

# Individual Test Point Fluctuations of Macular Sensitivity in Healthy Eyes and Eyes With Age-Related Macular Degeneration Measured With Microperimetry

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**Purpose:** To establish fluctuation limits, it was considered that not only overall macular sensitivity but also fluctuations of individual test points in the macula might have clinical value.

**Methods:** Three repeated measurements of microperimetry were performed using the Standard Expert test of Macular Integrity Assessment (MAIA) in healthy subjects ( $N = 12$ , age =  $23.8 \pm 1.5$  years old) and in patients with age-related macular degeneration (AMD) ( $N = 11$ , age =  $68.5 \pm 7.4$  years old). A total of 37 macular points arranged in four concentric rings and in four quadrants were analyzed individually and in groups.

**Results:** The data show low fluctuation of macular sensitivity of individual test points in healthy subjects (average =  $1.38 \pm 0.28$  dB) and AMD patients (average =  $2.12 \pm 0.60$  dB). Lower sensitivity points are more related to higher fluctuation than to the distance from the central point. Fixation stability showed no effect on the sensitivity fluctuation. The 95th percentile of the standard deviations of healthy subjects was, on average, 2.7 dB, ranging from 1.2 to 4 dB, depending on the point tested.

**Conclusion:** Point analysis and regional analysis might be considered prior to evaluating macular sensitivity fluctuation in order to distinguish between normal variation and a clinical change.

**Translational Relevance:** Statistical methods were used to compare repeated microperimetry measurements and to establish fluctuation limits of the macular sensitivity. This analysis could add information regarding the integrity of different macular areas and provide new insights into fixation points prior to the biofeedback fixation training.

## Introduction

Visual acuity (VA) alone has been generally considered a limited parameter for monitoring macular function since its impairment is usually preceded by neurofunctional alterations that may not initially affect VA.<sup>1</sup> Moreover, parafoveal lesions observed in some patients with normal VA may affect activities in daily life due to the impairment of other visual functions. For instance, age-related macular

degeneration (AMD) patients show macular dysfunction that precedes the reduction of VA.<sup>1,2</sup>

Alternatively, or in combination with VA measurement, clinicians have increasingly used microperimetry to measure macular function.<sup>3–7</sup> The advantage of current commercially available microperimetry instruments is that they allow compensation for eye movements, and therefore a precise measurement of the same retinal location is possible.<sup>8</sup> This compensation certainly reduces the fluctuations

of the test–retest sensitivity previously found in conventional automated perimetry.<sup>9</sup>

Intersession test–retest variability of different summative microperimetric parameters, such as average threshold, fixation stability, and macular integrity, has been recently reported within different populations.<sup>4,10–18</sup> Nevertheless, point-by-point intersession variability using Macular Integrity Assessment (MAIA) microperimetry has not been analyzed. We speculate that point-by-point sensitivity changes could be a more sensitive parameter than the above-mentioned summative parameters, especially in the case of localized dysfunction, as reported by Liu et al.<sup>5</sup>

Previous studies have demonstrated that diseased eyes (or specific macular areas with alteration) may have higher fluctuation in sensitivity across repeated microperimetric tests; however, extrafoveal fixation seems not to affect test–retest repeatability.<sup>19</sup>

In view of the growing importance of microperimetry in diagnosis and follow-up of macular diseases, the normal sensitivity fluctuation should be well-known. This would make it possible to consider an expected sensitivity fluctuation in macular dysfunction when performing biofeedback training for improvement of fixation stability.

Here, we evaluated macular sensitivities in response to repeated measurements for 37 macular points using MAIA microperimetry. The objective of the present study was to quantify fluctuations in sensitivity at different macular points in healthy and AMD eyes.

## Methods

The experiments adhered to the tenets of the Declaration of Helsinki and were approved by the ethics committee of Semmelweis University in Budapest, Hungary (registration number TUKEB 261/2015). Signed informed consent was obtained from each subject after explanation of the nature and possible consequences of the study.

The participants consisted of 23 untrained subjects. Group 1 comprised 12 healthy volunteers ages 21 to 27 ( $23.8 \pm 1.5$ ), and group 2 comprised 11 AMD patients ages 58 to 77 ( $68.5 \pm 7.4$ ). Detailed information about the participants is shown in the [Table](#).

Inclusion criteria were the absence of media opacity and any other known ophthalmic disease. In group 2, an additional inclusion criterion was the diagnosis of nonexudative AMD in both eyes.

Excluded from the study were patients with a history of any other macular disease (epiretinal fibrosis, diabetic maculopathy), exudative AMD, and earlier intravitreal anti-VEGF treatment.

For all participants, both eyes were undilated and monocularly tested three times. The second measurement was performed 1 hour after the first measurement, and the third measurement was performed 1 week later. In the healthy subjects (group 1), the right eye was always examined first. In AMD patients (group 2), the best eye was examined first, followed by the other eye. The same microperimeter system, MAIA (CenterVue, Padova, Italy), was used for all measurements. A single experienced examiner performed all the measurements, and identical instructions were given to each participant at all three examinations.

The system provides real-time eye-tracking through examinations performed using scanning laser ophthalmoscopy (SLO). For a detailed description of this technique, see Rohrschneider et al.<sup>2</sup> The Expert Protocol, used in the present study, consists of 37 macular points tested in three concentric circles; 2°, 6°, and 10° from the center point, with 12 points within each concentric circle (plus the measurement of the central point).

Foveal location was automatically set by the system considering the center of the SLO image captured when the observer was asked to look at the central target (red ring of ~1° of visual angle). The use of this central target may reduce the sensitivity of the central point (0 in the point analysis and ring 1 in the ring analysis) tested as it has been previously reported by Denniss and Astle<sup>20</sup> in normal observers.

Stimuli were set using standard parameters: Goldman-based size III stimuli against a background of 1.27 cd/m<sup>2</sup> for the 4-2 threshold strategy. The duration of the stimulus presentation was 200 milliseconds. The maximum stimulus luminance was 318 cd/m<sup>2</sup>, which allowed a stimulus presentation ranging from 0 to 36 decibels (dB). The observer's task was to press a button to indicate the presence of the light spot whenever it was detected. Visual field locations that required brighter stimuli to reach threshold had reduced sensitivity and had lower dB sensitivity values. Similarly, higher dB values represented more sensitive retinal locations.

The fixation stability index (%) considered the amount of fixation points recorded during the whole test within an area of 1° of eccentricity considering the center of the fixation area.

As shown in [Figure 1](#), we performed the following:

**Table.** Age, Average Threshold, and VA for Each Participant and Each Eye

	Age	Average Threshold		First Eye Tested	VA OD/OS
		First Eye Tested	Second Eye Tested		
<b>Group 1</b>					
1	27	30	30	OD	1.0/1.0
2	25	30	31	OD	1.0/1.0
3	21	32	31	OD	1.0/1.0
4	24	30	31	OD	1.0/1.0
5	25	32	32	OD	1.0/1.0
6	23	30	31	OD	1.0/1.0
7	23	31	31	OD	1.0/1.0
8	24	32	33	OD	1.0/1.0
9	23	28	30	OD	1.0/1.0
10	24	32	32	OD	1.0/1.0
11	24	32	31	OD	1.0/1.0
12	22	31	31	OD	1.0/1.0
Average	23.8	30.6	31.1		
Standard deviation	1.5	1.3	0.8		
<b>Group 2</b>					
1	58	27	12	OS	0.1/0.8
2	66	26	22	OS	0.6/0.8
3	66	24	15	OS	0.6/0.8
4	64	20	16	OD	0.1/0.6
5	82	20	16	OD	0.8/0.1
6	59	27	14	OD	1.0/<0.1
7	66	27	29	OD	0.7/0.8
8	68	26	25	OS	0.4/0.8
9	75	24	21	OD	0.8/0.3
10	72	25	16	OD	0.8/0.3
11	77	19	23	OS	0.3/0.5
Average	68.5	24.0	18.8		
Standard deviation	7.4	3.1	5.3		

(1) point analysis; (2) ring analysis: ring 1 = a single central point, ring 2 = points 1 to 12, ring 3 = points 13 to 24, and ring 4 = points 25 to 36; and (3) quadrant analysis for the superior, inferior, temporal, and nasal quadrants. To establish the expected fluctuation at each point, we calculated the 95th percentile of the individual standard deviations (group 1 = 12 values and group 2 = 11 values for each eye) of the three consecutive measurements. We also calculated the coefficient of variation (COV = standard deviation / average) of the three measurements for each of the 37 points tested. The choice of using COV, in addition to the mean and standard deviation, was the possibility of combining different sensitivity and fluctuation levels to one parameter in

order to verify its distribution as well as its relation to another parameter, for instance, the fixation stability.

Statistical analyses were performed with Excel (Microsoft, Redmond, WA) and with STATISTICA (StatSoft, Tulsa, OK) software. A *t*-test was used to compare results between the eyes and between the groups. The Pearson correlation coefficient was used to evaluate the effect of fixation stability on sensitivity fluctuation.

## Results

The results of the point analysis are shown in [Figure 2](#). The graphs show the average and standard deviation of the perimetric thresholds for each group

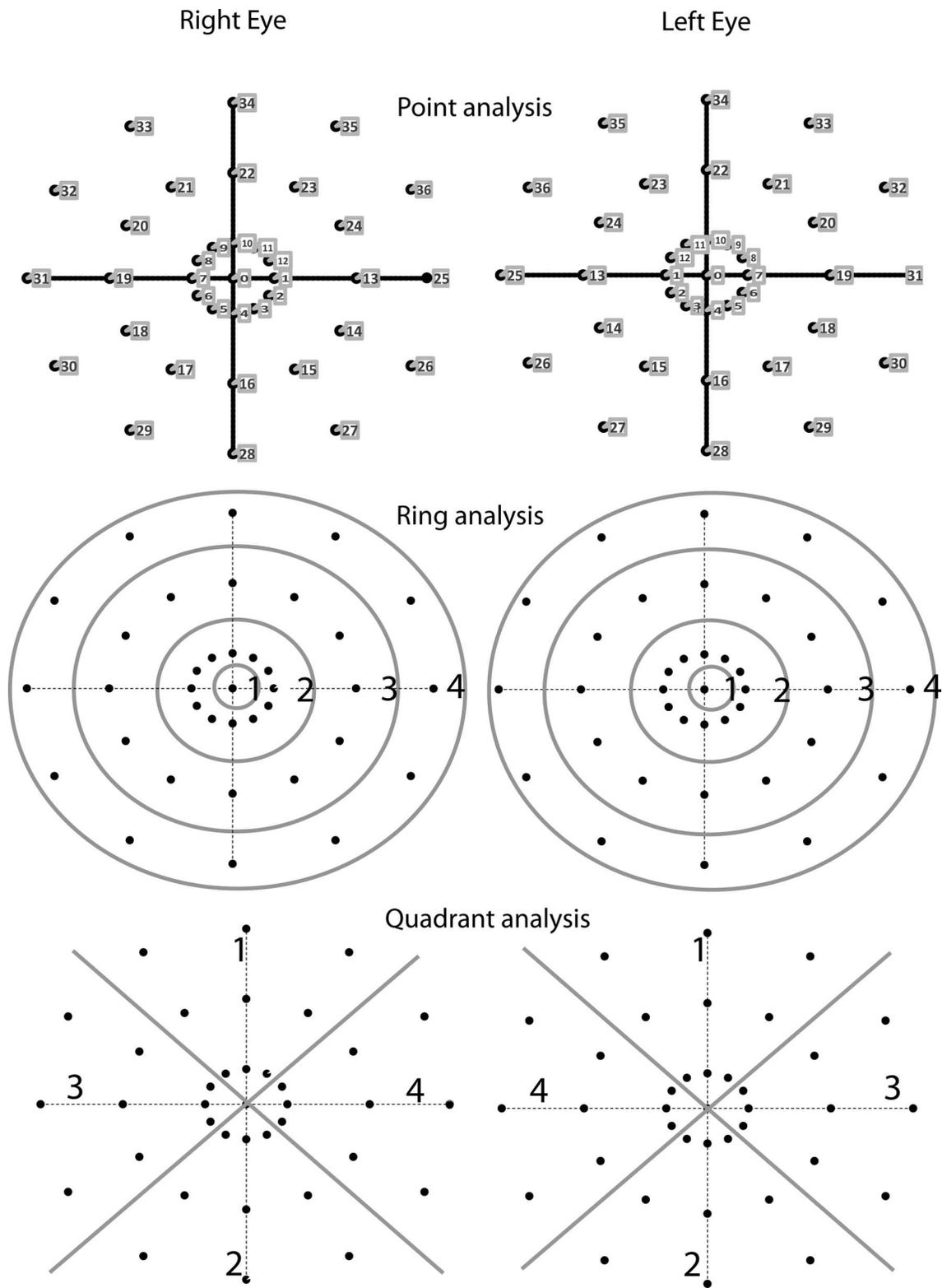
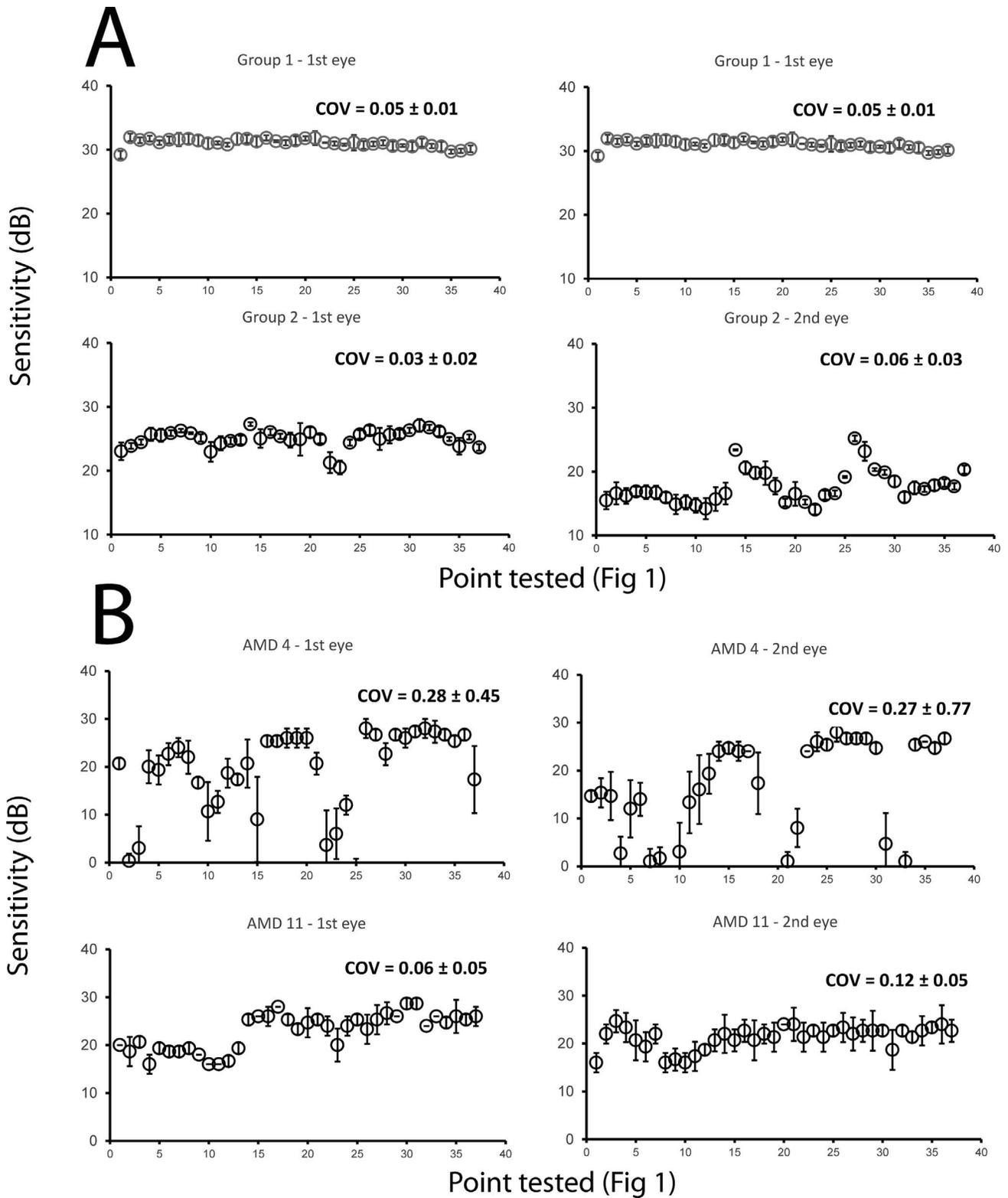


Figure 1. Points included in each of the three analyses performed: (1) point analysis (top), (2) ring analysis (middle), and quadrant analysis (bottom).



**Figure 2.** Control (A, upper graphs; group 1) and AMD (A, lower graphs; group 2) average sensitivity at 37 macular points according to Figure 1. The standard deviation is the variability among the three consecutive measurements. The COVs, calculated from the average and the standard deviation, are displayed above. Averages and standard deviations are constant among the macular points in group 1. B shows the individual results of AMD patients. AMD 4 shows higher standard deviation at the points with lower sensitivity. This was not found in patient AMD 11, who showed relatively constant sensitivities among the macular points.

(Fig. 2A; upper graphs = group 1 and lower graphs = group 2). Group 1 shows, in addition to very similar average sensitivities at all points tested and between the eyes, very small standard deviations (ranging from 0.7 to 2 dB) in both eyes. Group 2 shows standard deviations ranging from 1.3 to 3.9 dB in both eyes.

The COV of the sensitivity values was low for both group 1 (first eye and second eye =  $0.05 \pm 0.01$ ) and group 2 (first eye – better eye =  $0.03 \pm 0.02$  and second eye – worst eye =  $0.06 \pm 0.03$ ), with no significant differences between the eyes and between the groups ( $P > 0.05$ ).

Figure 2B shows the individual results of two subjects from group 2. Observe that AMD 4 shows more points with decreased sensitivity and higher standard deviation compared to AMD 11. Accordingly, the COV of AMD 4 is also higher. In AMD 4, the standard deviation is higher at points with lower sensitivities than at points with higher sensitivities.

Figure 3 shows the ring analysis (Fig. 3A, upper graphs = group 1 and lower graphs = group 2). The average and standard deviation of both groups are relatively constant, and the fluctuation does not seem to be influenced by lower sensitivity areas in the ring analysis. For example, in AMD 3 (Fig. 3B), the second (worse) eye tested showed lower sensitivity in rings 2, 3, and 4, although this did not significantly increase the fluctuation (based on the standard deviation) or the COV in ring 4.

Figure 4 shows the quadrant analysis for both group 1 and group 2 (Fig. 4A). The fluctuation seems to be more influenced by lower sensitivity areas in the quadrant analysis compared to the ring analysis. As was found for individual points, low sensitivity areas also display higher fluctuation than areas with high sensitivity, as shown in AMD 3 (Fig. 4B, upper graphs), compared to AMD 7 (Fig. 4B, lower graphs), which shows higher sensitivities. The COV in the inferior quadrant of AMD 3 ( $\text{COV} = 2.37$ ) was much higher than those of other patients and also higher than other quadrants for the same patient.

Figure 5A shows macular sensitivities scattered considering the 37 points tested. Data are interleaved in first and second eye measured in each of the three sessions (a total of six examinations). For each of the 37 points tested, the average results of group 1 (Fig. 5A, left graph) were constant throughout the six measurements. The average results of group 2 (Fig. 5A; right graph) showed a difference in sensitivity between the eyes, however, no learning effect was observed in either eye. Figure 5B shows the individual results of the sensitivities across all 37 points for each

of the three exams: one subject with lower sensitivity (AMD 10, left graph) and another subject with higher sensitivity (AMD 9, right graph). Note that neither patient showed a learning effect.

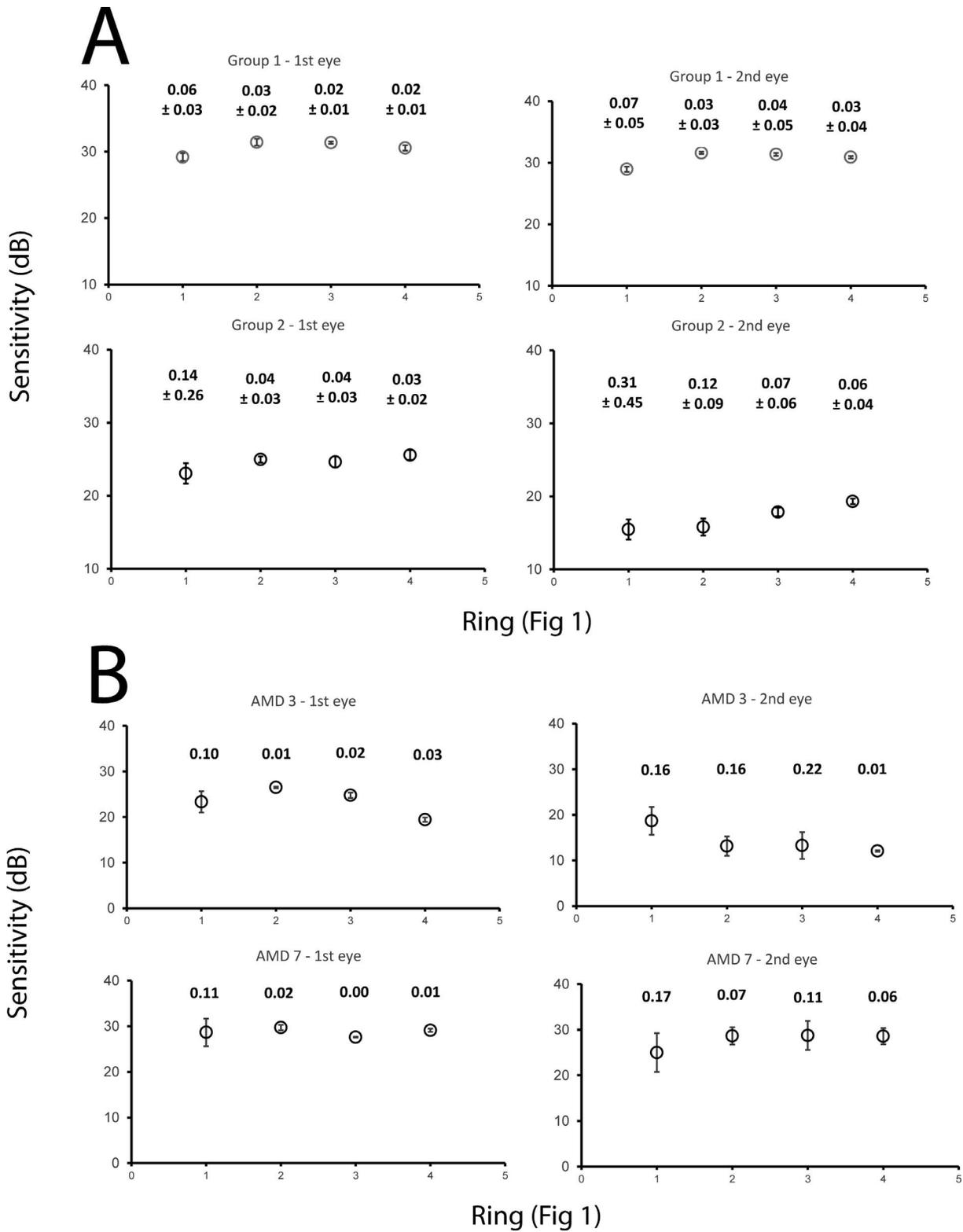
Although there were not significant differences in fixation stability between the eyes of both group 1 and group 2, fixation stability was significantly impaired ( $P = 0.004$  for the first eyes and  $P = 0.010$  for the second eyes tested) in group 2 (first eye =  $76.9 \pm 22.4\%$  and second eye =  $72.7 \pm 30.5\%$ ) compared with group 1 (first eye =  $97.7 \pm 3.2\%$  and second eye =  $97.8 \pm 2.2\%$ ). However, Figure 5C shows that there was no correlation between fixation stability and the average COV of the sensitivity values in group 1 ( $R = -0.20$ ;  $P = 0.30$ ) as well as in group 2 ( $R = -0.22$ ;  $P = 0.28$ ).

Finally, we calculated the 95th percentile based on the standard deviation of the three consecutive measurements. The goal of this analysis was to display a variation limit for fluctuation at each macular point tested. Figure 6 shows the variation limit at each point for the first and second eyes tested in group 1 (upper graphs) and group 2 (middle graphs). The variation limit was, on average,  $2.7 (\pm 0.6)$  dB, ranging from 1.2 to 4.0 dB depending on the point tested in group 1. Among the AMD patients (group 2), the variation ranged from 2.0 to 9.0 dB, or  $4.6 (\pm 1.6)$  dB on average among the 37 points analyzed.

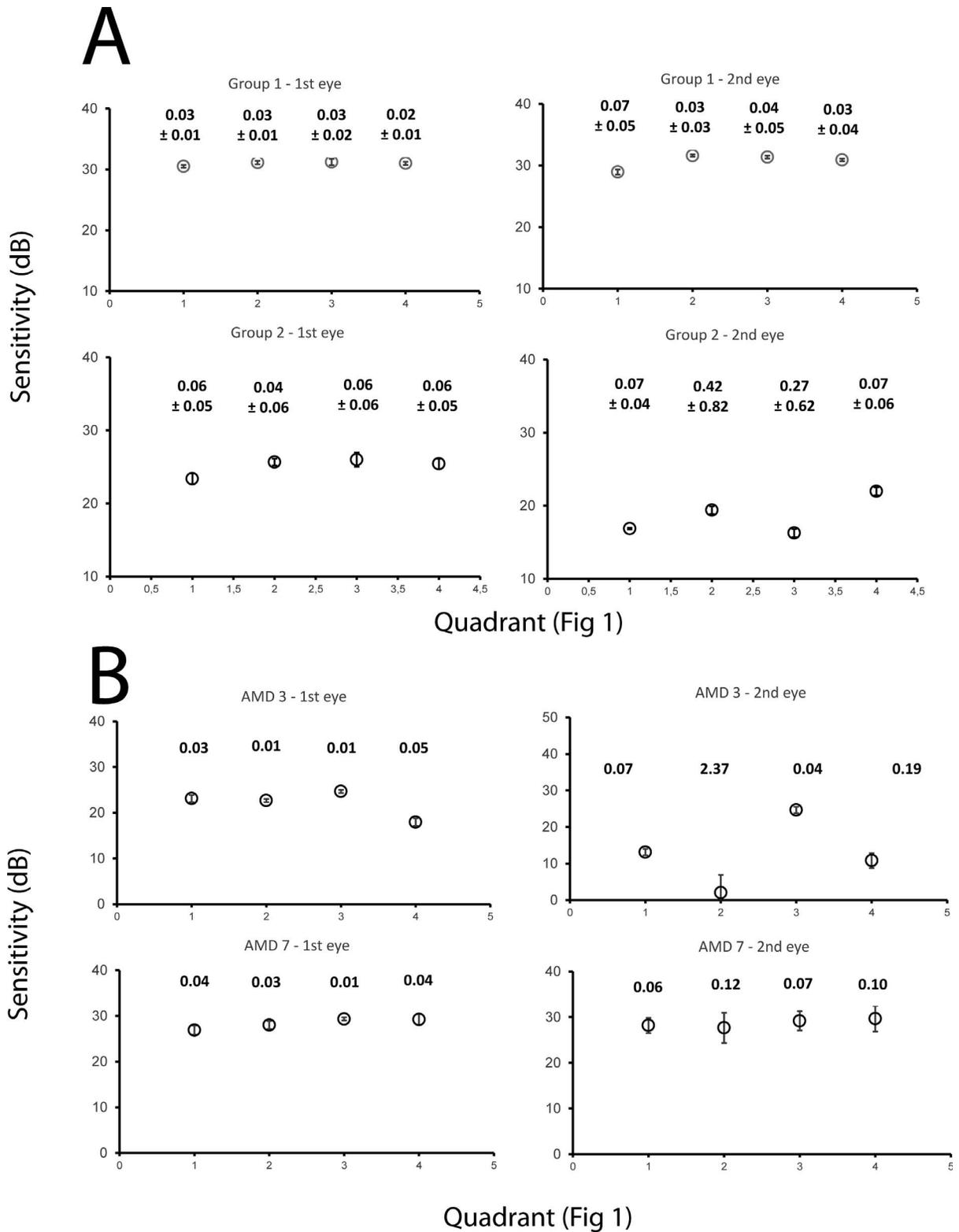
The variation limits calculated for the groups did not allow us to consider spared areas versus dysfunctional areas in AMD patients. Therefore, we also show the individual variation of AMD 3 (lower graphs). Note that the inferior quadrant in which we had previously shown lower sensitivities (see Fig. 4B, AMD 3, second eye) displayed higher fluctuation up to 13.3 dB, higher than the variation limit for the AMD group. On the other hand, other points displayed fluctuation within normal limits found in the healthy subjects.

## Discussion

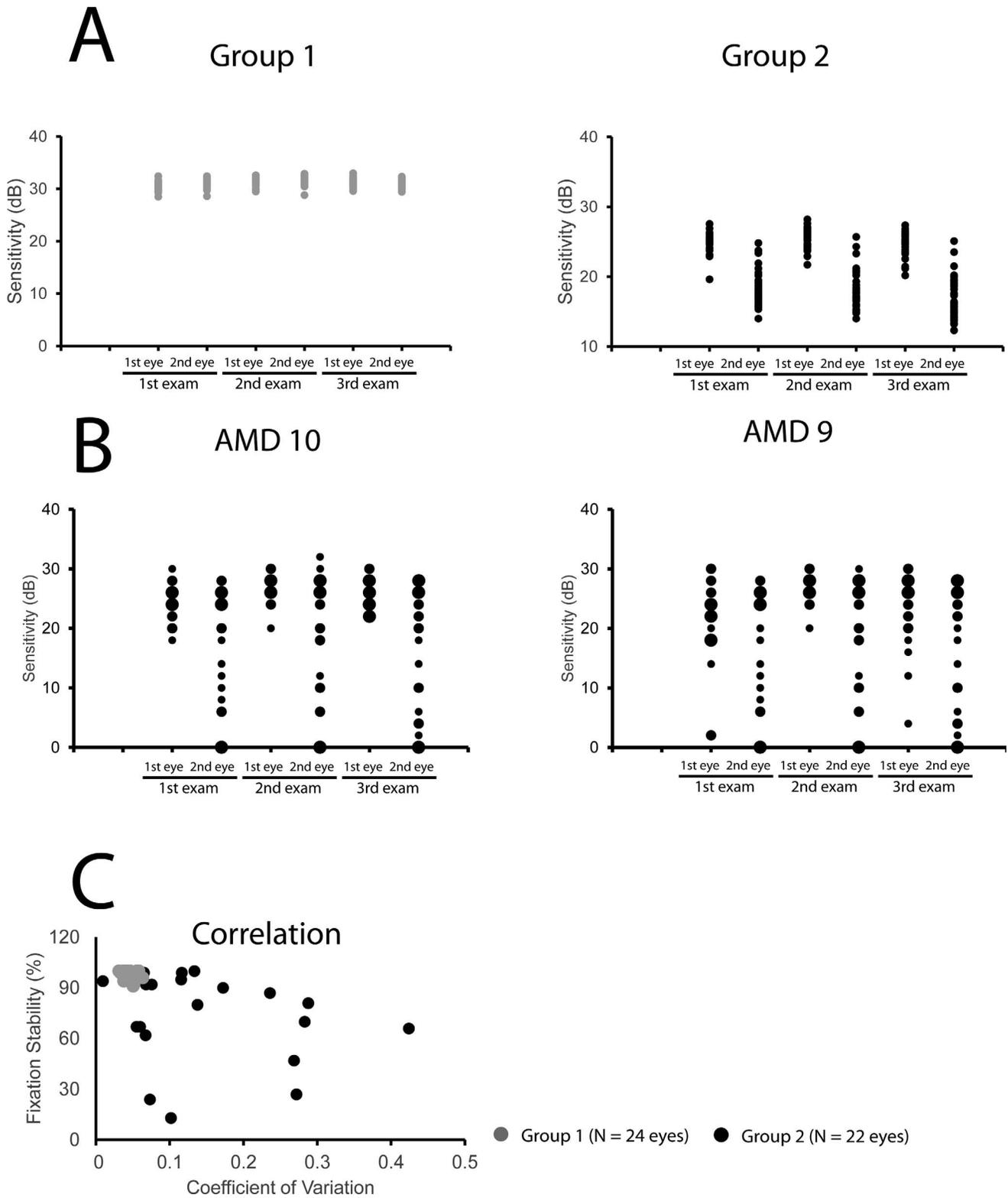
Previous summative findings<sup>4,5,7,10–18</sup> showed small fluctuations in macular sensitivity as measured with microperimetry. The present results provide new insights regarding point-by-point and regional fluctuation in healthy subjects and in patients with macular dysfunction. Macular sensitivity of healthy subjects shows an intersession fluctuation of less than 2 dB (average =  $1.38 \pm 0.28$  dB) with similar variation (ranging from 0.71 to 2.02 dB) at all 37 macular points tested. It is possible that well-controlled examinations (with a single examiner and identical instructions) along with real-time monitoring and



**Figure 3.** Control (A, upper graphs; group 1) and AMD (A, lower graphs; group 2) fluctuation of sensitivity at four rings according to Figure 1. The standard deviation is the variability among the three consecutive measurements. The COVs, calculated from the average and the standard deviation, are displayed above. Averages and standard deviations are constant among the rings in group 1. B shows the individual results of AMD patients. AMD 3 and AMD 7 show similar fluctuation.



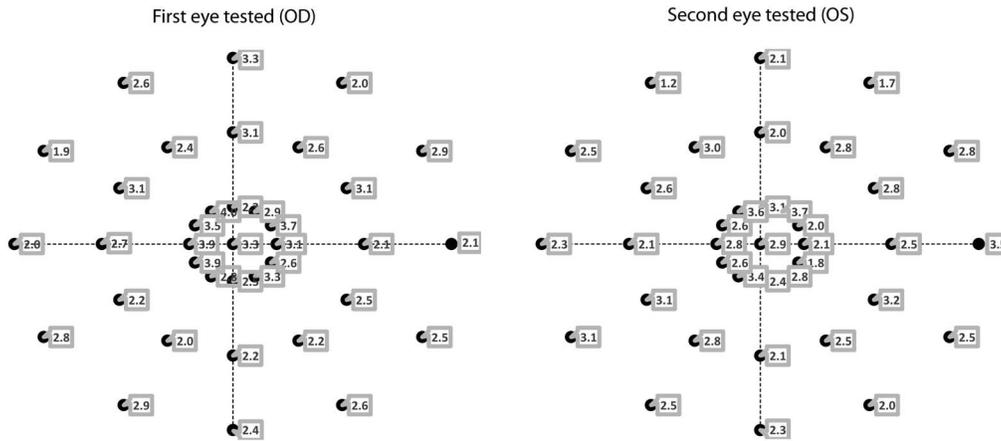
**Figure 4.** Control (A, upper graphs; group 1) and AMD (A, lower graphs; group 2) sensitivity at four quadrants (1 = superior; 2 = inferior; 3 = temporal; and 4 = nasal, according to Fig. 1). The standard deviation is the variability among the three consecutive measurements. The COVs, calculated from the average and the standard deviation, are displayed above. Averages and standard deviations are relatively constant among the quadrants in group 1. B shows the individual results of AMD patients. AMD 3 shows higher fluctuation for the areas with lower sensitivity. AMD 7 shows similar variability.



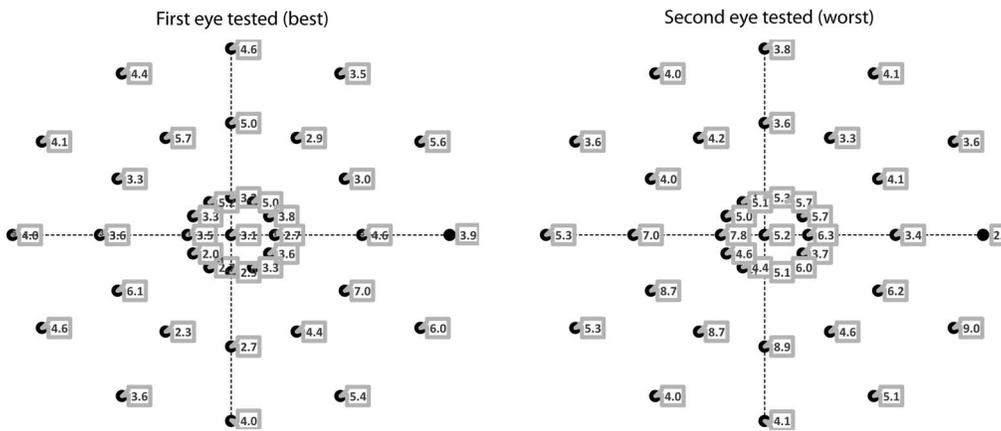
**Figure 5.** Sensitivity at the 37 points measured at each examination: first, second, and third examination for both the first eye tested and second eye tested (six examinations in total). A shows the group sensitivities for both group 1 (*left panel*) and group 2 (*right panel*). B shows the individual sensitivities of AMD patients over the six consecutive examinations. Observe that several individual points overlap. The size of the symbol represents the amount of points (small symbols = single result; medium symbol = two to five results; big symbols = more than five results) overlapping. We did not observe a learning effect in both groups. C shows the correlation between the fixation stability and the COV. In both, group 1 (*grey dots*) and group 2 (*black dots*), there was no significant correlation.

Variation limits (95% percentile)

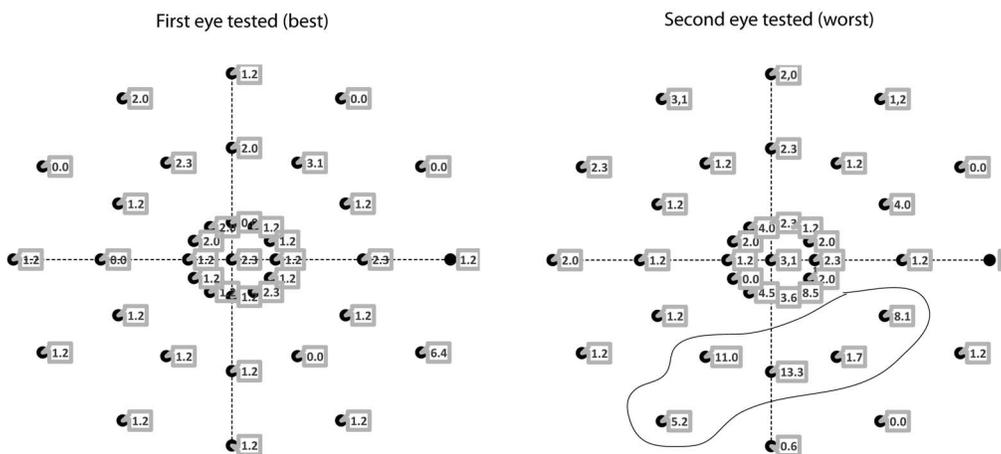
Group 1



Group 2



AMD 3



**Figure 6.** Variation limits calculated by the 95th percentile of the standard deviation. The upper graphs show the 95% variation limit at each point in group 1 for both the first eye tested and second eye tested. The variation limits varied among the points, ranging from 1.2 to 4 dB. In group 2, the variation was higher, ranging from 2 to 9 dB. The lower graphs show the results of one AMD patient. Note that in dysfunctional areas, such as the lower quadrant of AMD 3, there is a higher variation.

controlling of eye movement<sup>8</sup> using a microperimeter such as the one we used results in very low macular test–retest fluctuation of sensitivity.

The consistency of the results among the three consecutive examinations in healthy subjects is in accordance with previous findings that have reported an absence of significant variation in mean macular sensitivity using microperimetry.<sup>10,18</sup> The additional contribution of the present study is its finding that distance from the center and fixation stability appears to have no effect on sensitivity fluctuation. As previously reported by Wu et al.,<sup>14</sup> test–retest variability is greater at the border of a scotoma than in spared areas. This might explain the greater fluctuation of macular sensitivity in AMD patients versus healthy subjects found in the present study.

Surprisingly, the present data show that high fluctuation at low sensitivity areas does not seem to be an effect of short-term (1 hour later re-evaluation) learning. They instead appear to be characteristic of macular areas that have low sensitivity, which are evidently more dysfunctional.

We tested the effect of fixation stability on sensitivity fluctuation by comparing fixation stability and the average COV at the 37 points tested. Fixation stability is a pivotal parameter in patients with macular dysfunction that would be candidates for undergoing a visual training protocol to improve and/or replace their preferred retinal locus.<sup>21</sup> No correlation was found between fixation stability and the average COV.

We included, in the present study, a young group of healthy volunteers because we speculate that this group would provide the best possible fluctuation one would expect for the microperimetric thresholds. Although a healthy group accounts for the effects of the diseases, it does not account for the effects of the age. However, the relatively spared eyes of the AMD subjects (best or second tested eyes) with better VA (see [Table](#)) may be considered a group of relatively preserved eyes compared with the affected eyes of the AMD subjects. This comparison might account for the effects of the age. Fluctuation of sensitivity at different macular points resulted in a very low standard deviation in healthy subjects. Moreover, the average COV did not differ significantly between healthy subjects and AMD patients in the point-by-point analysis. When we analyze one area with lower sensitivity—for instance, the average sensitivity of the inferior quadrant in patient AMD 3 ([Fig. 4](#))—we find a much higher standard deviation and COV. This should be taken into account when microperimetry is used to follow up macular dysfunction and for therapeutic monitoring of patients with localized macular sensitivity loss.

Based on the present data, we would suggest that regional analysis, rather than average summative macular sensitivity, would be more reliable for establishing whether or not there is a macular sensitivity change within the variation limits we displayed in [Figure 6](#). The macular thresholds were measured in the “functional fovea,” which in advanced AMD eyes is mostly located outside of the anatomic fovea. This can be observed sometimes in healthy eyes as well, that the anatomic fovea does not correspond to the functional fovea. The best eyes of the AMD subjects were relatively spared. The [Table](#) shows that most AMD subjects (eight) had VA of 0.8 in their best eyes, one subject had normal VA = 1.0 and two subjects had lower VA, 0.6 and 0.5.

Considering the relatively preserved VA and macular sensitivity of the better eyes in group 2 (AMD subjects), we would expect these fluctuations to be more similar to those from elderly healthy eyes in which foveal fixation is assumed. Therefore, we suggest the fluctuation limits shown in [Figure 6](#) middle left panel, calculated from results of the better eyes of group 2 (AMD subjects), to be considered for AMD eyes with preserved central fixation.

Finally, we suggest that a significant change should be regarded as a difference that equals or exceeds 4 dB observed in normal sensitivity values (above 28 dB) or a difference that equals or exceeds 9 dB in decreased sensitivity values (below 26 dB) (Vujosevic S, et al. *IOVS*. 2010;51:ARVO E-Abstract 536).

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