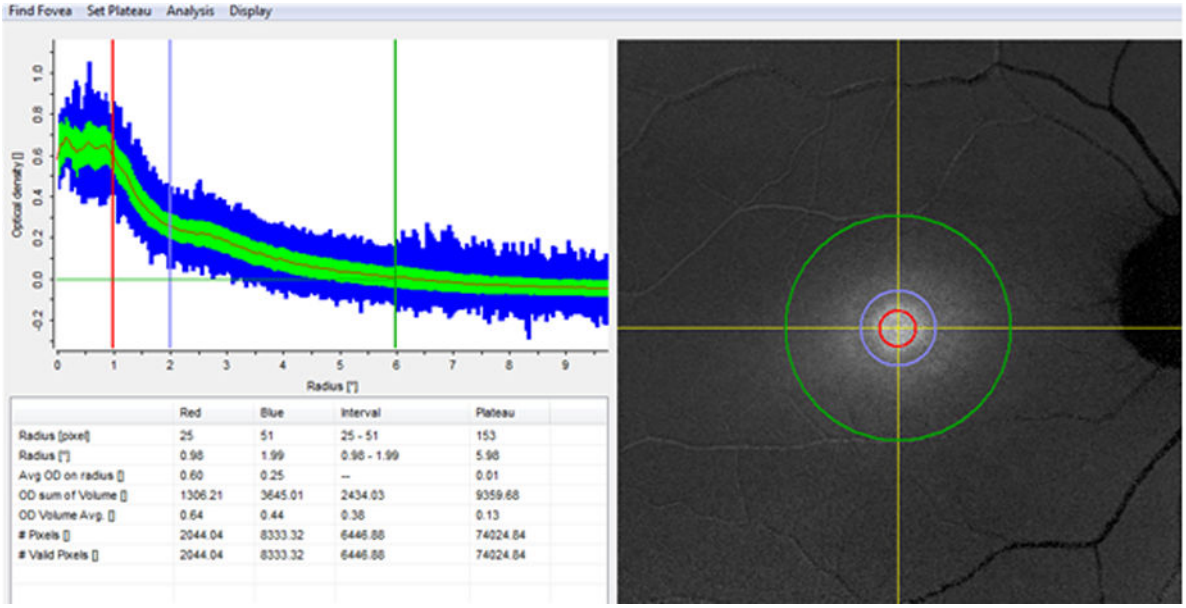

Figure 1.

A snap shot of the macular pigment optical density analyzed by Heidelberg Eye Explorer software.The red, purple and green circle is corresponding to the eccentricities of 1-, 2- and 6-degree radii.

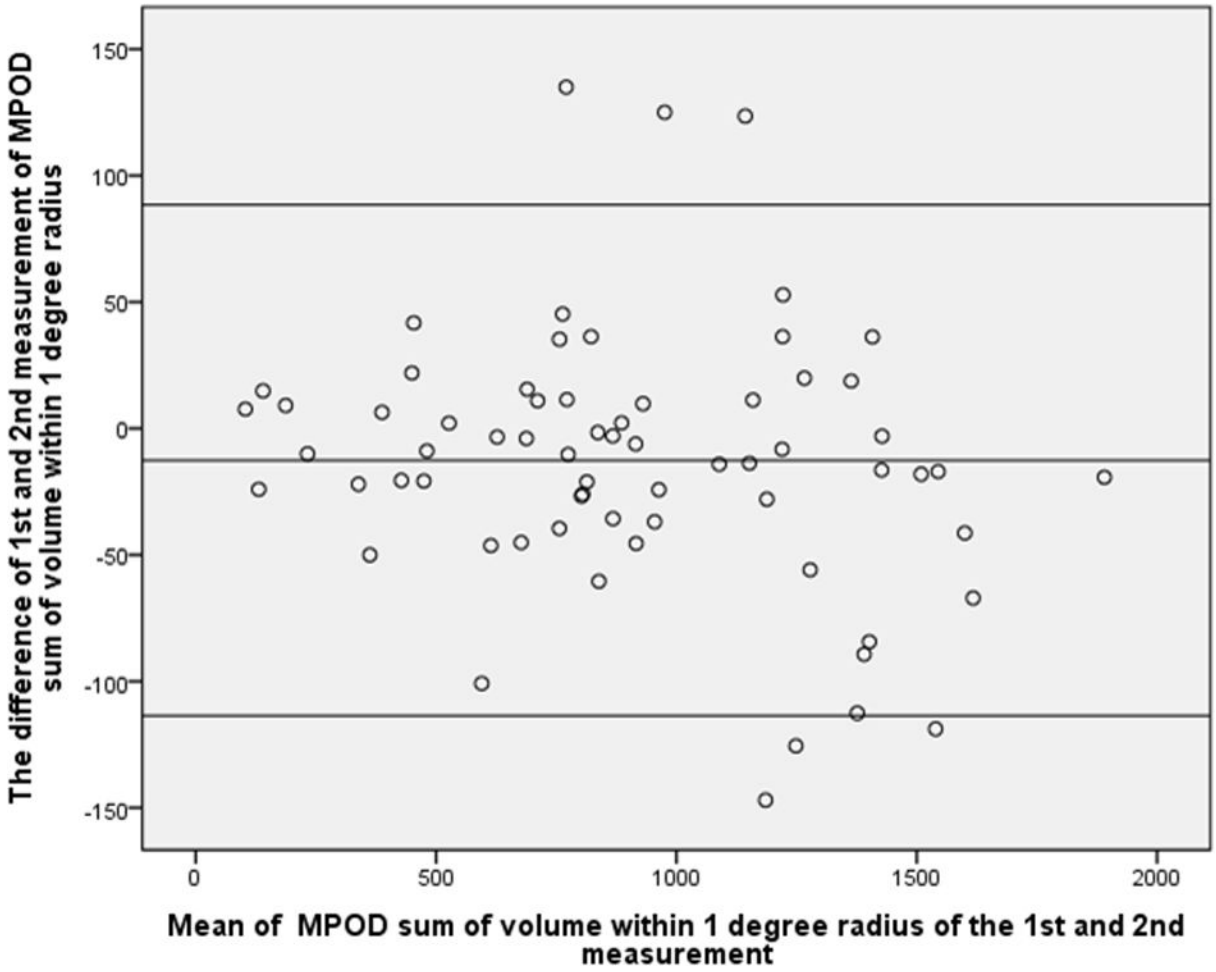

Figure 2.

Bland-Altman plot of MPOD within 1-degree radius of the 1st and 2nd measurement. X-axial defined as mean of the MPOD volume sum of the 1st and 2nd measurement. Y-axial defined as the MPOD value of the first measurement minus the value of second measurement. The mean differences and the 95% confidence limits of the bias are shown as three lines.

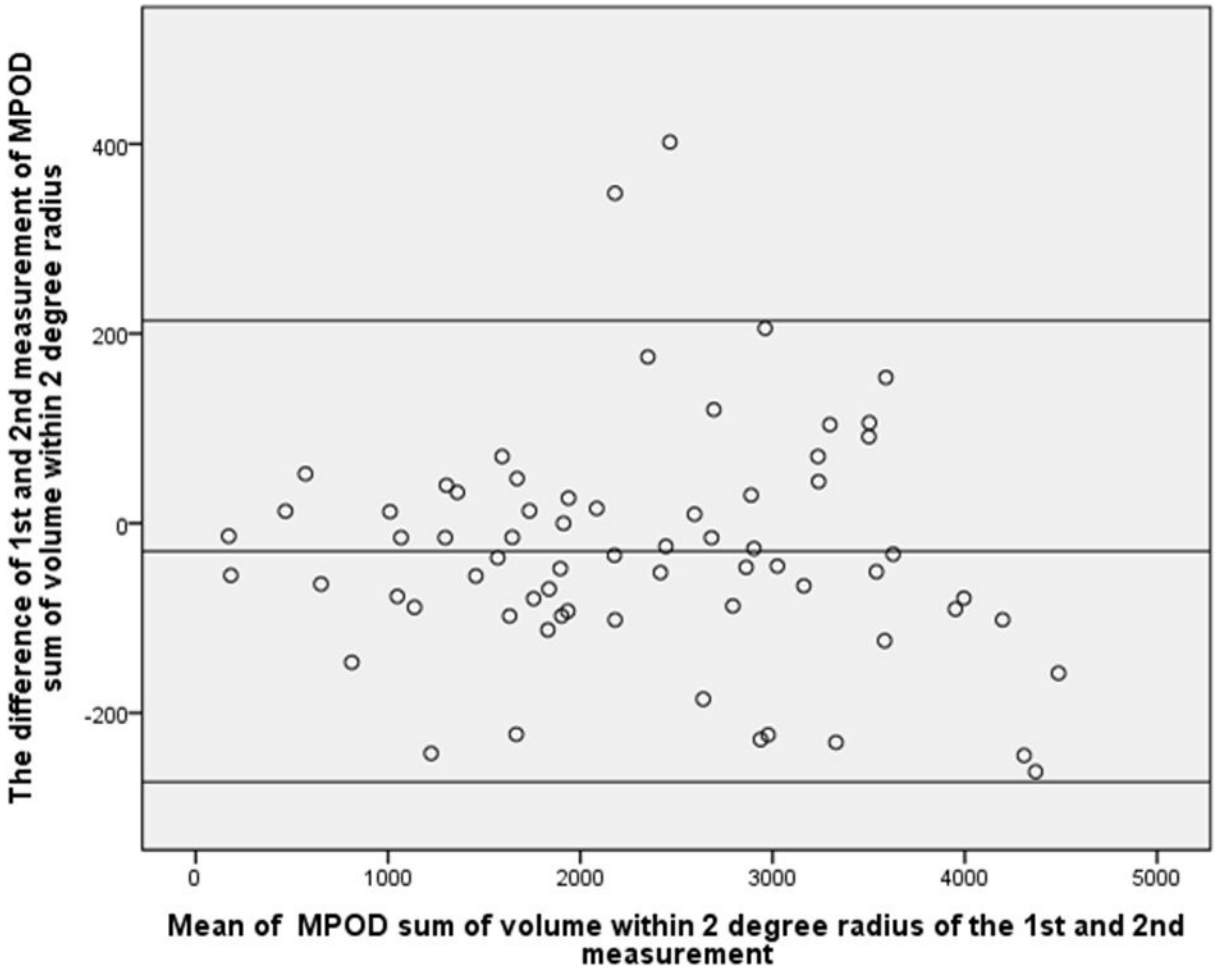

Figure 3.

Bland-Altman plot of MPOD within 2-degree radius of the 1st and 2nd measurement. X-axial defined as mean of the MPOD volume sum of the 1st and 2nd measurement. Y-axial defined as the MPOD value of the first measurement minus the value of second measurement. The mean differences and the 95% confidence limits of the bias are shown as three lines.

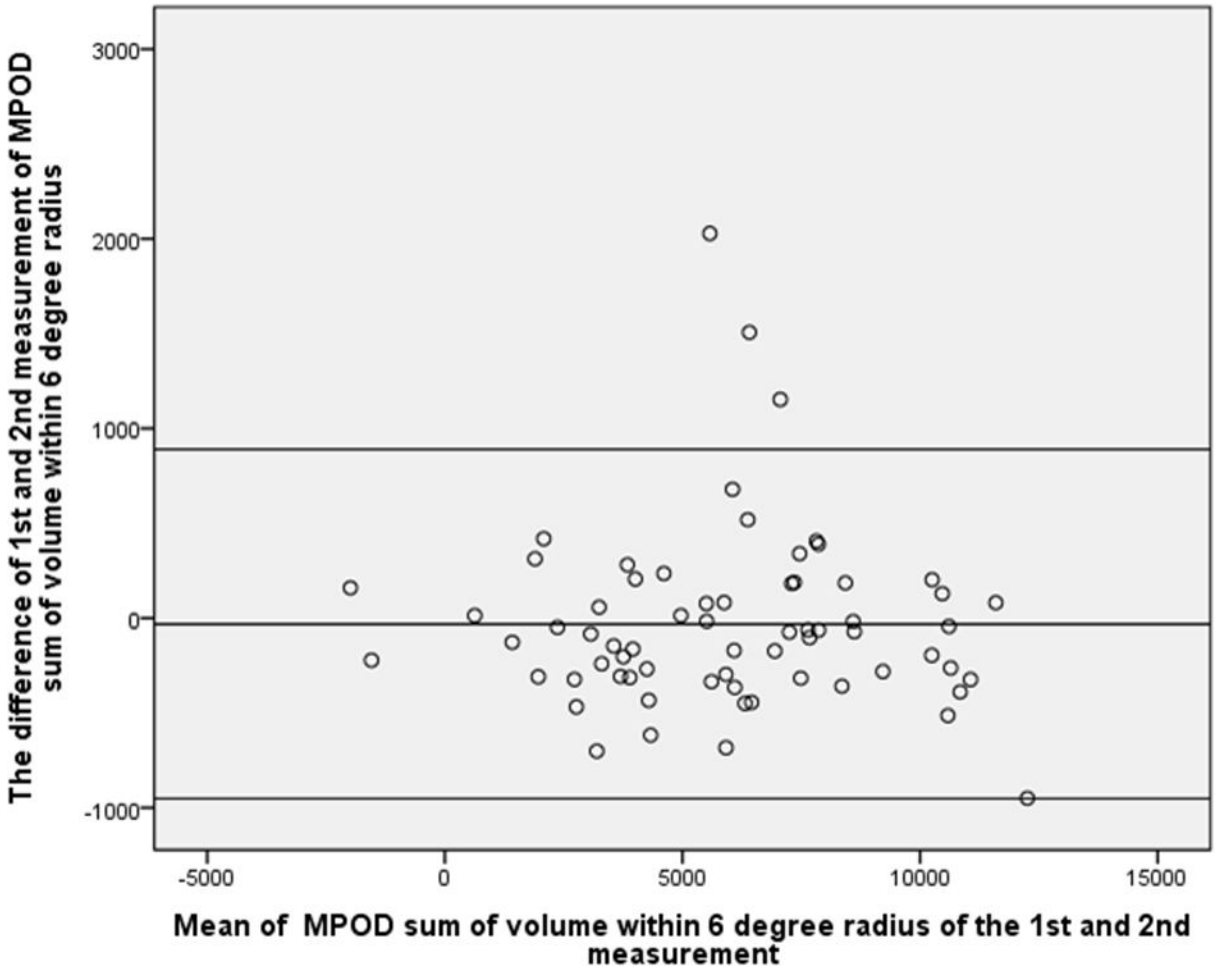

Figure 4.

Bland-Altman plot of MPOD within 6-degree radius of the 1st and 2nd measurement. X-axial defined as mean of the MPOD volume sum of the 1st and 2nd measurement. Y-axial defined as the MPOD value of the first measurement minus the value of second measurement. The mean differences and the 95% confidence limits of the bias are shown as three lines.

Table 1

The demographic characteristics of study participants.

Macular diseases	n	age	gender
		mean± SD (range)	male/female
Within normal limit	11	63.4±14.9 (34–77)	5/6
Wet AMD	16	83.3±9.2 (72–99)	10/6
Dry AMD	16	75.6±10.7 (54–89)	7/9
Macular edema due to retinal vessel diseases	11	62.8±13.0 (50–82)	9/2
Tractional maculopathy	14	68.4±8.4 (54–81)	8/6
Combined	68	71.9±13.4 (34–99)	39/29

Table 2

The reproducibility of MPOD measurements stratified by clinical diagnosis.

		N	the first measurement				the second measurement				mean difference of first and second measurement	P value
			Mean	standard deviation	minimum	maximum	Mean	standard deviation	minimum	maximum		
MPOD average at 1 degree radius eccentricity	normal	11	0.42	0.14	0.22	0.67	0.41	0.15	0.18	0.66	−0.01	0.29
	wet AMD	16	0.42	0.19	0.07	0.68	0.41	0.18	0.05	0.63	−0.01	0.28
	dry AMD	16	0.41	0.14	0.19	0.68	0.40	0.14	0.19	0.65	−0.01	0.06
	ME	11	0.30	0.10	0.17	0.44	0.30	0.11	0.15	0.44	0.00	0.91
	TM	14	0.25	0.19	0.04	0.69	0.24	0.19	0.02	0.68	0.00	0.19
	combined	68	0.36	0.17	0.04	0.69	0.35	0.17	0.02	0.68	−0.01	0.81
MPOD average at 2 degree radius eccentricity	normal	11	0.17	0.08	0.06	0.26	0.16	0.08	0.06	0.25	−0.01	0.11
	wet AMD	16	0.17	0.11	−0.03	0.35	0.17	0.10	−0.01	0.33	0.00	0.38
	dry AMD	16	0.15	0.08	0.01	0.34	0.15	0.08	0.02	0.32	0.00	0.38
	ME	11	0.16	0.07	0.07	0.29	0.15	0.07	0.06	0.29	0.00	0.71
	TM	14	0.11	0.06	0.00	0.25	0.10	0.07	0.00	0.26	−0.01	0.04
	combined	68	0.15	0.08	−0.03	0.35	0.15	0.08	−0.01	0.33	0.00	0.27

ME: macular edema due to retinal vascular diseases. TM: tractional maculopathy.