Supplementary Online Content

Steinberg JS, Fitzke FW, Fimmers R, Fleckenstein M, Holz FG, Schmitz-Valckenberg S. Scotopic and photopic microperimetry in patients with reticular drusen and age-related macular degeneration. *JAMA Ophthalmol*. Published online March 26, 2015. doi:10.1001/jamaophthalmol.2015.0477.

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Detailed Imaging Protocol and Microperimetry Assessment

Imaging protocol

Retinal imaging was performed according to standardized operating procedures. After dilation of the pupils with 1.0% tropicamide and 2.5% phenylephrine, color fundus camera photography of the central macula was obtained using the Visucam 500 (Carl Zeiss Meditec AG, Jena, Germany). High speed combined and simultaneous confocal scanning laser ophthalmoscopy (SLO)+spectral-domain optical coherence tomography (SD-OCT) imaging (768x768 pixel) was performed with the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) device and included acquisition of central 30° x 30° near-infrared reflectance (IR, λ = 820nm, ART (Automatic Real Time) at least 15 frames) and fundus autofluorescence (FAF, exc λ = 488nm, em λ = 500-800nm, at least 15 frames) images. In addition, single horizontal and vertical combined cSLO+SD-OCT scans through the fovea (30°, ART at least 15 scans) and a raster scan (30° x 25°, ART at least 4 frames, 61 B-scans, distance 120 µm) were recorded.

Microperimetry assessment

All subjects underwent at least two scotopic microperimetry examinations of the central retina in the study eye using the Nidek MP-1S (Nidek Technologies, Padova, Italy). This device represents a further development of the well-established MP-1 device.[1] While the latter is designed to measure cone function, the former is also capable to specifically assess rod function. The technique of the MP-1S device for fundus-controlled scotopic microperimetry assessment has been recently described and also validated.[2] Prior to testing, pupil dilatation and dark adaptation were performed. For the latter, the eyes were fully covered with an opaque eye patch, followed by a waiting period of 30 minutes in a dark room (< 0.1 lux). The anatomical position of the fovea was determined by uploading the combined central IR cSLO image and horizontal B-scan SD-OCT scan of the Spectralis (see above) to the MP-1S software. Using the optic nerve head and the major retinal vessels as landmarks for registration to the fundus real-time image of the MP-1S, test stimuli were then placed around the fovea (Goldmann size V, 200 msec, 4-2 strategy, background luminance 0.0032 cd/m^2, grid centered on the anatomical position of the fovea). As fixation target, a ring with a 3° radius and 1 pixel thickness was presented. Due to the testing under scotopic conditions, the fixation ring was not necessarily centered on the fovea.[3]

The Nidek MP-1S device allows for inserting different neutral density filters for scotopic microperimetry in order to overcome its limited dynamic range of test stimuli intensities (scale from 0 to 20 dB). Therefore, a filter selection exam was initially performed in each subject for establishment of the individual appropriate neutral density filter (either the 1.0 or the 2.0 log unit neutral density filter). This filter selection exam was performed with the 1.0 log unit neutral density filter. It consisted of 16 test points that were centered on the fovea within the central 20° in form of a cross [eFigure 1 *left*]. If > 60% of these 16 stimuli of the filter selection exam were within 3dB to 16 dB, the 1.0 log unit filter was used for the main

scotopic microperimetry assessment. If 60% of the 16 stimuli were equal or above 17 dB, the filter was changed to a 2.0 log unit neutral density filter.

Following the filter selection exam, the main scotopic microperimetry assessment was performed twice using a test pattern with 56 stimuli points, centered on the anatomical position of the fovea and within the central 20° [eFigure 1 *right*]. If the difference between the mean values of the 56 stimuli points of these two assessments was larger than 3 dB, a third main scotopic microperimetry assessment was conducted. If the difference of the mean values of all test points between the second and this third scotopic microperimetry assessment was again larger than 3 dB, the test results were not considered reproducible and the subject was excluded from the further analysis. All remaining subjects finally underwent photopic microperimetry assessment in the light-adapted condition and using no neutral density filters. Given the far better retinal sensitivity of cone function, the stimuli size was reduced from Goldmann V to Goldmann III. Otherwise, the same test pattern with 56 stimuli was applied (Goldmann size III, 200 msec, 4-2 strategy, background luminance 1.27 cd/m^2, ring with a 3° radius and 1 pixel thickness as fixation target, centered on the anatomical position of the fovea).

Depending on the performance and motivation of each individual – particularly considering the exhausting and time-consuming examination – the different scotopic and the photopic microperimetry assessments were either performed on one day or split in two to four visits. Furthermore, subjects could request breaks during individual assessments that were then continued after a short period of time. For the statistical analysis, the test results of the last scotopic and the photopic assessement were evaluated.

References

1. Midena E, Vujosevic S, Convento E, Manfre A, Cavarzeran F, Pilotto E. Microperimetry and Fundus Autofluorescence in Patients with Early Age-Related Macular Degeneration. Br J Ophthalmol. May 15 2007;91(11):1499-1503.

2. Crossland MD, Luong VA, Rubin GS, Fitzke FW. Retinal specific measurement of dark-adapted visual function: validation of a modified microperimeter. BMC Ophthalmol. 2011;11:5.

3. Von Noorden GK. Peripheral viewing in scotopic vision. Arch Ophthalmol. May 1963;69:547-550.

eResults. Microperimetry and Test results Representative Examples

Basic assessment data for microperimetry

The mean time interval between the first scotopic and the final photopic examination was 44.5 ± 21.5 days (range 0 - 74). Table 1 lists the total time, the tracked time (excluding breaks and periods of tracking loss) and fixation for the scotopic and the photopic microperimetry assessments.

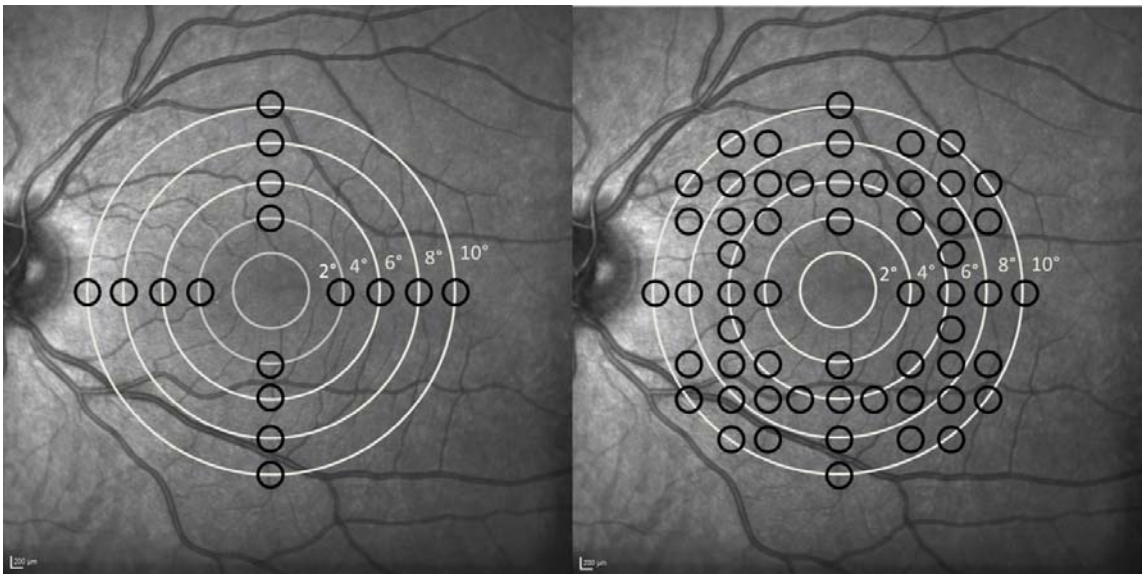
The filter selection exam that was initially performed before the main scotopic assessment determined in 18 eyes the 1.0 log unit and in 4 eyes the 2.0 log unit as the individual appropriate neutral density filter, respectively. Scotopic microperimetry results of the main test were only considered reproducible if the difference of the mean retinal sensitivity threshold values from two consecutive assessments was 3.0 dB or less. This criterion was met by 19 eyes using two exams, while three eyes required a third scotopic microperimetry assessment. The mean difference of the retinal sensitivity threshold values from two consecutive visits in the 22 eyes that were included was -0.6 ± 1.4 dB (range -1.9-3.0). The mean time interval between these two scotopic exams was 32.3 ± 20.0 days (range 0-69).

Description of test results for both representative examples

Figure 1 illustrates a representative example of the right eye of a 72 year old male subject with early AMD. There is a clearly distinguishable area affected by RDR that encompasses approximately 180° of the central macula towards the nasal-superior part (*upper right* – highlighted in blue color). For scotopic testing (*middle and lower row left)*, there is a clear and sharply demarcated relative loss at the site of RDR (category 1) with a mean sensitivity of 12.0 dB (95%CI [10.6;13.4]), while maximum or almost maximum thresholds are found at the site of no visible pathological alterations (category 2) with a mean sensitivity of 18.0 dB (95%CI [16.6;19.5]). As opposed, photopic testing (*middle and lower row right*) does not show any clear or sharply demarcated difference between both categories and no obvious relative threshold reduction with a mean sensitivity of 19.4 dB (95%CI [17.0;22.2]) at the site of RDR and of 19.3 dB (95%CI [17.2;22.1]) at the normal appearing retina, respectively.

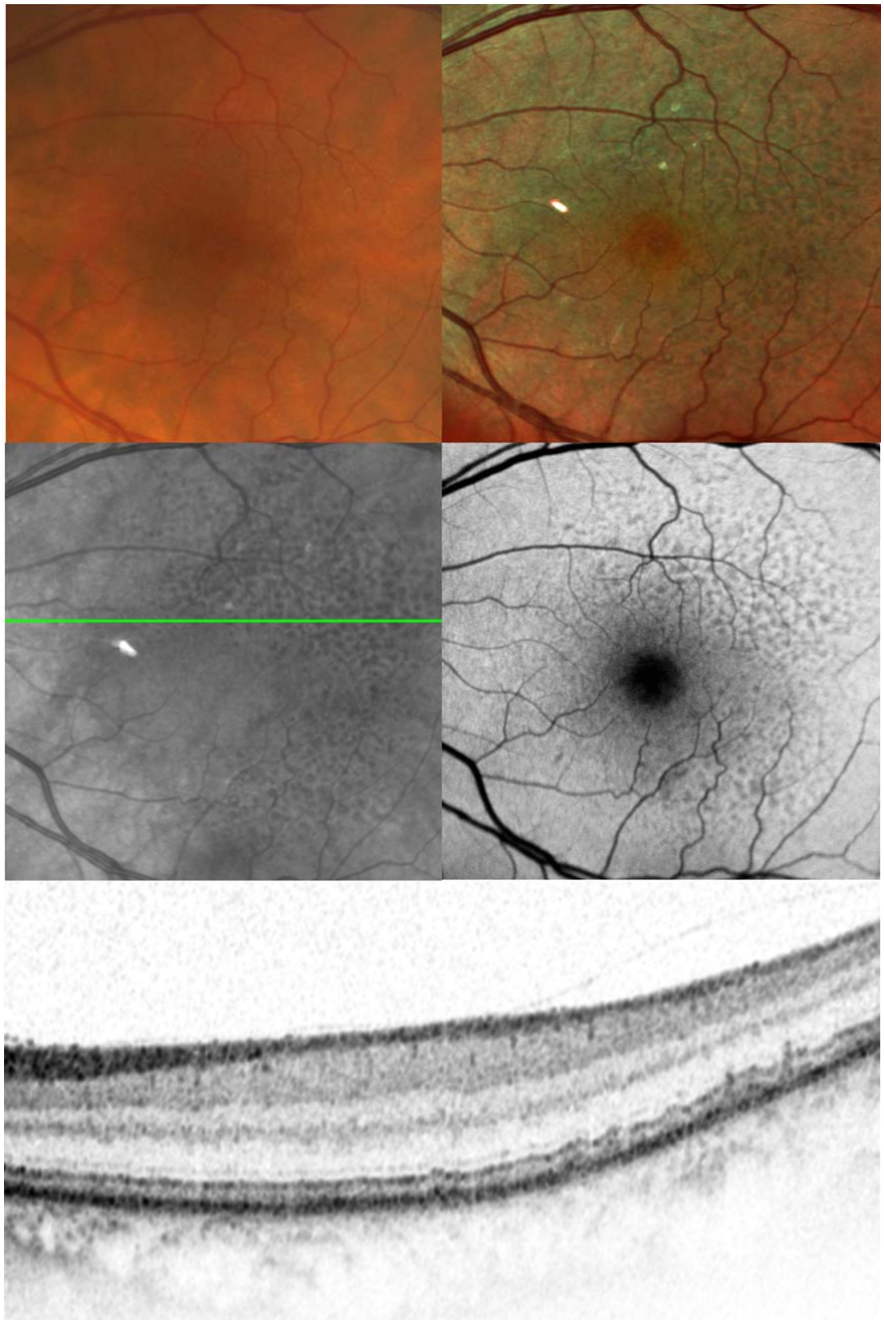
Figure 2 shows a second representative example of the left eye of a 74 year old female subject with intermediate AMD. In addition to a clearly distinguishable area with RDR towards the nasal superior part of the central macula, there is one area showing large drusen inferior nasally, while the remaining retina - as confirmed by reviewing the raster SD-OCT scans - does not show any visible pathological alterations (*upper row right*). Scotopic assessment (*middle and lower row left*) shows a relative reduction of retinal sensitivity over both areas with RDR (category 1, mean 8.5 dB (95%CI [7.0;10.0])), while there are (almost) maximum values measured over areas with no visible pathological alterations (category 2, mean 18.2 dB (95%CI [17.2;19.2)])). By contrast, photopic testing (*middle and lower row right*) only shows mild reduction at category 1 (mean 17.4 dB (95%CI [16.0;18.8])) as compared to category 2 (mean 19.5 dB (95%CI [16.8;22.2])).

eFigure 1. Test patterns for microperimetry



Ilustration of the test patterns for the filter selection exam (16 stimuli points in form of a cross, centered on the fovea, *left*) and the main scotopic assessment (56 stimuli points centered on the fovea, *right*) within the central 20°.

eFigure 2. Characteristics of reticular drusen by multimodal retinal imaging



Upper left – color fundus photopgraphy, *upper right* - multi-spectral (“Multicolor”) confocal scanning laser ophthalmoscopy (cSLO) reflectance imaging, *middle left* - cSLO near-infrared imaging, *middle right* - cSLO fundus autofluorescence, *lower row* - spectral-domain optical coherence tomography (SD-OCT) imaging.

