Online Appendix

Macular Pigment Optical Density (MPOD) Testing in the Elderly: Development of a Simplified and Abbreviated Testing Protocol to Minimize Fatigue and Participant Burden

The most commonly and most extensively validated method used to measure MPOD is a psychophysical test based on heterochromatic flicker photometry (HFP).[1, 2] This method determines psychophysically the ratio between the visual sensitivity for a flickering test light that is maximally absorbed by MPs alternating in counterphase with a suppressing background at a wavelength that is not absorbed by the MP. The ratio between two measurements, one at the fovea and one parafoveally (where the MP density is minimal) provides an estimate of the MPOD. Subjects taking the test with this technique are asked to find the point at which the sensation of flicker disappears typically by adjusting a knob that controls the intensity of the flickering test target. The range of intensity within which flicker is no longer appreciated –also termed the "no-flicker zone"– varies in width with the rate at which the test target is flickering, such that the flickering frequency needs to be individually optimized based on an initial determination of the subject's flicker fusion frequency and subsequent fine-tunings performed by the examiner. As previously reported,[3] subjects undergoing MPOD determinations are typically asked to identify empirically the middle of the no-flicker zone with progressively finer adjustments of the knob and to push a button at the subjectively determined point.

Figure S1 illustrates the testing apparatus used in our study (Macular Metrics Corp., Rehoboth, MA). The appearance of the test targets from a participant's perspective is illustrated in Panels C and D. By using this testing protocol typically used in younger adults, our initial experience in the older adults (mean age 79) participanting in ARMA was that of

unacceptably long test durations (in excess of 2 hours), which jeopardized its use in epidemiologic research. In this appendix, we detail our rationale and strategies to adapt the testing protocol to be suitable for use in geriatric populations, and present the results of a pilot study that led to the development of the protocol used in ARMA.

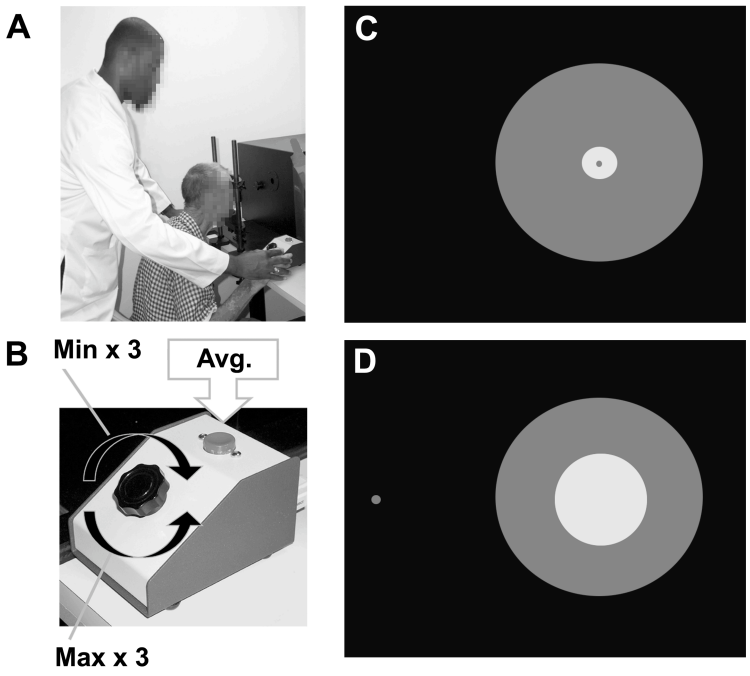

Figure S1. Illustration of the HFP-based densitometer used in the study. *Panel A*, Study staff explains to a participant how to correctly perform knob adjustments to identify the limits of the no-flicker zone (faces have been scrambled digitally to render them unrecognizable). *Panel B*, Close-up view of the knob utilized to identify the limits of the no-flicker zone: the standard technique used in the study consisted in three pairs of clockwise ("minimums") and counter-clockwise ("maximums") progressive rotations of the knob; the average of each pair of values was determined and entered by the examiner by pressing the red button atop the knob box. *Panels C and D*, Sketch of the appearance of the foveal and parafoveal flickering test stimuli, respectively, as seen from a participant's perspective through the opening on the front of the instrument box. The 5-minute central fixation target is shown in the middle of foveal stimulus, whereas a red LED provides a fixation target for the parafoveal stimulus (7-deg eccentricity) through a side opening. In each case, the test stimuli are shown surrounded by the suppressing background (see main text for further details).

To minimize test results' variability and participants' fatigue, we systematically instructed participants to identify the limits of the no-flicker zone for each target, which were termed the "minimum" and the "maximum" intensity values for the test target in question. This was done by adjusting first clockwise and then counter-clockwise the knob controlling the intensity of the test targets (Fig. S1B), going always from flickering to not flickering and starting at the low intensity end (i.e., minimums first, clockwise motion of the knob). Participants were always encouraged to blink several times when they first thought they reached a no-flicker point, and to continue adjusting the knob until the blinking no longer allowed the sensation of flickering in the test targets to resume. Different from standard testing protocols, the examiner then calculated the exact mathematical average of these two numerical values, which identified the middle of the no-flicker zone, and entered it on the subjects' behalf. This procedure was followed for both test targets for all subjects. In so doing, the middle of the no-flicker zone was the result of a precise average, rather than the subjective location thereof identified by the participant, and was attained faster than by letting the participants find it on their own by subsequent fine-tunings of the knob's position.

In subjects who had difficulties performing the adjustment of the knob on their own also to identify the limits of the no-flicker zone (e.g., in case of tremor), the examiner performed this task on their behalf as well, instructing the subject to notify immediately the examiner about cessation of flicker sensation in the test targets. A similar strategy has been recently used successfully also by others.[4, 5]

In previous studies with HFP-based methods, each determination of the no-flicker zone was replicated typically five, and up to 8 times. Aleman et al. and Duncan et al.,[4, 5] though, had already noted that some subjects could provide reliable MPOD estimates with as few as 3 replicates. As we sought to reduce test duration as much as possible without compromising accuracy, we first re-analyzed MPOD data obtained with a Maxwellian view-

based system by Hammond and Fuld in 1992,[6] in which MPOD values were provided for each replicate. A re-analysis of the data in their series indicated that five repetitions yielded the same results as the first three repetitions with respect to both average MPOD values (0.31 in both cases, p=0.834 by paired two-tailed student's t-test), and standard deviation (SD) values (0.14 in both cases). Therefore, prior to study onset, we performed a pilot study to test whether also with our aforementioned technique and our free-view LED-based system three repetitions would yield the same estimates as five.

This pilot study was conducted on 13 subjects, 48.8 ± 12.2 years old (Tables 1 and 2). The study confirmed that, at least with our testing protocol that did not require subjects to find empirically the middle of their no-flicker zone, three repetitions yielded raw measurements and MPOD estimates indistinguishable from those obtained with five repetitions (12 out of 13 estimates within 0.01 units of each other) and with virtually identical SDs for the averages of each target. None of the paired t-tests for these comparisons were significant. Since the use of three repetitions instead of five had the potential to result immediately in a reduction in testing time by as much as 40% in each eye, three repetitions were designated as the standard protocol throughout our investigation.

Table S1. Raw measurements, resulting MPODs, and differences between 5-repetition testing vs. abbreviated protocol (3 repetitions)

Subj. No.	Target 2 (Avg. 1-5)	Target 2 (Avg. 1-3)	Difference	Target 5 (Avg. 1-5)	Target 5 (Avg. 1-3)	Difference	MPOD (Avg. 1-5)	MPOD (Avg. 1-3)	Difference
1	708.4	721.3	-12.9	234.8	237.3	-2.5	0.55	0.56	-0.01
2	283.2	284.0	-0.8	186.6	187.3	-0.7	0.21	0.21	0.00
3	351.0	346.3	4.7	222.0	222.0	0.0	0.23	0.22	0.01
4	468.0	462.7	5.3	322.4	338.0	-15.6	0.19	0.16	0.03
5	595.0	595.0	0.0	302.8	301.7	1.1	0.34	0.34	0.00
6	371.0	369.3	1.7	242.2	242.0	0.2	0.21	0.21	0.00
7	631.2	630.7	0.5	265.2	266.0	-0.8	0.43	0.43	0.00
8	299.0	297.3	1.7	190.0	190.3	-0.3	0.23	0.22	0.01
9	367.2	367.0	0.2	280.6	283.0	-2.4	0.13	0.13	0.00
10	451.0	449.7	1.3	398.4	397.3	1.1	0.06	0.06	0.00
11	562.2	561.7	0.5	317.2	317.7	-0.5	0.29	0.28	0.01
12	310.6	307.3	3.3	200.2	200.0	0.2	0.22	0.21	0.01
13	490.2	482.3	7.9	223.6	224.7	-1.1	0.39	0.38	0.01
Total	452.9	451.9	1.0	260.5	262.1	-1.6	0.27	0.26	0.01

Table S2. Comparison between standard deviations for the raw measurements and MPOD values presented in Table 1.

Subj. No.	Target 2 (Avg. 1-5)	Target 2 (Avg. 1-3)	Difference	Target 5 (Avg. 1-5)	Target 5 (Avg. 1-3)	Difference
1	20.8	14.6	6.2	5.4	5.5	-0.1
2	5.5	7.0	-1.5	2.9	3.5	-0.6
3	38.3	53.4	-15.0	5.7	5.3	0.4
4	10.2	9.7	0.5	25.7	20.0	5.7
5	13.2	15.1	-1.9	5.8	7.6	-1.7
6	7.6	10.3	-2.6	1.3	1.0	0.3
7	7.0	5.7	1.3	2.2	2.6	-0.5
8	3.9	4.5	-0.6	1.2	1.5	-0.3
9	32.9	39.0	-6.0	8.3	10.8	-2.5
10	24.2	34.0	-9.8	7.0	9.2	-2.2
11	2.3	2.9	-0.6	3.8	5.1	-1.3
12	5.3	3.2	2.1	1.6	1.7	-0.1
13	11.8	3.5	8.3	10.9	15.3	-4.4
Total	14.1	15.6	-1.5	6.3	6.9	-0.6

Appendix References

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