REVIEW

# Scotopic microperimetry: evolution, applications and future directions

Laura J Taylor *

Amandeep S Josan *

Maximilian Pfau †

Matthew P Simunovic §

Jasleen K. Jolly*‡

*Nuffield Laboratory of Ophthalmology, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

† Ophthalmic Genetics and Visual Function Branch, National Eye Institute, National Institutes of Health, Bethesda, MD, USA

§ Save Sight Institute, Discipline of Ophthalmology, University of Sydney, Sydney, Australia

‡ Vision and Eye Research Institute, Anglia Ruskin University Medical School, Young Street, Cambridge CB1 2LZ

Running Title: Scotopic Perimetry: Methods and Applications

Key Words: scotopic microperimetry, microperimetry, fundus-controlled perimetry, rods, dark-adapted

## Abstract

For many inherited and acquired retinal diseases, reduced night vision is a primary symptom. Despite this, the clinical testing options for spatially-resolved scotopic vision have until recently been limited. Scotopic microperimetry is a relatively new visual function test that combines two-colour perimetry with fundus-controlled perimetry performed in scotopic luminance conditions. The technique enables spatially-resolved mapping of central retinal sensitivity alongside the ability to distinguish between rod and cone photoreceptor sensitivities. Two companies produce commercially available scotopic microperimeters – Nidek (Nidek Technologies Srl, Padova, Italy) and CenterVue (CenterVue S.p.A., Padova, Italy). Scotopic microperimetry is a promising technology capable of detecting changes in retinal sensitivity before changes in other measures of visual function. Scotopic microperimetry is a promising functional biomarker that has potential as a useful clinical trial outcome measure. In this review, we summarise the evolution and applications of scotopic microperimetry, discuss testing options, including testing grid selection and dark-adaptation time and threshold sensitivity analyses.

## *Introduction*

The investigation of scotopic (i.e. luminance <$10^{-3}$ cd.m$^{-2}$) visual function, mediated by rod photoreceptors, is becoming increasingly relevant.[1] Reduced scotopic vision is an early symptom in many conditions, including rod-cone degenerations,[2,3] chorioretinal degenerations and maculopathies.[4,5] With promising new therapies on the horizon, it is important to have appropriate visual function markers to identify suitable patients and to monitor localised treatment effects. Traditional methods for investigating scotopic retinal function include dark adaptometry, which is performed at pre-determined loci. As well as global functional measures such as full-field stimulus threshold testing and the International Society for Clinical Electrophysiology of Vision standard flash scotopic full-field electroretinography (ERG). Although such spatially integrated testing is useful to aid diagnosis, particularly in the presence of poor fixation and very low vision, such methods cannot provide detail on spatial variations in retinal function. Furthermore, scotopic full-field ERG is often insensitive to low levels of rod photoreceptor function.

Three fifths of the visual cortex (V1) is dedicated to the central 20 degrees of visual field. As a result the human visual system is biased towards processing information received from the central retina under both photopic and scotopic conditions (i.e. the cortical representation of the macula is larger than that of the periphery). For this reason, assessing central retinal function is arguably most critical.[6] Fundus-controlled perimetry, or microperimetry, allows spatially-resolved mapping of central retinal sensitivity. It can capture disease severity and disease progression outside of the fovea (or preferred retinal locus), which can be missed with visual acuity (VA) testing alone.[7,8] Microperimetry has been extensively used for over two decades and is now an established clinical trial outcome measure.[9] However, microperimetry is generally performed under mesopic conditions, which maximises target detection redundancy, since multiple retinal mechanisms contribute to the detection of achromatic stimuli.[10] This prevents the ability to isolate the target detection system responsible for the threshold, e.g. rod or cone.

The development of two-colour perimetry coupled with dark-adapted fundus controlled microperimetry (known as scotopic microperimetry) has been driven by the need to improve the efficacy of measuring rod dysfunction and loss. Two companies currently produce commercially available fundus-controlled perimeters with scotopic capabilities: Nidek (Nidek Technologies Srl, Padova, Italy) and CenterVue (CenterVue S.p.A., Padova, Italy). The need for scotopic perimetry in clinical trials has previously been highlighted.[11] This review summarises the evolution and applications of scotopic microperimetry, discussing testing options, including testing grid selection and dark-adaptation time and threshold sensitivity analyses.

*Two-Colour Perimetry*

Two-colour perimetry is a psychophysical test designed to isolate and quantify rod and cone function perimetrically at fixed background luminance. The technique exploits differences in spectral sensitivities between rod (peak sensitivity of 505nm) and the cone-mediated luminance mechanisms (peak sensitivity at the cornea of about 555nm). Typically, short-wavelength targets are used to probe rod dominant sensitivity under scotopic conditions, whilst long-wavelength targets presented on a neutral (white) photopic background are used to suppress rods and isolate responses from the additive medium (M) plus long (L) wavelength sensitive cone mechanisms.[12]

Two-colour perimetry has a long-standing history, with its earliest incarnations implemented using manual perimeters.[13-16] Jacobson et al.[17], in 1986, were the first to combine the technique with automated perimetry. They modified a commercially available perimeter (Humphrey Visual Field Analyser) to perform dark- and light-adapted two-colour full-field static perimetry to examine patients with retinitis pigmentosa. The initial aim of the technique was to topographically assess both rod and cone function (at a time when multifocal ERG techniques were not available). Latterly, the method has been applied to evaluate the effects of emerging treatments such as gene therapy for Leber's Congenital Amaurosis.[18]

In scotopic microperimetry, threshold is assessed using short-wavelength stimuli (typically 480-500nm) at various retinal locations. The same locations can then be tested using a long-wavelength stimulus (typically 640-660nm) while still under scotopic conditions, which is in contrast to conventional two-colour perimetry. Testing this way, with a long-wavelength target under scotopic conditions enables evaluation of dark-adapted spectral sensitivity difference. In healthy subjects, rod dominant responses are isolated using a short-wavelength stimulus and mixed rod/cone responses are probed using a long-wavelength stimulus (except for foveally presented targets). The lack of photopic testing limits its ability to isolate cone function.[19,20] The cyan and red stimuli luminosity are calibrated so that in healthy individuals the difference between cyan and red sensitivity should be 0dB beyond the rod free zone. In patients with retinal disease, the difference between cyan and red sensitivity needs to be elucidated to understand the extent of rod dysfunction.[21]

The development of commercially available two-colour perimeters has been slow. Previous research has been limited by the need for specialised modifications to existing perimeters. More recently, a dedicated device has become commercially available: the dark-adapted chromatic perimeter (Medmont International Pty Ltd; Victoria, Australia),[22] while another device, the MonCVOne (MetroVision, Perenchies, France), is available with scotopic perimetry capabilities built in. Several studies using the Medmont dark-adapted chromatic perimeter have investigated wider visual field rod function in retinitis pigmentosa and age-related macular degeneration (AMD) to improve the characterisation of localised rod photoreceptor function. This has significance particularly in the early detection of disease and monitoring the effects of potential therapeutic agents.[23-26] However, the dark-adapted chromatic perimeter, although useful for wide-field perimetry, does not incorporate eye-tracking capabilities to mitigate errors due to unstable fixation. Currently available microperimeters address this shortcoming with scotopic and two-colour perimetry functions.

## *Commercially Available Microperimeter Machines with Scotopic Capabilities*

### *Nidek Microperimeter-1/1S*

The MP-1S was launched in 2012 for scotopic testing.[9] It includes a slider attached to the machine, allowing a neutral density filter (usually 2.0) combined with a short pass filter (≤ 500nm) to be inserted into the stimulus optical pathway, without affecting the infrared fundus camera, eye tracking and fixation control system.[27] The 2.0 log unit neural density filter reduces the internal luminance of liquid crystal display (LCD) to a background luminance of 0.0025cd/m$^2$, while the short pass filter attenuates longer wavelengths to optimise the stimuli for assessment of rod dominant function.[9] However, the internal LCD specification limits the stimuli range to 20 dB making it prone to ceiling effects.[27] Furthermore, unlike two-colour perimetry, only a single short-wavelength stimulus was employed, limiting the isolation of rod photoreceptor dysfunction.

Using different neutral density filters to increase the dynamic testing range has been recommended, although this alters the background and stimuli luminance. Steinberg et al.[28] recommended performing a short perimetry 'filter' test to determine the most appropriate ND filter to use for a patient. However, it is still common that some test-points will be outside of the selected filter dynamic range (<0 dB or >20 dB). Furthermore, typically less dense (lighter) ND filters are required for follow-up visits due to overall decline in retinal sensitivity with disease progression (e.g., 1 Log Unit instead of 2 Log Units). This limits longitudinal assessment since the variable background and stimuli intensities inhibits measurement of true sensitivity change over time.[28,29]

An alternative modification involves adding filters externally to the MP-1 to reduce stimuli luminance and stimulus wavelength. Such as a Schott long-pass filter (RG780; 590nm; red) and a Schott short pass filter (BG3; <502nm; blue) to isolate long and short spectral sensitivity, respectively.[30] However, as the filters are placed externally, they affect both the optical path for stimulus projection and the infrared

camera used by the eye tracking system. The impact of reduced camera visibility on the eye tracking system is unknown.

Despite these limitations, the MP-1S has proven to be a suitable tool to map central scotomata and correlate functional defects to fundus topography (Figure 1).[27] It can detect greater functional defects than photopic perimetry in patients with macula-involving disease processes, including non-exudative AMD and Stargardt's disease.[28,31] In addition to highlighting reduced visual function prior to structural changes being detectable with optical coherence tomography and infrared imaging at specific retinal loci.[31,32]

[Figure 1 near here]

*Nidek MP-3 type S*

An updated Nidek microperimeter, the MP-3 type S (launched in 2019), incorporates scotopic microperimetry with an extended stimulus intensity range (0-24 dB, increased from 20 dB in the MP-1S), generating a maximum stimulus intensity of 0.097cd/$m^2$ with a background luminance of 0.00095cd/$m^2$.[9] However, this is still less than the S-MAIA stimuli luminance range (discussed below). On the other hand, the stimulus size of the MP-3S can be increased to Goldman V, increasing the dynamic range in 'practical terms', by making the target 'easier' to detect. At the time of writing, there are two studies reported in conference proceedings using this device[33,34] but to our knowledge, there are no peer-reviewed publications.

*Scotopic Macular Integrity Assessment (S-MAIA)*

The S-MAIA (MAIA, CenterVue S.p.A., Padova, Italy) enables scotopic testing (at background luminance <0.001 cd/$m^2$) with two projection LEDs with peak wavelengths of 505nm (cyan) and 627nm (red). The current S-MAIA model has a dynamic stimuli range of 36dB, consisting of stimulus luminance levels between 0.00064 scotopic cd/$m^2$ and 2.545 scotopic cd/$m^2$. The early S-MAIA prototype

versions had a dynamic range of 20dB, this was extended to 36dB by increasing the intensity range of the stimulus targets.[35] Test-retest variability is similar to mesopic microperimetry in both healthy controls and patients with macular disease.[36,37] Although the maximum variability is seen within the cyan testing in the central 1 degree (the rod-free region).[38]

Subsequent studies have demonstrated that both mesopic and scotopic microperimetry techniques can detect retinal dysfunction due to exudative and non-exudative AMD, even when VA is well-preserved.[5,39,40] Similarly, the S-MAIA scotopic microperimetry has been shown to detect greater cyan dysfunction, suggesting greater rod then cone photoreceptor dysfunction for several maculopathies.[5,21,40,41] On the other hand, patients with macular telganectisa type 2 who had a greater loss of macular pigments showed reduced red stumuli sensitivity.[21] More recently, the S-MAIA has demonstrated the patterns of rod and cone loss in a heterogeneous group of rod-cone dystrophies.[42] In addition, subtle changes in the outer nuclear layer and retinal pigment epithelial thickness have been associated with a marked change in cyan sensitivity.[5,38] Scotopic sensitivity also appears to be associated with drusen volume.[43] Overall S-MAIA scotopic microperimetry has potential as an early disease marker that is associated with early retinal structure change in several diseases. S-MAIA scotopic microperimetry may enable a greater understanding of the patterns of disease progression. The use of the S-MAIA for AMD will be further validated in large scale studies, including an EU-funded MACUSTAR study,[44] and the ALSTAR2 study, which is currently ongoing at the University of Alabama.[45]

The S-MAIA has not yet been standardised. In particular, mesopic MAIA with its Maxwellian view system does not require pupillary dilatation for pupils ≥ 2.5mm as the exit pupil of the optical system is 2.5mm: this has been confirmed by empirical assessment in patients.[46] Whilst this has so far not been validated under scotopic conditions, we foresee no reason why this should differ for S-MAIA scotopic microperimetry, particularly as eyes will normally dilate in lower light levels in all

patient groups, apart from those with paradoxical pupillary constriction (e.g. rod monochromats/achromats) or iris abnormality.

## *Testing Methodologies and Considerations*

### *Testing Grid Selection*

Both the MP-1S and S-MAIA have in-built and customised grid capabilities; the latter is useful when tailoring testing to a particular research question. There are no standardised testing grids, which limits comparisons of results between studies. As with standard automated perimetry, grid design, including total size and stimulus spacing, requires a careful balance between the extent of retinal sensitivity assessment and testing duration and subsequent patient or participant fatigue. Scotopic test grid patterns used to date include rectilinear arrangements (such as the 10-2), radial patterns (Figure 2), and horizontal single or double meridian patterns applied to simplify structure-function analyses.[47] In the first validation study of scotopic microperimetry using the MP-1, Crossland et al. used a grid containing 100 testing points across a 10-degree square.[27] Radial patterns appear to be the most frequently used approach with both devices; however there are often differences in the total number of testing locations (between 33 and 56 point locations), the arrangements and size of central visual field assessment, ranging from 6 to 14 degrees.[31,32,39,48] Similarly, caution should be exercised with the mean sensitivity index in radial patterns due to the effects of spatial weighting. In these instances, the higher concentration of stimuli within the foveal region creates a higher sampling density, influencing the validity of averaging indices that may not be relevant to scotopic testing where the rod rich parafovea is of greater interest.[9]

[Figure 3 near here]

A standardised grid pattern may seem attractive, such as the 10-2 rectilinear grid that enables a ‘threshold’ visual field assessment of the Amsler grid, that was designed to assess macular cone function.[49] However, predefined grid patterns do not consider the individual distribution of scotomata and have a limited spatial resolution in areas of interest;[38] extra testing time is ‘wasted’ assessing areas with no

recordable visual function.[9] Pfau et al.[35,38] described using patient-tailored perimetry grids to maximise testing density in regions of interest using custom-built software for individuals with geographic atrophy. The software places test points along contour lines surrounding the area of atrophy at predefined distances (typically a total of 60 tests points per eye corresponding to an average exam time of 10 minutes). The approach can also be applied in other diseases where precise monitoring of scotoma boundaries is of interest (e.g. Stargardts disease). By focusing on the scotoma boundary of specific lesions, this approach is not designed to provide “global” function assessment but rather a more localised assessment on an area of interest.[9]

*Dark adaptation time*

Scotopic testing aims is to determine representative thresholds in scotopic conditions: the time taken to adapt to this extent depends upon several variables, chief of which is the retinal illuminance prior to dark adaptation. In short, the greater the retinal illuminance prior to testing, the longer it will take to reach absolute threshold. There are discrepancies in dark adaptation duration undertaken prior to scotopic microperimetry testing in the literature. Most studies have utilised adaptation times of between 30-45 minutes, in keeping with the rod adaptation plateau in healthy individuals.[31,41,50] However, the kinetics of dark adaptation slow with age and in certain ocular diseases, including AMD and many inherited retinal degenerations.[51-53]

In theory, without extensive dark adaptation times, it is impossible to ensure that all individuals (particularly those with retinal disease) are sufficiently adapted to achieve absolute threshold results. However, two studies have compared 10, 20 and 30 minutes dark adaptation before testing healthy individuals and found 20 minutes sufficient, with little change in sensitivity between 20 and 30 minutes.[54,55] Similarly, in a mixed cohort of patients with rod/cone degenerations, a comparison of results obtained after 20 and 40 minutes also found that 20 minutes was sufficient to obtain reliable results.[42] Dark adaptation curves using the Espion Visual Electrophysiology System dark adaptometry module (Diagnosys, LLC, Cambridge, UK) in choroideremia have shown a linear correlation, the decline in sensitivity at one time

point is predictive of the sensitivity at a later time point regardless of the severity of absolute sensitivity loss.[56] This work suggests that it is not necessary for the eye to reach absolute sensitivity levels to understand scotopic dysfunction. A dark-adaptation time of 20 minutes is less arduous on patients or study participants, and the reduced testing duration would make the scotopic microperimetry clinically more practical.

Twenty minutes may be satisfactory in healthy individuals or those with cone-rod degenerations without variants in visual cycle related enzymes. However, dark adaptation is often impaired in AMD, and it may take up to 40 minutes for the rods to recover sensitivity to enable assessment of absolute threshold.[57] Furthermore, in diseases with a direct impairment of the visual cycle including: *RPE65*-associated Leber congenital amaurosis, congenital stationary night blindness, Sorsby's macular dystrophy, late-onset retinal degeneration, overnight dark-adaptation by patient self-patching would optimise assessment of absolute threshold.[58] Overnight adapted sensitivity results could be useful to aid diagnosis and understanding of disease mechanisms; however, they would have limited use as a marker for everyday visual function. Furthermore, if dark adaptation duration is optimised for healthy participants, in patients with delayed dark adaptation due to their eye condition, this could increase the sensitivity of scotopic microperimetry to identify decrements of disease.

The decision on the length of dark adaptation time used should balance the accuracy of uniform adaptational state and associated improvement in responses. Coupled with a pragmatic approach based on what is clinically viable and the purpose of the functional assessment e.g. an assessment to understand visual function in everyday living vs an assessment of absolute thresholds to understand disease mechanisms.

### ***Analysis Techniques***

*Scotopic Microperimetry Indices*

Microperimetry indices are related and influenced by the testing grid selection. The standard scotopic microperimetry output, with the Nidek and MAIA devices is a pointwise sensitivity for each test location and an overall mean sensitivity. Further analyses of visual field irregularities, including total deviation, localised mean sensitivity and pattern standard deviation, require reference to normative data and spatial interpolation.[38,59 60] However, these analyses are limited in microperimetry due to variability in the spatial locations being tested in different individuals, limiting reliable comparisons.[59] Currently, there are no standardised reference data sets built into either the MP-1S or S-MAIA software to enable automatic analysis. Pfau et al. applied spatial interpolation modelling previously described by Dennis and Artle,[59,60] to scotopic microperimetry data from 40 healthy control participants. These interpolated reference maps could subsequently be applied to calculate pointwise sensitivity loss values for any test pattern, including the patient-tailored patterns for patients with geographic atrophy.[35,38]

Further microperimetry parameters include measurement of relative and absolute scotoma, zonal analyses, cluster mean sensitivity of responding-, perilesional- and extra-lesional loci, as well as changes in these regions.[8,9,61] These indices may be helpful, although they have yet to be widely applied to scotopic microperimetry.

Hill of vision volumetric analyses has recently been applied to mesopic microperimetry to overcome the issues of spatial weighting in averaged indices, hindering identification of localised sensitivity changes and reduced validity with irregular or centrally condensed grid testing patterns. These analysis models follow on from static full visual field modelling involving custom software by Weleber et al.[62] Volumetric indices quantify the magnitude of visual field sensitivity by modelling a hill of vision from point sensitivity data, therefore permitting meaningful comparisons between different grid patterns obtained with consistent testing conditions. This may help reduce the testing times involved in high-resolution mapping by combining examinations from multiple short-interval visits.[63,64] The total volume or a specific field subset volume can be calculated. The future scope could include using mixed-effects models to combine volume measures with other factors such as: age or

participant variability. These analyses are yet to be applied to scotopic microperimetry but could help analyse subtle sensitivity defects, localised regions of hyposensitivity or more global binocular visual function. In addition, techniques like volumetric analysis can also combine monocular testing grids to create binocular retinal sensitivity maps. These may correlate more strongly with everyday patient experiences and reported functional vision, supporting health economic analyses.

### *Scotopic Microperimetry Limitations*

Scotopic microperimetry is time-consuming, requiring approximately 25 minutes of testing plus dark adaptation time.[48] It requires very dark (<0.1 lux) testing facilities, which may not always be feasible. Two-colour scotopic testing (i.e. using a short- and a long-wavelength target) has the advantage of assessing absolute threshold and rod dysfunction; however, the isolation of cone function is inherently limited.[10] In scotopic two-colour microperimetry photoreceptor adaptational asymmetries (i.e. where rods are assessed at absolute threshold and cones are assessed where Weber's law holds) may artefactually favour the detection of rod abnormalities. This asymmetry may be further increased by 'filter effects' such as pupil size and media opacities increasing the absorption of short wavelengths and crucially receptor defects.[10,20]

Research to determine precise two-colour perimetry procedures, variability, confounding factors, and reliability of scotopic testing are ongoing[11,22-25,65]. Differences between current scotopic microperimetry devices include projected stimulus size, fixation target size, background luminance levels, stimulus intensity range and maximum stimulus intensity, which limit any meaningful comparison of results between devices (table 1). The minimum stimulus intensity of both the S-MAIA and Nidek machines is so low, the dynamic ranges are dictated by the maximum stimulus intensity, which is most relevant for patients with retinal disease and minimizes the floor effect. The minimum can be calculated from the information provided using the equation dB = 10 * log (Lmax/L).

[Table 1 near here]

A comparison of the S-MAIA and Nidek MP-1S was conducted for AMD by Steinberg et al. Both machines could detect changes in the patient population, but thresholds were not directly comparable.[48] The initial S-MAIA studies were performed on an S-MAIA prototype model with a maximum of 20 dB stimulus intensity range for scotopic testing, resulting in ceiling and floor effects. Subsequently, the S-MAIA was upgraded to include a 36 dB stimulus intensity range for scotopic testing mode. Although floor effects have still been reported and the S-MAIA remains limited in the upper bounds of the stimulus intensity range (i.e., unsuitable to evaluate severe degrees of rod dysfunction).[38] The MP-1S is limited by the need for different neutral density filters to extend the stimulus intensity range. This prevents accurate longitudinal progression analysis since there is no reliable mathematical conversion calculation to track sensitivity with different density filters.[48,66] The lack of compatibility interpreting the output from both machines limits the application of scotopic microperimetry across multi-centre patient registries, who may have different devices.

*Scotopic Fixation Stability.*

Fixation target size and type, and level of VA are critical factors affecting fixation stability. Fixation is often impaired when foveal-mediated vision such as VA is affected, which typically occurs in AMD.[9] Since vision in low light is also particularly difficult for these patients, reduced fixation ability in scotopic tests (including perimetry and dark adaptometry) was previously a significant limiting factor. The fixation target brightness on the S-MAIA can be increased to improve visibility and subsequently fixation stability. The fixation tracking properties of microperimetry minimise fixational errors, enabling the unique ability to reliability map the scotopic fovea and scotopic scotoma.[27] Steinberg et al. reported similar fixation for both the Nidek MP-1S and the S-MAIA when similar fixation targets were used, although in their study all participants had good VA, 6/7.5 (0.1 LogMAR) or better.[48] Supplementary figures 1 & 2 detail examples of the fixation tracking maps and results provided by each microperimeter.

Although the eye-tracking capabilities minimise testing errors associated with poor fixation stability, they are limited by the eye-tracking system frequency. Both the Nidek MP-1S and S-MAIA have a 25Hz eye tracking frequency camera.[38,67] This is likely insufficient relative to the speed of a saccade, which is up to 700 degrees per second depending on amplitude, velocity and other visual conditions.[68] The new MP-3 has an improved tracking speed of 30Hz but would still likely be insufficient to capture a rapid eye movement fixational errors.[9] The optimum eye-tracking camera frequency to accurately monitor fixation stability is unknown. A patient with normal visual function and good fixation stability may require very little grid positional adjustment from the retinal tracking feature. In contrast, a patient with nystagmus or poor central fixation would likely require far higher tracking capability. Furthermore, there are limited data available concerning the visual tracking performance and the impact of micro-saccades on microperimetry stimulus placement. In a study by Cideciyan and co-workers, it was concluded that most stimulus placement errors are < 0.25° even in patients with unstable fixation.[69] However, this study was on a small sample size (n=4) and in a significant minority of stimulus measurements, eye movements of up to 100 degrees/sec were recorded, leading to potential stimulus placement errors of up to 4 degrees. Further research to investigate the range of eye movements during microperimetry and scotopic microperimetry in both normal and those with visual impairment is warranted to deduce the precise effects on the variability of results.

## Final Discussion

Scotopic microperimetry with both the Nidek MP-1S and S-MAIA devices appears to be a promising technique to detect early disease changes. Early results have demonstrated the utility of using scotopic microperimetry to understand visual capabilities under low light levels. Further research is needed to investigate scotopic microperimetry changes over time in different retinal diseases. Scotopic perimetric testing at absolute threshold is attractive as it may elucidate functional defects not apparent once receptor mechanisms adapt.[70] This may provide insights into poorly understood or unknown disease mechanisms. Current microperimetry systems are relatively unaffected by pupil size variation but would benefit from improved

standardized testing across systems to enable comparable results, greater application of patient-tailored perimetry grids, as well as improved threshold analysis techniques. Scotopic microperimetry has a promising future and is likely to become an invaluable early disease marker and outcome measure in retinal disease clinical trials.

## REFERENCES


1 Jolly JK, Bridge H, MacLaren RE. Outcome Measures Used in Ocular Gene Therapy Trials: A Scoping Review of Current Practice. *Front pharmacol* 2019; 10: 1076-1076.

2 Dimopoulos IS, Freund PR, Knowles JA et al. The Natural History of Full-Fuield Stimulus Threshold Decline in Choroideremia. *Retina* 2018; 38: 1731-1742.

3 Verbakel SK, van Huet RAC, Boon CJF et al. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res* 2018; 66: 157-186.

4 Tahir HJ, Rodrigo-Diaz E, Parry NRA et al. Slowed Dark Adaptation in Early AMD: Dual Stimulus Reveals Scotopic and Photopic Abnormalities. *Invest Ophthalmol Vis Sci* 2018; 59: AMD202-AMD210.

5 Hess K, Gliem M, Charbel Issa P et al. Mesopic and Scotopic Light Sensitivity and Its Microstructural Correlates in Pseudoxanthoma Elasticum. *JAMA Ophthalmology* 2020; 138: 1272-1279.

6 Gloriani AH, Schütz AC. Humans Trust Central Vision More Than Peripheral Vision Even in the Dark. *Curr Biol* 2019; 29: 1206-1210 e1204.

7 Midena E, Pilotto E. Microperimetry in age: related macular degeneration. *Eye (Lond)* 2017; 31: 985-994.

8 Jolly JK, Xue K, Edwards TL et al. Characterizing the Natural History of Visual Function in Choroideremia Using Microperimetry and Multimodal Retinal Imaging. *Invest Ophthalmol Vis Sci* 2017; 58: 5575-5583.

9 Pfau M, Jolly JK, Wu Z et al. Fundus-controlled perimetry (microperimetry): Application as outcome measure in clinical trials. *Prog Retin Eye Res* 2020: 100907.

10 Simunovic MP, Moore AT, MacLaren RE. Selective Automated Perimetry Under Photopic, Mesopic, and Scotopic Conditions: Detection Mechanisms and Testing Strategies. *Transl Vis Sci Technol* 2016; 5: 10.

11 McGuigan DB, 3rd, Roman AJ, Cideciyan AV et al. Automated Light- and Dark-Adapted Perimetry for Evaluating Retinitis Pigmentosa: Filling a Need to Accommodate Multicenter Clinical Trials. *Invest Ophthalmol Vis Sci* 2016; 57: 3118-3128.

12 Jacobson SG, Apáthy PP, Parel J-M. Rod and cone perimetry: computerized testing and analysis. . In: *Heckenlively JR, Arden GB, eds Principles and Practice of Clinical Electrophysiology of Vision*. St. Louis, MO: Mosby, Inc, 1991. p. 475-482.

13 Wald G, Zeavin BH. Rod and cone vision in retinitis pigmentosa. *Am J Ophthalmol* 1956; 42: 253-269.

14 Massof RW, Finkelstein D. Rod sensitivity relative to cone sensitivity in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1979; 18: 263-272.

15 Gloor BP. Franz Fankhauser: The Father of the Automated Perimeter. *Surv Ophthalmol* 2009; 54: 417-425.

16 Ernst W, Faulkner DJ, Hogg CR et al. An automated statis perimeter/adaptometer using light emitting diodes. *Br J Ophthalmol* 1983; 67: 431-442.
17 Jacobson SG, Voigt WJ, Parel J-M et al. Automated Light- and Dark- Adapted Perimetry for Evaluating Retinitis Pigmentosa. *Ophthalmology* 1986; 93: 1604-1611.
18 Bainbridge JW, Smith AJ, Barker SS et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med* 2008; 358: 2231-2239.
19 Heckenlively JR, Arden GB. *Principles and Practice of Clinical Electrophysiology of Vision*: Mosby Year Book, 1991.
20 Simunovic MP, Hess K, Avery N et al. Threshold versus intensity functions in two-colour automated perimetry. *Ophthalmic Physiol Opt* 2021; 41: 157-164.
21 Heeren TFC, Tzaridis S, Bonelli R et al. Dark-Adapted Two-Color Fundus-Controlled Perimetry in Macular Telangiectasia Type 2. *Invest Ophthalmol Vis Sci* 2019; 60: 1760-1767.
22 Fraser RG, Tan R, Ayton LN et al. Assessment of Retinotopic Rod Photoreceptor Function Using a Dark-Adapted Chromatic Perimeter in Intermediate Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci* 2016; 57: 5436-5442.
23 Tan RS, Guymer RH, Luu CD. Repeatability of Retinal Sensitivity Measurements Using a Medmont Dark-Adapted Chromatic Perimeter in Healthy and Age-Related Macular Degeneration Cases. *Transl Vis Sci Technol* 2018; 7: 3.
24 Bennett LD, Klein M, Locke KG et al. Dark-Adapted Chromatic Perimetry for Measuring Rod Visual Fields in Patients with Retinitis Pigmentosa. *Transl Vis Sci Technol* 2017; 6: 15.
25 Nguyen CT, Fraser RG, Tan R et al. Longitudinal Changes in Retinotopic Rod Function in Intermediate Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci* 2018; 59: AMD19-AMD24.
26 Stingl K, Stingl K, Nowomiejska K et al. Clinical protocols for the evaluation of rod function. *Ophthalmologica* 2021; 244: 396-407.
27 Crossland MD, Luong VA, Rubin GS et al. Retinal specific measurement of dark-adapted visual function: validation of a modified microperimeter. *BMC ophthalmology* 2011; 11: 5.
28 Steinberg JS, Fitzke FW, Fimmers R et al. Scotopic and Photopic Microperimetry in Patients With Reticular Drusen and Age-Related Macular Degeneration. *JAMA ophthalmology* 2015; 133: 690-697.
29 Sassmannshausen M, Pfau M, Thiele S et al. Longitudinal Analysis of Structural and Functional Changes in Presence of Reticular Pseudodrusen Associated With Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci* 2020; 61: 19.
30 Bowl W, Stieger K, Lorenz B. Fundus-controlled two-color dark adaptometry with the Microperimeter MP1. *Graefe's Arch Clinl Exp Ophthalmol* 2015; 253: 965-972.
31 Salvatore S, Fishman GA, McAnany JJ et al. Association of dark-adapted visual function with retinal structural changes in patients with Stargardt disease. *Retina (Philadelphia, Pa)* 2014; 34: 989-995.
32 Nebbioso M, Barbato A, Pescosolido N. Scotopic microperimetry in the early diagnosis of age-related macular degeneration: preliminary study. *BioMed Res Int* 2014; 2014: 671529.
33 Quintana NPL, Fernandez-Nunez C, Alberto-Pestano M et al. Retinal sensitivity and foveal fixation, measured by photopic and scotopic microperimetry, in patients with diabetic retinopathy and healthy subjects. *Invest Ophthalmol Vis Sci* 2020; 61.
34 Carrasco OED, Fernandez-Nunez C, Alberto-Pestano M et al. Usefulness of photopic and scotopic microperimetry in the early diagnosis of macular toxicity secondary to hydroxychloroquine use. *Invest Ophthalmol Vis Sci* 2020; 61.
35 Pfau M, Müller PL, von der Emde L et al. Mesopic and Dark-Adapted Two Colour Fundus-Controlled Perimetry in Geographic Atrophy Secondary to Age-Related Macular Degeneration. *Retina* 2020; 40: 169-180.

36 Pfau M, Lindner M, Fleckenstein M et al. Test-Retest Reliability of Scotopic and Mesopic Fundus-Controlled Perimetry Using a Modified MAIA (Macular Integrity Assessment) in Normal Eyes. *Ophthalmologica* 2017; 237: 42-54.

37 Pfau M, Lindner M, Steinberg JS et al. Visual field indices and patterns of visual field deficits in mesopic and dark-adapted two-colour fundus-controlled perimetry in macular diseases. *Br J Ophthalmol* 2018; 102: 1054-1059.

38 Pfau M, von der Emde L, Dysli C et al. Determinants of Cone and Rod Functions in Geographic Atrophy: AI-Based Structure-Function Correlation. *Am J Ophthalmol* 2020; 217: 162-173.

39 Welker SG, Pfau M, Heinemann M et al. Retest Reliability of Mesopic and Dark-Adapted Microperimetry in Patients With Intermediate Age-Related Macular Degeneration and Age-Matched Controls. *Invest Ophthalmol Vis Sci* 2018; 59: AMD152-AMD159.

40 von der Emde L, Pfau M, Thiele S et al. Mesopic and Dark-Adapted Two-Color Fundus-Controlled Perimetry in Choroidal Neovascularization Secondary to Age-Related Macular Degeneration. *Transl Vis Sci Technol* 2018; 8: 7.

41 Nassisi M, Tepelus T, Corradetti G et al. Relationship Between Choriocapillaris Flow and Scotopic Microperimetry in Early and Intermediate Age-related Macular Degeneration. *Am J Ophthalmol* 2021; 222: 302-309.

42 Jolly JK, Buckley TMW, Pfau M et al. Assessment of Scotopic Function in Rod-Cone Inherited Retinal Degeneration With the Scotopic MAIA. Forthcoming 2022.

43 Pondorfer SG, Heinemann M, Wintergerst MWM et al. Detecting vision loss in intermediate age-related macular degeneration: A comparison of visual function tests. *PLoS One* 2020; 15: e0231748.

44 Terheyden JH, Holz FG, Schmitz-Valckenberg S et al. Clinical study protocol for a low-interventional study in intermediate age-related macular degeneration developing novel clinical endpoints for interventional clinical trials with a regulatory and patient access intention-MACUSTAR. *Trials* 2020; 21: 659.

45 Curcio CA, McGwin G, Sadda SR et al. Functionally validated imaging endpoints in the Alabama study on early age-related macular degeneration 2 (ALSTAR2): design and methods. *BMC Ophthalmology* 2020; 20: 196.

46 Han RC, Jolly JK, Xue K et al. Effects of pupil dilation on MAIA microperimetry. *Clin Exp Ophthalmol* 2017; 45: 489-495.

47 Birch DG, Wen Y, Locke K et al. Rod sensitivity, cone sensitivity, and photoreceptor layer thickness in retinal degenerative diseases. *Invest Ophthalmol Vis Sci* 2011; 52: 7141-7147.

48 Steinberg JS, Sassmannshausen M, Pfau M et al. Evaluation of two systems for fundus-controlled scotopic and mesopic perimetry in eye with age-related macular degeneration. *Transl Vis Sci Technol* 2017; 6: 7.

49 Marmor MF. A brief history of macular grids: from Thomas Reid to Edvard Munch and Marc Amsler. *Surv Ophthalmol* 2000; 44: 343-353.

50 Locke KG, Duwel S, Cideciyan AV et al. Progressive loss of rod sensitivity in patients with autosomal dominant retinitis pigmentosa (adRP) due to RHO Pro23His mutation. *Invest Ophthalmol Vis Sci* 2017; 58.

51 Owsley C, Jackson GR, White M et al. Delays in rod-mediated dark adaptation in early age-related maculopathy. *Ophthalmology* 2001; 108: 1196-1202.

52 Jackson GR, Owsley C, McGwin G. Aging and dark adaptation. *Vision Research* 1999; 39: 3975-3982.

53 Yang G-Q, Chen T, Tao Y et al. Recent advances in the dark adaptation investigations. *Int J Ophthalmol* 2015; 8: 1245-1252.

54 Naska KTK, Hogg R, Morales MU et al. Impact of dark adaptation time on the Scotopic microperimeter S-MAIA. *Invest Ophthalmol Vis Sci* 2018; 59: 1273-1273.

55 Montesano G, Naska TK, Higgins BE et al. Determinants of Test Variability in Scotopic Microperimetry: Effects of Dark Adaptation and Test Indices. *Transl Vis Sci Technol* 2021; 10: 26.
56 Jolly JK, Dubis AM, Hogg C et al., Local versus global assessment of dark adaptation and scotopic function in retinal dystrophy. Forthcoming 2022.
57 Chen KG, Alvarez JA, Yazdanie M et al. Longitudinal Study of Dark Adaptation as a Functional Outcome Measure for Age-Related Macular Degeneration. *Ophthalmology* 2019; 126: 856.
58 Fitzke FW. Dark adaptation in retinal abnormalities. *Br J Ophthalmol* 1994; 78: 426.
59 Denniss J, Astle AT. Spatial Interpolation Enables Normative Data Comparison in Gaze-Contingent Microperimetry. *Investigative Ophthalmology & Visual Science* 2016; 57: 5449-5456.
60 Astle AT, Ali I, Denniss J. Central visual field sensitivity data from microperimetry with spatially dense sampling. *Data brief* 2016; 9: 673-675.
61 Wong EN, Mackey DA, Morgan WH et al. Intersession test-retest variability of conventional and novel parameters using the MP-1 microperimeter. *Clin Ophthalmol* 2015; 10: 29-42.
62 Weleber RG, Smith TB, Peters D et al. VFMA: Topographic Analysis of Sensitivity Data From Full-Field Static Perimetry. *Transl Vis Sci Technol* 2015; 4: 14.
63 Georgiou M, Singh N, Kane T et al. Long-Term Investigation of Retinal Function in Patients with Achromatopsia. *Invest Ophthalmol Vis Sci* 2020; 61: 38.
64 Josan AS, Buckley TMW, Wood LJ et al. Microperimetry Hill of Vision and Volumetric Measures of Retinal Sensitivity. *Transl Vis Sci Technol* 2021; 10: 12
65 Bennett LD, Metz G, Klein M et al. Regional Variations and Intra-/Intersession Repeatability for Scotopic Sensitivity in Normal Controls and Patients With Inherited Retinal Degenerations. *Invest Ophthalmol Vis Sci* 2019; 60: 1122-1131.
66 Steinberg J, Sassmannshausen M, Fleckenstein M et al. Longitudinal analysis of structural and functional changes in patients with reticular drusen and age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2017; 58.
67 Cideciyan AV, Roman AJ, Jacobson SG et al. Developing an Outcome Measure With High Luminance for Optogenetics Treatment of Severe Retinal Degenerations and for Gene Therapy of Cone Diseases. *Invest Ophthalmol Vis Sci* 2016; 57: 3211-3221.
68 Wilson SJ, Glue P, Ball D et al. Saccadic eye movement parameters in normal subjects. *Electroencephalogr Clin Neurophysiol* 1993; 86: 69-74.
69 Cideciyan AV, Charng L, Swider M et al. Comparative measurements of rod function in retinal degenerations with two-color dark-adapted perimetry. *Invest Ophthalmol Vis Sci* 2016; 57.
70 Hood DC, Greenstein V. Models of the normal and abnormal rod system. *Vision Res* 1990; 30: 51-68.

Corresponding author: Vision and Eye Research Institute, Anglia Ruskin University Medical School, Young Street, Cambridge CB1 2LZ Jasleen.jolly@aru.ac.uk.

## Tables

Table 1

| Scotopic microperimeter | Background luminance (cd/m$^2$) | Maximum stimulus intensity (cd/m$^2$) | Stimuli intensity range (dB) |
|---|---|---|---|
| Nidek MP-1S | 0.0025 | 0.257 | 0-20* |
| Nidek MP-3S | <0.001 | 0.097 | 0-24 |
| S-MAIA | <0.001 | 2.545 | 0-36 |

*Extended with neutral density filters

Table 1: Summarises the background and maximum stimuli luminance for each of the microperimeters in scotopic mode testing

## Figure Captions

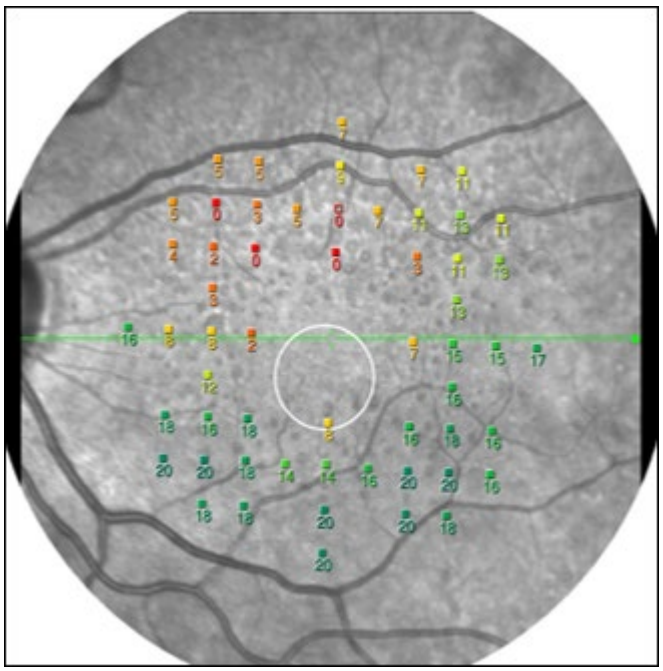


Figure 1: The Nidek scotopic microperimetry results in a patient with age-related macular degeneration and reticular pseudodrusen showing reduced cyan stimulus sensitivity in the superior macular, corresponding to increased density of reticular pseudodrusen.

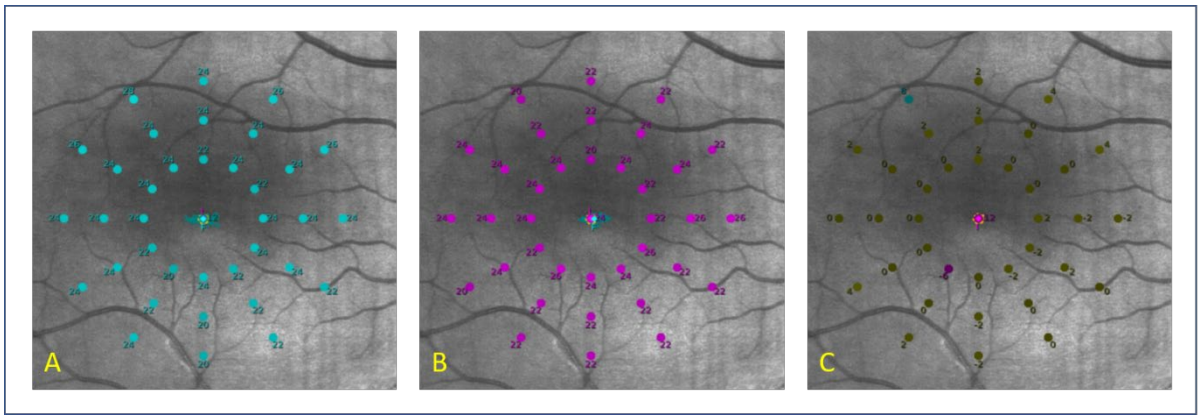



Figure 2: The S-MAIA pointwise scotopic microperimetry central retinal sensitivity results after testing with a radial grid. A: Cyan stimulus threshold results for a healthy participant with 'normal' visual function. B: Red stimulus threshold results for the same healthy participant with 'normal' visual function. C: Calculated cyan and red stimulus threshold differences for the healthy participant.

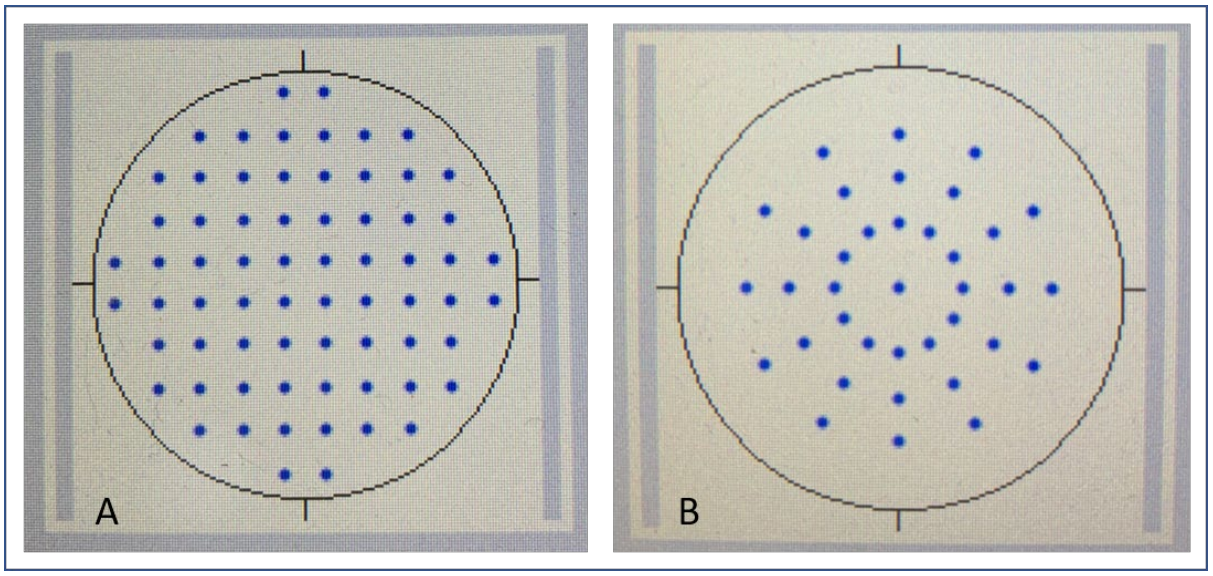
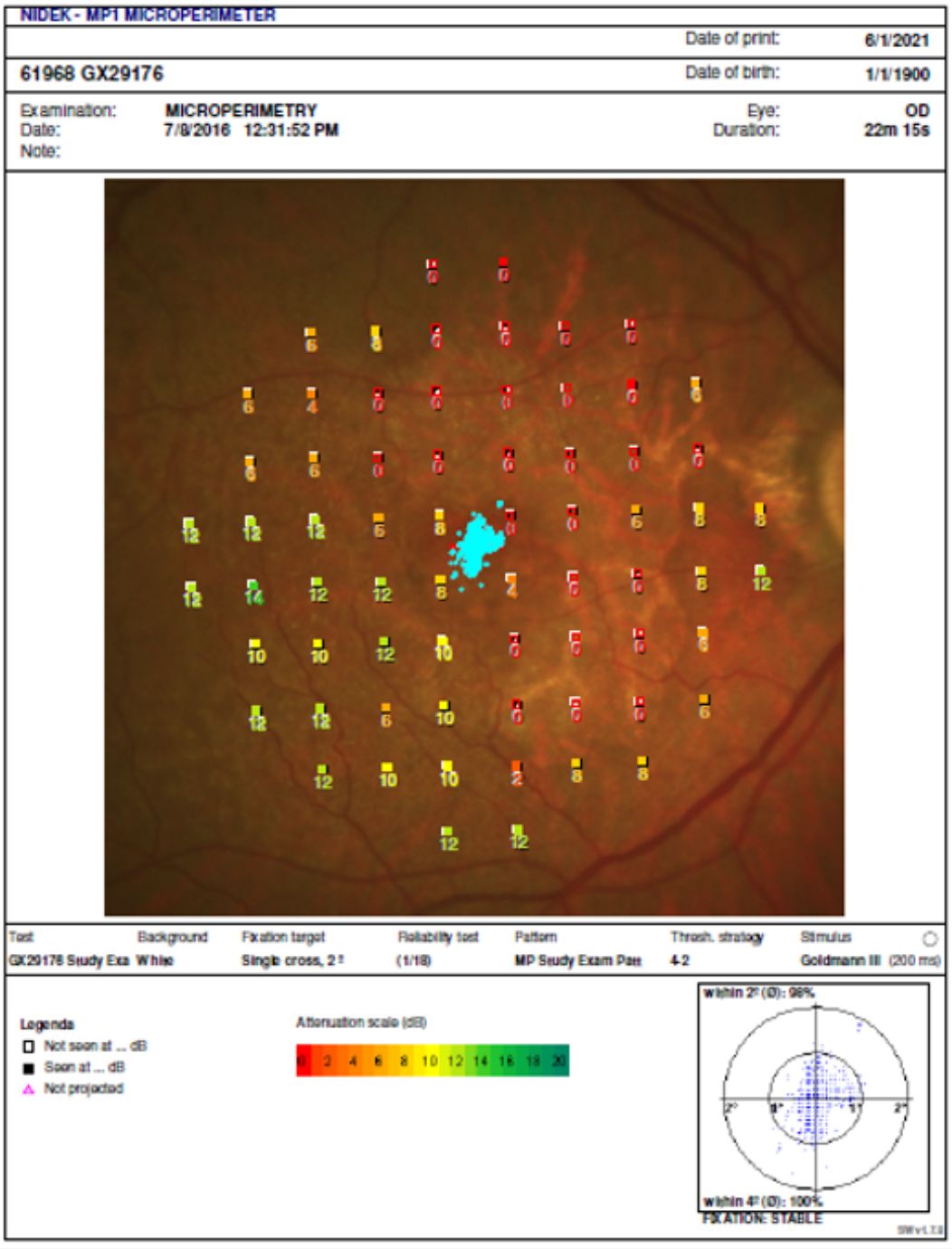
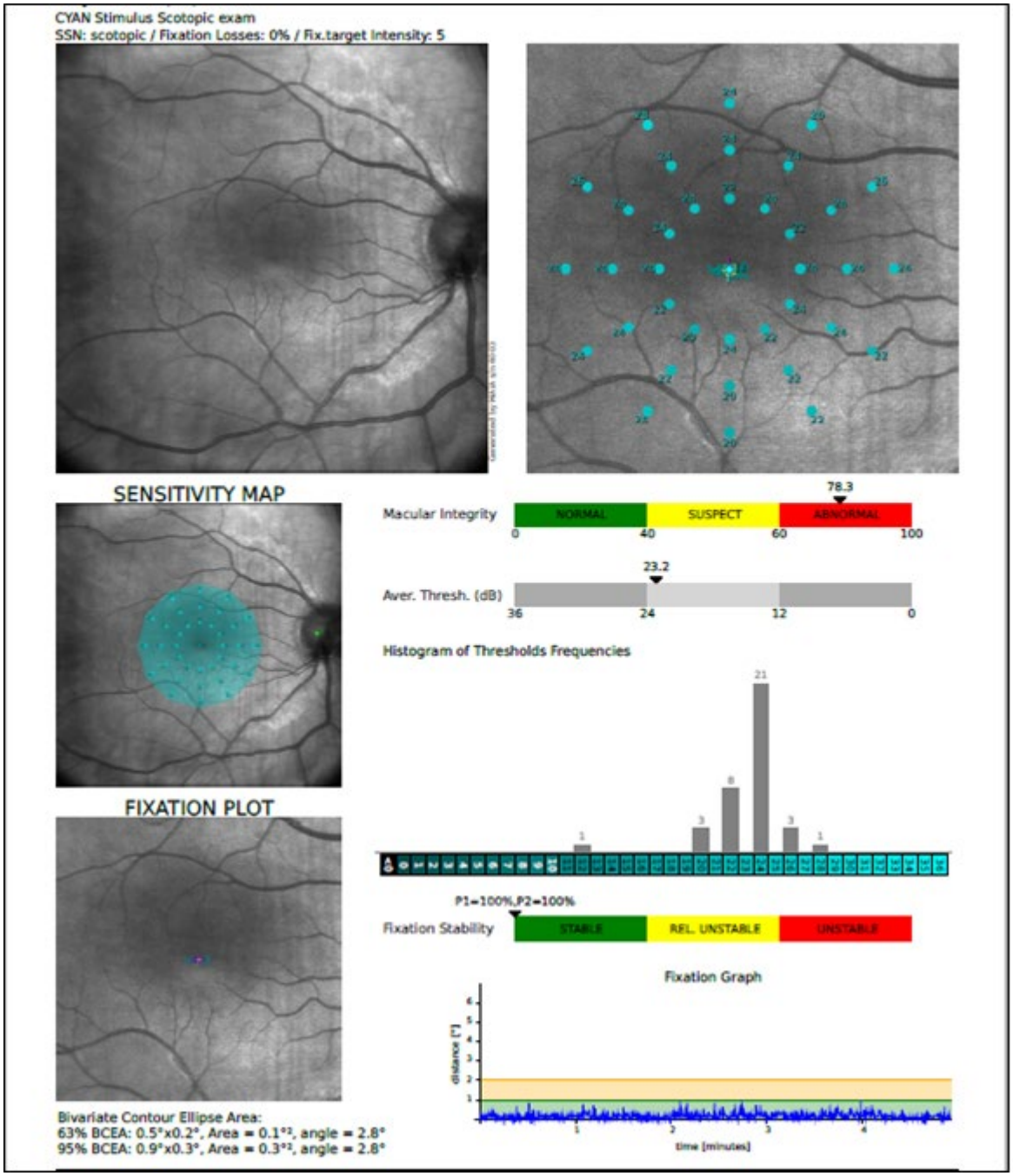



Figure 3: Details two grid options available on the S-MAIA. A: Is an example of the linear 10-2 grid made up of 68 points. B: Is an example of a radial plot with 37 points.




Supplementary Figure 1




Supplementary Figure 2

Financial: This study is supported by the kind donation made by Jean Williams (known as the Winstanley donation) to the University of Oxford for research into inherited macular diseases, and the NIHR Oxford Biomedical Research Centre. JKJ

682 was funded by a College of Optometrists Clinical Research fellowship. The views
683 expressed are those of the authors and not necessarily those of the NHS, the NIHR
684 or the Department of Health and Social Care. The sponsor and funding organization
685 had no role in the design or conduct of this research.

686 No authors have a conflict of interest.

687

688