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Exploring visual function outcome measures in inherited retinal disease: developing evidence-based recommendations

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A critical analysis report submitted to City St George's,
University of London for the degree of Doctor of
Philosophy



Department of Optometry and Vision Science.

January 2026

I, Laura Taylor, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed Declaration..........

Abstract

Inherited retinal diseases are the leading cause of irreversible visual impairment in the working-age population in the United Kingdom, with rod-cone degenerations a common subphenotype. Treatments are limited, but research to develop new therapies is ongoing. This critical analysis synthesises eleven published research papers that investigate alternative visual function assessments for use as clinical trial outcome measures in such patients. The aim is to provide outcome measure evidence-based recommendations to support ongoing translational research.

Chapter 1 introduces inherited retinal diseases and provides an overview of the visual function tests explored in this critical analysis. Chapter 2 outlines the critical analysis and evidence synthesis structure, data collection and outcome measure evaluation approaches, including test standardisation, reliability and repeatability. Chapter 3 synthesises and evaluates the key publication results grouped by test type: acuity tests, mesopic microperimetry and scotopic microperimetry. This includes five publications that evaluate measures of visual acuity; they explore sources of variability, test performance, reliability and sensitivity. One publication focuses on microperimetry central retinal sensitivity testing, exploring sensitivity indices and endpoint selection. A further three publications evaluate a modified version of microperimetry, called scotopic microperimetry, for use in patients with inherited retinal disease and healthy controls. Chapter 4 makes recommendations for the use of these alternative vision tests based on disease severity and identifies future research directions.

This critical analysis emphasises that outcome measures selection should consider clinical phenotype as well as stage of disease. Scotopic microperimetry testing showed poor reliability and significant floor effects in patients with moderate-stage choroideremia. Further validation is required to determine its suitability for patients with very early-stage disease with preserved central function. Mesopic microperimetry appears to be suitable for most patients with early-to-moderate rod-cone degeneration. Testing has been standardised, and evidence is provided to support the use of volumetric hill-of-vision sensitivity indices. Standardisation recommendations were made for low luminance visual acuity testing. However, its ability to detect subtle sensitivity changes appears limited; low luminance visual acuity may instead be a useful pre-screening tool to determine the onset of late-stage disease. The Moorfields Acuity Chart did not show any clinically significant improvements in reliability compared to other visual acuity testing and so is not recommended. Further research is needed to determine whether low-contrast visual acuity testing provides a more sensitive marker of disease progression, to evaluate the utility of electronic letter charts, and to clarify how different vision assessments relate to everyday visual function.

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Abbreviations

AUC: Area under the curve	LoA: Limits of Agreement
BCEA: Bivariate Contour Ellipse Area	LogMAR: Log Minimum Angle of Resolution
BCVA: Best Corrected Visual Acuity	MAIA: Macular Integrity Assessment
BAF: Blue Autofluorescence	MLMT: Multi-Luminance Mobility Test
CI: Confidence Interval	MP: Microperimetry
CIE: International Commission on Illumination	MPMS: Microperimetry Mean Sensitivity
COMET: Core Outcome Measures in Effectiveness Trials	MRDQ: Michigan Retinal Degeneration Questionnaire
CoR: Coefficient of Repeatability	NCT: National Clinical Trial
DA: Dark Adaptation	NHS: National Health Service
dB: Decibels	NICE: National Institute of Care and Excellence
EMA: European Medicines Agency	OCT: Optical Coherence Tomography
EDTRS: Early Treatment Diabetic Retinopathy Study	ORF: Opening Reading Frame
ERG: Electroretinography	PRL: Preferred Retinal Locus
FDA: Food and Drug Administration	RE: Right Eye
FST: Full-field Stimulus Threshold	ROC: Receiver Operator Curve
HOVER: Harmonisation of Outcomes and Vision Endpoints in Vision Restoration Trials	RP: Retinitis Pigmentosa
IQR: Interquartile Range	RPE: Retinal Pigment Epithelium
IRD: Inherited Retinal Disease	SD: Standard Deviation
ISRCTN: International Standard Registered Clinical/sociAl sTudy Number	SLO: Scanning Laser Ophthalmoscope
LE: Left Eye	ULoA: Upper Limit of Agreement
LLoA: Lower Limits of Agreement	USA: United States of America
LLD: Low Luminance Deficit	VA: Visual Acuity
LLVA: Low Luminance Visual Acuity	VFIRD: Visual Function in Retinal Degeneration

Chapter 1: Introduction

1.1 Inherited Retinal Disease: An Overview

Collectively, inherited retinal diseases (IRDs) are the leading cause of severe visual impairment in the working-age population within the United Kingdom (Liew, Michaelides and Bunce, 2014). Global and national epidemiological data reporting IRD prevalence are limited. This is partly due to the absence of national disease registries in many countries, variable access to genetic testing, incomplete or inaccurate clinical diagnoses, and differing disease definitions arising from IRD heterogeneity. Furthermore, many epidemiological studies are limited to European and North American populations. Consequently, prevalence estimates vary between studies. A recent review reported a combined IRD prevalence of approximately 1 in 3450 individuals, affecting an estimated 1.5 million people worldwide (Hanany et al., 2024).

IRDs are a complex, clinically and genetically heterogeneous group of diseases, with over 332 associated single-gene disorders (RetNet, <https://retnet.org/> accessed on 19th May 2025), which can be subclassified by Mendelian inheritance (autosomal dominance, autosomal recessive, X-linked and mitochondrial). They are also classified by syndromic presentations, whereby clinical manifestations occur elsewhere in the body, and non-syndromic presentations, where clinical manifestations are localised to the eye or retina (Karuntu *et al.*, 2025a). Classification of IRDs can also be divided into stationary and progressive subtypes. Stationary retinal disorders are congenital or early-infantile onset and predominantly cause cone or rod dysfunction, but show little or no disease progression with time (Georgiou, Fujinami and Michaelides, 2021). Progressive retinal disorders are typically of later onset and are classified by the primary affected cell type or location. They include rod-cone degeneration, cone-rod degeneration, cone-only degeneration, maculopathies and generalised associated Retinal Pigment Epithelium (RPE) disease (Berger, Kloeckener-Gruissem and Neidhardt, 2010). It is believed that cone photoreceptors cannot survive without rod photoreceptors. Therefore, rod-only disease phenotypes do not exist (Ait-Ali *et al.*, 2015).

1.2 Presentation of Rod-Cone Degeneration

This critical analysis is focused primarily on the most common type of IRD, those causing rod-cone degeneration, where rod photoreceptors degenerate primarily, followed by cones (Daiger, Sullivan and Bowne, 2013). Patients typically present with nyctalopia (difficulty seeing in low light conditions) and peripheral vision loss due to rod photoreceptor degeneration (Buckley *et al.*, 2022). As the disease progresses, degeneration advances radially (inward and outward), encroaching on the macula, affecting central vision. Eventually, patients enter late-stage disease and experience severe visual impairment. Traditionally, rod-cone degenerations were characterised by electroretinographic measures that were either severely reduced or demonstrated absent a⁻ and b⁻ waves (Berger, Kloeckener-Gruissem and Neidhardt, 2010). Age of onset and disease progression have been shown to vary considerably with different rod-cone degenerations; even within the same genotype, a significant degree of individual phenotypic variation often exists (Berger, Kloeckener-Gruissem and Neidhardt, 2010; Aylward *et al.*, 2018).

Retinitis pigmentosa (RP) traditionally represents a disease group of clinically similar phenotypes that typically present as a rod-cone degeneration, which are associated with over 60 different genotypes (Schneider *et al.*, 2022). RP is the most common IRD, with an estimated prevalence of between 1 in 3000 and 1 in 7000 (Daiger, Bowne and Sullivan, 2007). Loss of photoreceptors and degeneration of Bruch's membrane enable RPE cells to migrate into the retina, causing pigmentary retinopathy (Figure 1.1). As degeneration progresses, attenuation of retinal vessels and a waxy, pale optic nerve head are typically observed.

Choroideremia is an X-linked IRD that results from RPE degeneration with secondary degeneration of rods, followed by cones; despite this, it is typically considered a rod-cone degeneration since the initial symptoms overlap with RP. As a result, choroideremia is sometimes misdiagnosed as RP. However, choroideremia does not present with the characteristic pigmentary retinopathy observed in RP (Figure 1.1) (Nanda *et al.*, 2018). In later disease stages, retinal appearances diverge significantly.

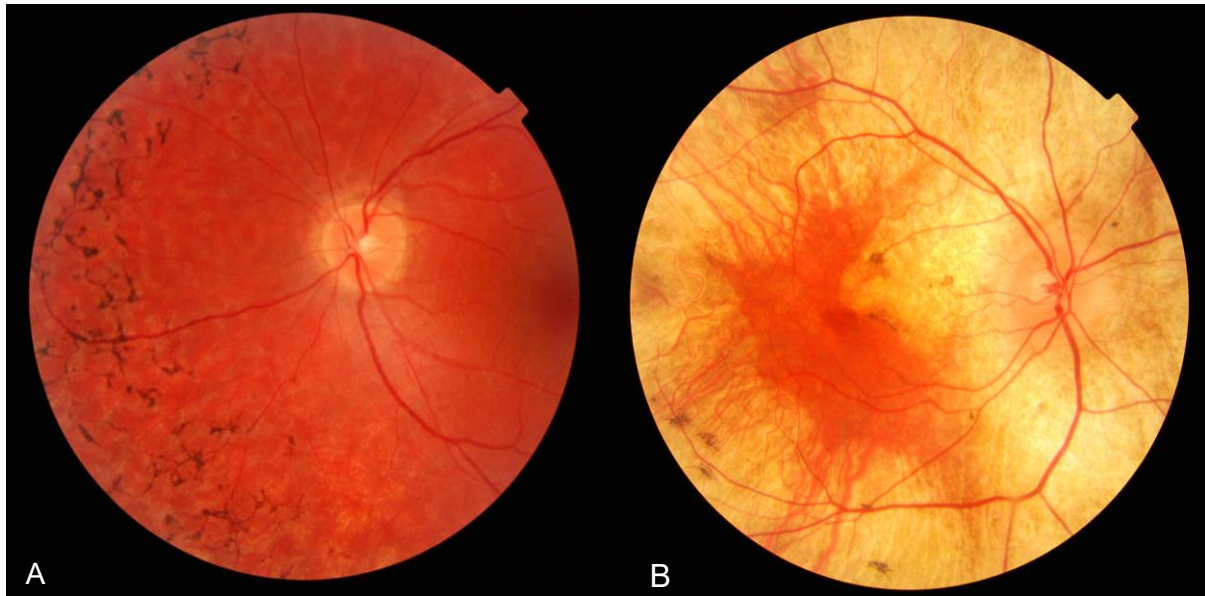


Figure 1.1: A) A colour fundus photo of a patient with X-linked RPGR-associated RP, showing the classical pigmentary retinopathy. B) Colour fundus photo of a patient with moderate stage choroideremia; the preserved central seeing island is surrounded by degenerated retina and RPE.

1.3 Inherited Retinal Disease Treatments and Clinical Trials

For the majority of IRDs, there are no approved treatments. In November 2019, the first ocular gene therapy was approved in the United Kingdom by the National Institute of Care and Excellence (NICE) for use in National Health Service (NHS) patients with *RPE65*-associated Leber congenital amaurosis, a rare but severe early-onset retinal dystrophy (Mahase, 2019). Due to the severity of the sight loss in these patients, standard vision measures were unsuitable to detect sufficient visual function detail and sensitivity changes. Furthermore, many trial participants were children, and so traditional visual function and psychophysical testing were too complex to achieve reliable results. This led to the development and regulatory validation of the Multi-Luminance Mobility test (MLMT), a navigational maze, used as the primary outcome measure, which ultimately supported the approval of the gene therapy (Russell *et al.*, 2017; Chung *et al.*, 2018). The MLMT assessed mobility function at nine controlled light levels (ranging from 400-1 lux). Alternative mobility maze tests have since been created for use as clinical trial endpoints for IRDs (Kumaran *et al.*, 2020; Zhou, 2023;

Jung *et al.*, 2024). The original MLMT was designed for patients with *RPE65*-associated Leber Congenital Amaurosis, an IRD causing severe visual impairment, to detect changes in rod photoreceptor function and impacts on low light mobility. The relevance and validation of MLMT or similar multi-luminance maze tests in other IRDs is yet to be determined. However, the case highlights the need for alternative visual function measures beyond visual acuity (VA) and standard perimetry. A call for alternative outcome measures was made at an international Endpoints Workshop by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Csaky *et al.*, 2017).

Numerous clinical trials for IRDs are ongoing, including gene and stem cell therapies, neuroprotection therapies and optogenetics (Fenner *et al.*, 2021; Brar *et al.*, 2024). In 2015 and again in 2020, the Monaciano Consortium, a group of specialist clinicians and researchers, established ambitious research priorities to support the development of new treatments for IRDs. One of the priorities was the development of effective and standardised disease-specific outcome measures that are non-invasive, safe, easy and quick to undertake with minimal patient burden, highly repeatable, reproducible, permit standardisation and are clinically meaningful (Thompson *et al.*, 2015; Thompson *et al.*, 2020). At the most recent Monaciano Symposia, expanding and standardising clinically relevant outcome measures was again identified as a research priority (Thompson *et al.*, 2025). Despite these recommendations, disease-specific outcome measures are an unrealistic short-term goal given the number of genotypes found within IRD and their rarity (discussed in section 1.1). A more realistic goal is to develop outcome measures relevant to clinical phenotype, whilst also considering disease severity and residual visual function.

The subsequent sections in this introduction chapter introduce the vision tests, which may be used as outcome measures in clinical trials, and are included in this series of work.

1.4 Acuity Tests

1.4.1 Standard Visual Acuity

Standard VA assessment using the Early Treatment Diabetic Retinopathy Study Chart (ETDRS) and protocol, designed by Ferris *et al.* (1982), is a well-established outcome measure in ophthalmic clinical trials and an indicator of central visual function (Figure 1.2). The test uses the principal design features of the Bailey-Lovie LogMAR (log minimum angle of resolution) chart, with logarithmic progression of letter sizes, enabling equal numbers of letters per line and equal spacing, to improve VA testing consistency (Bailey and Lovie, 1976). The ETDRS testing protocol uses a cumulative scoring system for VA (i.e., the number of letters read), making it more amenable to statistical analyses than traditional Snellen or LogMAR scoring systems. Furthermore, the ETDRS chart, compared to the more familiar Snellen chart, provides a greater range of larger letters, enabling more reliable VA assessment in individuals with low vision. It is robust, straightforward to implement, and low-cost; as a result, the testing procedures have remained unchanged for over 30 years. However, it is well accepted that standard VA is largely insensitive to subtle changes in visual function, particularly in the early stages of retinal disease (Cedrun-Sanchez *et al.*, 2025). Standard VA is also limited, as it reflects only the central foveal macular function. This is particularly problematic in patients with rod-cone degeneration, where patients' peripheral vision is typically affected first, and central visual function is preserved. Therefore, standard VA remains intact until extremely late in disease stages. In clinical trials, to assess the therapeutic efficacy of treatment administered within the macular region, alternative, more sensitive visual function measures are required. In addition, although standard VA using the ETDRS protocol assessments is commonly used in clinical trials for IRD, few studies have specifically examined the application and limitations in these patients.

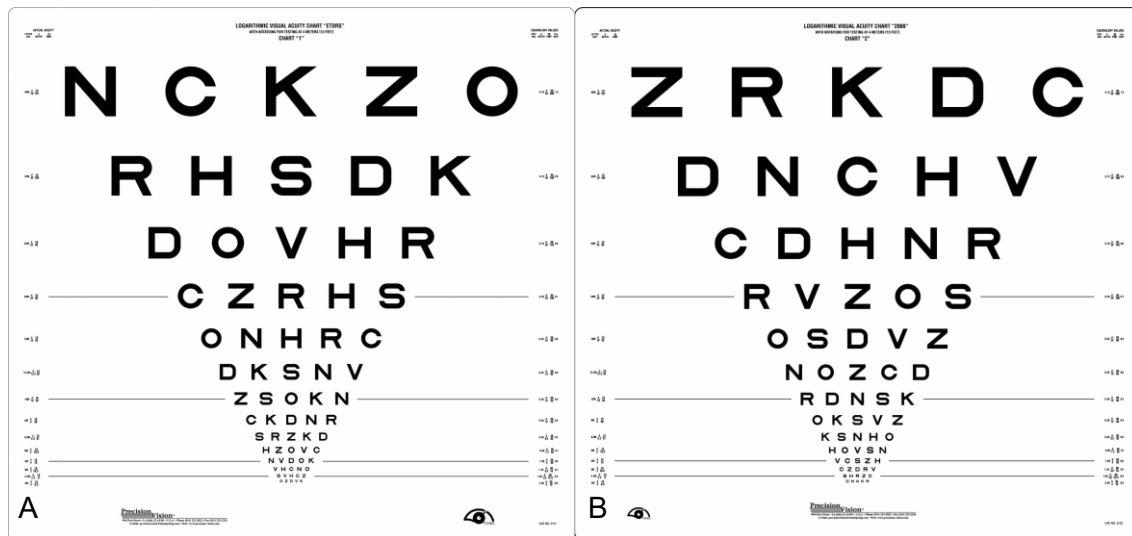


Figure 1.2: The standard ETDRS charts designed by Ferris et al.(1982). Chart 1 is used for right eye testing and chart 2 for left eye testing.

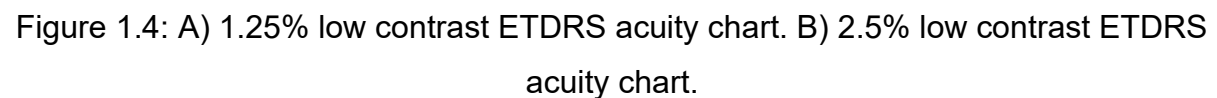
1.4.2 Low Luminance Visual Acuity and Low Luminance Deficit

Low luminance VA (LLVA) is the assessment of VA in lower background luminance conditions. It has become a promising outcome measure used in ophthalmic clinical trials, as it has demonstrated the ability to detect earlier changes in visual performance in patients with age-related macular degeneration (Sunness *et al.*, 2008). Various methodologies have been employed to test VA at different reduced luminance levels. However, the test, described by Sunness *et al.* (1997), uses the standard ETDRS letter chart, but with the addition of a 2.0 log unit neutral density filter placed in front of the patient's testing eye. Low luminance deficit (LLD), defined as the difference between standard VA and LLVA (units: number of letters), was first proposed by Sunness *et al.* (2008) as a functional indicator of disease progression in patients with geographic atrophy. The greater the LLD (i.e. the greater the disparity between LLVA and standard VA), the greater the risk of subsequent disease progression. See Paper 3 for a scoping literature review on LLVA and LLD; this is also discussed further in section 3.1.2 (Wood *et al.*, 2021b).



Figure 1.3: A representative view of the ETDRS chart as seen through a 2.0 log unit neutral density filter simulating LLVA.

Low-contrast acuity tests have also been designed using the basic principles of the standard ETDRS chart, but with letters printed at varying contrast levels. These are available at 1.25%, 2.5%, 5%, 10% and 25% contrasts (Precision Vision, Illinois, USA) (Figure 1.). The first reported use of lower contrast Snellen acuity charts was by Regan and Neima (1983). Subsequently, reduced low contrast acuity (before significant changes in standard VA were detected) was reported in early diabetic retinopathy, ocular hypertension, glaucoma and Parkinson's disease (Regan and Neima, 1984). Low contrast ETDRS charts have been commercially available since the 1990s and used to detect early visual dysfunction in neurological conditions. They have become a popular visual function measure in studies of multiple sclerosis with associated optic neuritis, Down Syndrome and Cerebral Palsy (Little *et al.*, 2013; Balcer *et al.*, 2017; Park *et al.*, 2020). However, use in IRDs is currently limited.



21

sclerosis. It is unknown how low contrast acuity correlates with early outer retinal changes, as seen in choroideremia and *RPGR*-associated RP.

1.4.4 The Moorfields Acuity Chart

The Moorfields Acuity Chart (MAC) was designed by Shah *et al.* (2016) and Anderson (2017) to be a more sensitive and repeatable alternative to the standard ETDRS VA test, to improve the detection of early age-related macular degeneration changes (Figure). The chart combines the design principles of the standard ETDRS chart and Cardiff Acuity Cards (Ferris *et al.*, 1982; Adoh and Woodhouse, 1994). It uses ‘pseudo-high-pass filtered’ letters to remove low-frequency content. This is achieved using black letters with a white surround and a grey background. The mean luminance of the black letters and white surround is almost equal to the grey background, resulting in the letter detection and recognition acuity thresholds, previously classified by Thibos and Bradley (1993), being almost equal under foveal viewing conditions in normal subjects. As a result, the letters disappear as soon as the resolution limit is reached, leading to ‘vanishing optotypes’. The rationale is that test-retest repeatability will be improved since patients are no longer required to guess and work out letters that they can just detect but not resolve. It also aims to eliminate ‘effort variability’ where some patients are likely to try harder to make out barely resolvable letters, and variability from different termination rules (Shah *et al.*, 2014).

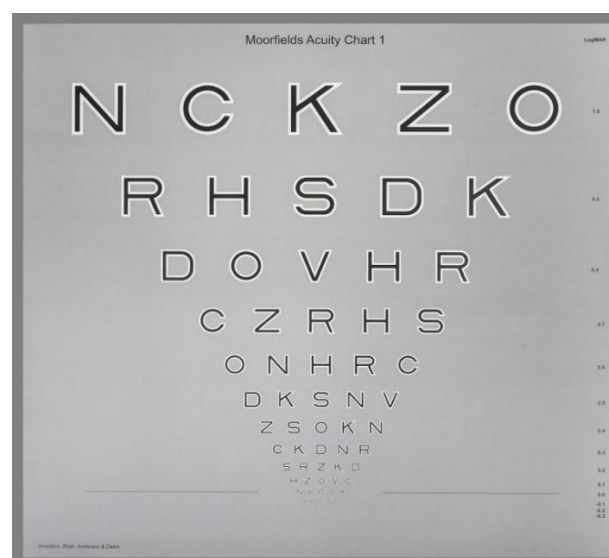


Figure 1.5: The Moorfield Acuity Chart (MAC) 1.

Shah *et al.* (2016) reported generally lower MAC VA scores compared to standard VA in both healthy controls and patients with age-related macular degeneration. This was attributed to loss of low-spatial-frequency optotype content, although it was also argued that this makes it more robust against subtle impacts of optical defocus such as uncorrected refractive error. The same study reported a proportional bias with greater VA versus MAC VA difference (-0.45 logMAR, ~ 22 letters) in early disease and a smaller difference (-0.26 logMAR, ~ 13 letters) in later disease stages. The study concluded that the MAC can detect early-stage functional loss due to age-related macular degeneration when standard VA remains relatively unaffected. The MAC is being explored as an outcome measure in the MACUSTAR study for use in intermediate age-related macular degeneration (Dunbar *et al.*, 2022). Until now, use of the MAC in IRD had been unexplored.

1.5 Mesopic Microperimetry

Mesopic microperimetry (also known as fundus-controlled perimetry) enables assessment of central macular sensitivity while using live retinal imaging and fundus tracking software to monitor participant fixation during testing. This enables more precise measurements of visual function at specific retinal locations, thereby facilitating more accurate structure-function relationships, when compared to traditional static perimetry, which does not include fundus tracking. This fundus tracking feature enables quantification of fixation stability as well as the theoretical assessment of central retinal sensitivity in patients with a complete absence of foveal fixation. A thorough review of microperimetry was published by Pfau *et al.* (2021), describing the theoretical and technical development, its use in clinical and research settings, as well as summarising future developments. The Macular Integrity Assessment Device (MAIA, CenterVue S.p.A., Padova, Italy) has emerged as the leading microperimetry testing device and is the focus of this work. It has improved fundus tracking capability, image quality and testing dynamic range in comparison to older first-generation devices (Pfau *et al.*, 2021). Figure 1.6: Standard MAIA microperimetry results output from a healthy patient after threshold testing with the 10.2 grid. A) Scanning laser ophthalmoscope (SLO) image. B) Zoomed SLO image with pointwise threshold sensitivity results and fixation mapping overlaid. C)

Interpolated sensitivity heat map overlaid onto SLO image; the brighter the colour, the greater the sensitivity. D) Average mean sensitivity result (dB). E) Locations of fixation during the test overlaid onto a zoomed SLO image and preferred retinal locus mapping. F) Histogram of pointwise thresholds with normative age-matched reference distributions (green). H) Bivariate Contour Ellipse Area (BCEA) Fixation tracking indices. G) Fixation time series graph showing the amplitude of fixational eye movements throughout testing..6 shows the standard MAIA microperimetry output of results.

Test Test - OD

01-Jan-1900 (119)

Expert exam #9397 [4-2 strategy] / 15-Oct-2019, 09:19 / Duration: 8' 20"

Fixation Losses: 0%

maia
Macular Integrity Assessment

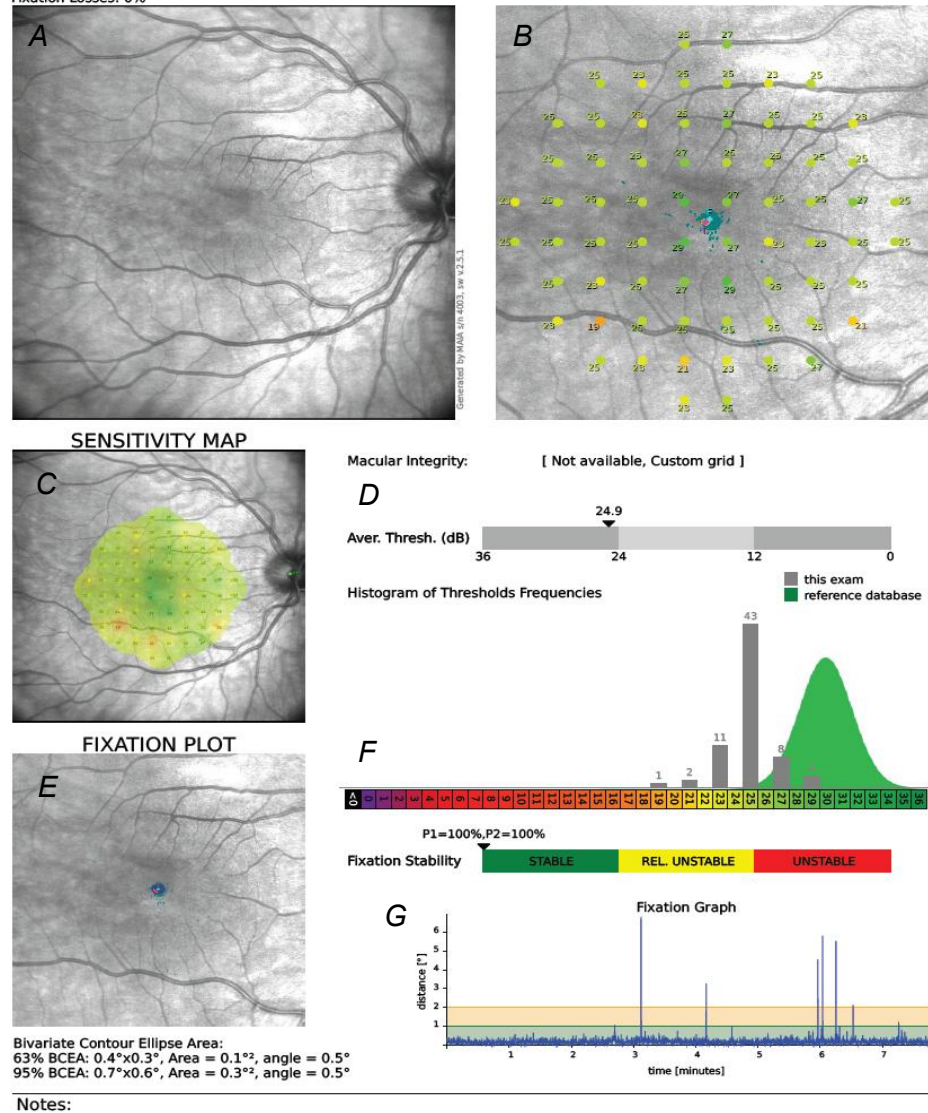


Figure 1.6: Standard MAIA microperimetry results output from a healthy patient after threshold testing with the 10.2 grid. A) Scanning laser ophthalmoscope (SLO) image.

B) Zoomed SLO image with pointwise threshold sensitivity results and fixation mapping overlaid. C) Interpolated sensitivity heat map overlaid onto SLO image; the brighter the colour, the greater the sensitivity. D) Average mean sensitivity result (dB).

E) Locations of fixation during the test overlaid onto a zoomed SLO image and preferred retinal locus mapping. F) Histogram of pointwise thresholds with normative age-matched reference distributions (green). H) Bivariate Contour Ellipse Area (BCEA) Fixation tracking indices. G) Fixation time series graph showing the amplitude of fixational eye movements throughout testing.

1.5.1 Mesopic Microperimetry Testing Parameters

Mesopic conditions refer to vision in low-to-moderate lighting (between 0.01-3.0 cd/m²). Mesopic microperimetry is the assessment of central retinal sensitivity in these dim conditions. A detailed testing protocol specifically for MAIA microperimetry assessment was published in an attempt to standardise testing (Talib, Jolly and Boon, 2018). The MAIA has various testing grid options and testing algorithms. The 10-2 grid with 68 testing points has emerged as the grid of choice for many clinical trials. It covers the central 20° in diameter, with 2° spacing between points. The device has supra and full threshold testing options. The 4-2 staircase bracketing strategy is used for determining thresholds with high accuracy in a reasonable timeframe. Standard sensitivity indices include pointwise sensitivity and mean sensitivity, which is an average of all pointwise loci, measured in decibels (dB) (Figure 1.6).

1.5.1.1 Pupil dilation

Microperimetry uses a Maxwellian view optical system, where a lens focuses the light directly on the pupillary plane. Retinal illumination should remain constant irrespective of pupil size. A minimum 2.5mm pupil is required to ensure the light passes through without hindrance and to permit fundus tracking (Westheimer, 1966; Pfau *et al.*, 2021). Since the test is performed in darkened (mesopic) conditions where the pupil naturally dilates to enable more light to enter the eye, the minimum pupil size should be reached without pharmacological pupil dilation. The reported mean mesopic pupil diameter is 6.4mm (± 0.9) in healthy subjects with a mean age of 44.6 ± 10.9 years (range, 12–70), which is well within the 2.5mm minimum (Yang, Thompson and Burns, 2002). Despite this, there is often inconsistency regarding pupil dilation before microperimetry testing in clinical trial protocols.

Han *et al.* (2017) investigated the effects of pupil dilation and microperimetry test sensitivity, they reported no significant differences in average threshold sensitivity, pointwise thresholds (peripheral macula and central), or fixation stability, using microperimetry with and without pupil dilation in both patients with choroideremia and healthy controls (post-operative cataract patients). The study also investigated the

effects in 'younger' and 'older' cohorts and found no significant effects on sensitivity with and without pupil dilation. Nevertheless, pupil dilation before testing is still routinely used in patients with geographic atrophy (Wu *et al.*, 2024; Alibhai *et al.*, 2025), which is typically an age-related disease. Since pupil diameter naturally reduces with age, this may be to ensure all participants reach the 2.5mm minimum pupil size and are able to perform the test.

1.5.1.2 Dark Adaptation

To understand the effects of dark adaptation on microperimetry testing, a study by Han *et al* (2019) investigated the effects of varying dark adaptation times on retinal sensitivity and fixation. They showed no significant differences in threshold sensitivity or fixation performance with increasing dark adaptation in either healthy controls or patients with choroideremia. In a somewhat extreme case, healthy controls were exposed to a bright light (direct retinal ophthalmoscope) and then split into a group that underwent 10 minutes of dark adaptation and a group that did not. The results showed a statistically significant sensitivity difference (mean increased sensitivity of 0.3 dB) in the dark adaptation group. However, since this increase is small and is likely to be within test-retest variability, it was deemed not clinically significant. Further dark adaptation, beyond 10 minutes, did not show any increases in central retinal sensitivity. The study concluded that no formal dark adaptation before microperimetry testing is required unless there has been exposure to extreme bright light, such as retinal photography or slit lamp examination; in these cases, 10 minutes of dark adaptation is sufficient in healthy controls. However, it was noted in discussions that participants with choroideremia verbally reported that they found testing more difficult without dark adaptation (Han *et al.*, 2019). Therefore, it has been suggested for improved participant comfort to allow 5 to 10 minutes of informal dark adaptation for all participants (Talib, Jolly and Boon, 2018).

1.5.2 Mesopic Microperimetry Reliability Indices

1.5.2.1 Fixation losses

The MAIA device measures responses to suprathreshold stimuli presented in the physiological blind spot, termed fixation losses (or Heijl-Krakau fixation catch-trial errors) (Heijl and Krakau, 1975). Assuming accurate fundus tracking (permitted by microperimetry testing) and correct setup (selection of the optic nerve head and correct patient alignment), these responses are generally assumed to represent false positive catch trials. These catch trials are presented approximately 1/minute (Pfau *et al.*, 2021). Thirty per cent fixation losses (albeit, arbitrarily) are often used as an acceptable cut-off for test reliability (Talib, Jolly and Boon, 2018). Given the average test takes around 6-9 minutes (Pfau *et al.*, 2021), this implies that greater than 2-3 responses to the 6-9 stimuli presented in the blind spot, the test is deemed unreliable. No formal false positive or false negative catch trials are incorporated into the testing algorithm.

1.5.2.2 Fixation stability.

Given the fundus tracking capability of the MAIA, fixation location and stability (of gaze on fixation target) data are collected throughout testing. The fixation location is termed the preferred retinal locus (PRL). The MAIA device measures this PRL initially during the first 10 seconds of testing (denoted as PRL_i , i for initial) and PRL_f (final) for the centre of the remainder of the test. Differences between PRL_i and PRL_f indicate large deviations in fixation locations throughout testing, thus, unstable fixation (Morales *et al.*, 2013). Fixation stability outputs include P1 and P2, which correspond to the percentage of fixation points falling inside 1° and 2° radii of the final preferred retinal locus, respectively (Morales *et al.*, 2016). Alongside a categorical classification of the fixation stability described by Fujii *et al.* (2002) where for stable fixation, P1 must include $>75\%$ of fixation points. For relatively unstable fixation classification, P1 must include $<75\%$ and P2 must include $>75\%$ of fixation points. An unstable fixation classification occurs when $<75\%$ of fixation points are within P2. Bivariate contour ellipse area (BCEA) indicates the area and orientation of a two-dimensional ellipse encompassing a given proportion (either 95% or 63%) of the fixation points. The

smaller the BCEA value, the better the fixation stability. BCEA is the recommended metric for accurate fixation stability assessment (Crossland, Dunbar and Rubin, 2009).

1.5.3 Mesopic Microperimetry: A Test of Cone Function?

In mesopic conditions, retinal sensitivity as measured by microperimetry is thought to reflect non-opponent M+L and S cone function within the central 10-degree radius of fixation (Simunovic, Moore and MacLaren, 2016). This notion is further supported by the absence of an increase in central retinal sensitivity following periods of dark adaptation (10 minutes), which indicates that responses are mediated by cone photoreceptors with negligible input from rod photoreceptors, as rod sensitivity would typically show an enhancement in retinal sensitivity with extended dark adaptation (Han *et al.*, 2019). Nevertheless, other studies have proposed that rod photoreceptor input can modify cone photoreceptor responses through either rod activation, inducing cone suppression or through the summation of rod and cone responses via cone bipolar cells and the lateral geniculate nucleus. Consequently, central sensitivity that reflects cone responses may be influenced by diminished or abnormal rod inputs (Zele, Maynard and Feigl, 2013; Zele and Cao, 2014).

1.5.4 Mesopic Microperimetry in Rod-Cone Degeneration

Yang and Dunbar (2021) have published a comprehensive review of microperimetry as a clinical trial outcome measure in IRD. The review included publications from six choroideremia trials, one *RPGR*-associated RP trial and five RP trials with nonspecific genotypes. The review details testing devices, strategies and reported metrics. Here, more recent applications of microperimetry, in rod-cone degenerations and its appropriateness at various stages of the disease, are discussed.

In a natural history and outcome measure exploration study focused on patients with *USH2A*-associated RP (RUSH2A), microperimetry was concluded to be superior to static perimetry due to greater precision of stimulus placement, enabling a higher percentage of eyes showing reduced sensitivity with time (Maguire *et al.*, 2024).

Microperimetry has been used to explore the characteristics and natural history of retinal degeneration in females carrying mutations in the *CHM* gene. Statistically significant reductions in central sensitivity have been shown in female *CHM* gene carriers with coarse, geographic and male-pattern retinal changes (Thobani, Anastasakis and Fishman, 2010; Gocuk *et al.*, 2024). However, microperimetry showed no significant difference in central sensitivity in those with fine retinal changes, observed with fundus autofluorescence imaging, compared to healthy controls, suggesting it is unable to detect any reduced function in those with the most subtle of changes (Gocuk *et al.*, 2024). Although the article did not specifically define what ‘fine’ retinal changes are.

In *RPGR*-associated RP, information on the mean sensitivity rate of change is limited. XOLARIS, a prospective natural history study, reported the mean retinal sensitivity change from baseline to month 24 was -0.7 dB (standard deviation ± 1.8 dB) in the lower VA subgroup and -0.4 dB (standard deviation ± 2.0 dB) in the higher VA subgroup. However, these differences were not found to be statistically significant. They are likely reflective of the slowly progressive nature of the disease. In a different *RPGR*-associated RP natural history study reported by Anikina *et al.* (2022) an annual mean sensitivity rate of progression of 0.8 dB (SD ± 0.9 dB) and 0.7 dB (SD ± 0.9 dB) was reported for the right and left eyes, respectively. This data was collected using the Nidek MP1 microperimeter, which used a limited dynamic testing range of 0-20 dB. The study used a linear best-fit analysis due to the short follow-up interval (mean follow-up 2.8 years); ideally, a longer follow-up period is required to understand the exact degenerative trend. Despite this, the study showed that microperimetry has a high degree of reproducibility and good interocular correlation (Anikina *et al.*, 2022).

Microperimetry was used as a secondary outcome measure in a phase I/II dose escalation clinical trial (XIRIUS, [https://clinicaltrials.gov/":NCT03116113](https://clinicaltrials.gov/)), targeting patients with moderate *RPGR*-associated RP (VA greater than or equal to 34 letters, equivalent to Snellen 6/120). MAIA microperimetry testing was performed using the

10-2 68-point rectilinear grid. The 12-month results for part one (dose escalation phase) of this trial reported mean sensitivity microperimetry gains of 5.1 dB in the six responders, compared with a reduction of -0.9 dB in the untreated eyes. In addition to an average of 4.7 central 16 loci increasing by ≥ 7 dB, as opposed to 1.2 loci increasing in untreated eyes. Thirty-three per cent of participants showed a sustained gain and met the predefined microperimetry endpoint (≥ 7 dB improvement from baseline at ≥ 5 of the 16 central loci) (von Krusenstiern *et al.*, 2023). Despite these promising results, the 12-month part two (high dose, low dose and control group phase) study results showed no statistically significant differences in the proportion of participants meeting the predefined microperimetry endpoint between treatment groups (low-dose group, 3/8 [37.5%; $P = 0.3181$]; high-dose group, 2/8 [25.0% $P = 0.5177$]) and the control group (2/9 [22.2%]). This was attributed to limited recruitment and underpowered sampling resulting from early study termination due to the coronavirus 19 pandemic (Lam *et al.*, 2024). A post-hoc analysis across all 68 loci, however, found that a statistically significant mean sensitivity change from baseline in the low-dose group versus the untreated group was observed at month 12 (2.79 dB [95% CI: 1.27-4.32 dB] vs. 0.11 dB [95% CI: -0.63 to 0.85 dB]; $P = 0.04$). Despite the significance of the mean sensitivity change from baseline results, this was not considered the primary endpoint, and so the study was deemed unsuccessful.

In a third *RGPR*-associated RP gene therapy clinical trial, also using patients with moderate-stage retinal disease defined by an entry criterion of VA less than 78 letters but still able to complete visual function tests, microperimetry was used as a secondary endpoint. This trial reported at 12 months, more than half (4/7) of the participants at the highest tolerated dose showed an improvement of at least 7.0 dB from baseline in ≥ 5 of the 68 test loci (Yang *et al.*, 2025). This has led to an ongoing phase II/III clinical trial (NCT04850118), in which microperimetry is a secondary outcome measure in the USA and a primary outcome measure in Europe. The trial uses a change from baseline in mean sensitivity across the whole grid with the proportion of responders with an improvement of ≥ 7 dB in at least 5 loci, and a change from baseline in mean sensitivity across the central 4 loci at months 12, 18, and 24 used as secondary outcome measures.

Microperimetry has also played a key role in the deep phenotyping of *RPGR*-associated RP patients, showing how the phenotype as measured by microperimetry changes as the genetic variant location approaches the distal part of the open reading frame (ORF) 15 region (Cehajic-Kapetanovic *et al.*, 2022). This is consistent with the current understanding that microperimetry is associated with cone photoreceptor function and has specifically been shown to detect reduced function from shortened photoreceptor outer segment (Micevych *et al.*, 2023).

In choroideremia studies, microperimetry has been extensively researched and significant retinal sensitivity correlations with retinal structural changes have been demonstrated, such as with the area of remaining choriocapillaris and preserved fundus autofluorescence ($r=0.81$, $p=0.001$ and $r=0.60$, $p=0.02$, respectively)(Hagag *et al.*, 2021). Furthermore, microperimetry mean sensitivity was shown to be statistically significantly greater in areas of smooth appearance (26.1 ± 2.0 dB) versus mottled appearance (20.5 ± 4.2 dB) ($P < 0.0001$) with short-wavelength fundus autofluorescence imaging (Poli *et al.*, 2023). A survival analysis of microperimetry mean sensitivity showed that the median survival age for detectable retinal sensitivity was 39 years, with a half-life of 13.6 years, and with significant inter-eye symmetry (Jolly *et al.*, 2017). Microperimetry has also been used as a secondary outcome measure, using mean sensitivity change from baseline, in several early-phase choroideremia gene therapy trials (MacLaren *et al.*, 2014; Dimopoulos *et al.*, 2018; Fischer *et al.*, 2019; Lam *et al.*, 2019; Cehajic-Kapetanovic *et al.*, 2024). More recently, microperimetry using mean sensitivity indices emerged as the leading ranked outcome measure for patients with early-moderate stage choroideremia in a dominance analysis. However, wide 95% confidence intervals (CI) suggest significant variability (Josan *et al.*, 2024). Despite this promising data, a phase 3 choroideremia gene therapy trial targeting later-stage patients, which included microperimetry as a secondary outcome measure, found its results from this cohort to be inconsistent. In this trial, many participants were unable to perform the test accurately or with any detectable sensitivity. This was attributed to the advanced disease stages of the cohort (MacLaren *et al.*, 2023) and shows that microperimetry is not suitable in all instances.

In a recent study examining a mixed cohort of patients with IRD, microperimetry was found to be significantly associated with all functional domains of the Michigan Retinal Degeneration Questionnaire (MRDQ), except for photosensitivity. Suggesting it is highly reflective of vision-related quality of life (Karuntu, Nguyen and Boon, 2024).

Overall, these results suggest that microperimetry has great potential to detect therapeutic changes in early-moderate disease patients with detectable central sensitivity. However, further research is needed to support the selection of suitable microperimetry endpoints for use in IRD clinical trials.

1.5.5 Volume Sensitivity in Mesopic Microperimetry

As an alternative to the standard pointwise and mean sensitivity indices, volume sensitivity uses pointwise sensitivities connected by interpolation techniques to multiply retinal sensitivity within a region by the area covered by that region. It uses the 'hill of vision' concept, which was first described by Traquair (1927), reflecting the total amount of sensitivity within a given visual field area. This approach has been previously used in standard static perimetry (using data collected from the Octopus 900 [Haag-Streit AG, Köniz, Switzerland]), alongside a custom program termed the visual field modelling and analysis (VFMA) (Weleber *et al.*, 2015). The program has subsequently been used as an outcome measure in a gene therapy clinical trial for *RPE65*-associated Leber Congenital Amaurosis, in a natural history study for childhood-onset Stargardt disease and an investigation into structure-function correlations between optical coherence tomography and standard automated perimetry in patients with autosomal dominant RP (Smith *et al.*, 2016; Weleber *et al.*, 2016; Tanna *et al.*, 2018). Since the visual field area in static perimetry testing is much larger and the perimetry bowl is curved, the VFMA uses decibel-steradians (Weleber *et al.*, 2015). Following this, volumetric analyses have been used in microperimetry using a microperimetry-adapted version of the VFMA program, investigating childhood-onset Stargardt disease, as an outcome measure in a stem cell clinical trial for Stargardt disease and in a cohort of patients with *RPGR*-associated RP. However,

this data was collected using the Nidek MP1 with a limited dynamic range of 0-20 dB. Volumetric analyses using the MAIA microperimeter had only been used to investigate the use of customised grids in patients with choroideremia (Dimopoulos, Tseng and MacDonald, 2016). To the best of our knowledge, MAIA microperimetry volume sensitivity indices had not been more broadly explored.

1.6 Scotopic Microperimetry

Scotopic microperimetry (also known as dark-adapted microperimetry or two-colour fundus-controlled perimetry) is a modified version of mesopic microperimetry performed under scotopic background luminance levels ($<0.001 \text{ cd/m}^2$). It has been suggested as a strategy to spatially assess isolated rod and cone function and enable earlier detection of IRD progression (Thompson *et al.*, 2020). The S-MAIA (Scotopic Macular Integrity Assessment, CenterVue S.p.A., Padova, Italy) is the only device with in-built two-colour scotopic microperimetry testing capabilities, and uses the same fundus imaging and eye tracking capabilities as mesopic microperimetry. Other scotopic microperimetry devices are available, such as the NIDEK MP-3, but these are only set up for single colour stimulus testing unless additional filters are added. Overall, scotopic microperimetry is considerably less validated compared to mesopic microperimetry. Paper 9 provides a more comprehensive literature review of scotopic microperimetry, and is discussed in more detail in section 3.3 (Taylor *et al.*, 2022).

1.6.1 Scotopic Microperimetry Testing Parameters and Reliability Indices

There is no published scotopic microperimetry testing protocol. The testing setup and process are very similar to mesopic microperimetry; however, dark adaptation before testing and the order of coloured stimuli testing are significant considerations. Pupil dilation, which is discussed in detail in section 2.2.1.1, is not considered necessary as long as participants have a minimum pupil diameter of 2.5mm (Pfau *et al.*, 2021). The average natural pupil size under scotopic conditions is between 6.02 (± 0.93) mm and 6.19 (± 0.86) mm in individuals aged 23 to 43 years (Liu *et al.*, 2017), which indicates most individuals would achieve the minimum required diameter without pharmacological dilation. However, no studies have explored the effects of pupil

dilation on scotopic microperimetry. The S-MAIA uses the same fixation stability and reliability indices used in mesopic microperimetry and described in section 2.2.2. The output of results is the same as for mesopic microperimetry, although the contouring of the coloured heat maps is not equivalent (Figures 1.6 & 1.7). A novel reliability marker termed 'wrong pressure events' using the raw fixation tracking data collected by the S-MAIA device was proposed by Montesano et al. (2021) and showed high predictability with test-retest variability, unlike other reliability markers. However, its reliance on computational post-hoc analyses limits its current usability in a clinical setting; also, it is unclear how delays in testing due to unstable or loss of fixation are compensated for.

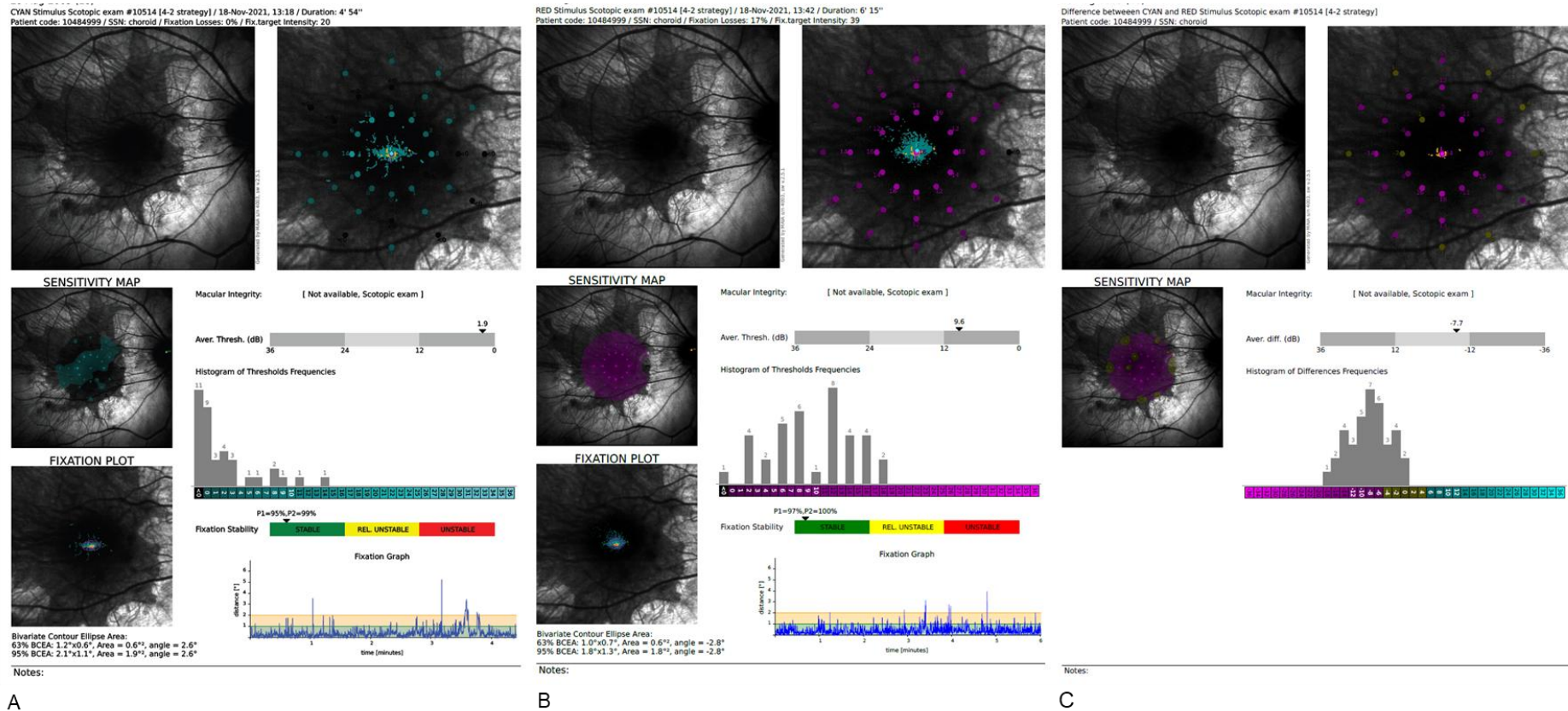


Figure 1.7: S-MAIA scotopic microperimetry results output for a patient with choroideremia. A) depicts the scotopic cyan results. B) illustrates the scotopic red results and C) details the cyan-minus red differences (discussed in section 1.6.2). The sensitivity and reliability indices are comparable to mesopic microperimetry discussed in section 1.5.

1.6.2 Scotopic Microperimetry: Rod versus Cone Photoreceptor Function.

In traditional two-colour perimetry, short-wavelength stimuli under scotopic conditions are used to preferentially stimulate rod responses, while long-wavelength stimuli under photopic conditions are used to preferentially stimulate medium wavelength (M) and long wavelength (L) cone responses. The S-MAIA two-colour stimulus testing uses scotopic conditions for both short (505nm, cyan) and long (627nm, red) wavelength stimuli, which enables evaluation of dark-adapted spectral sensitivities (Figure 1.7). Cyan stimuli responses are considered rod dominant; however, red stimuli responses under scotopic background conditions reflect mixed rod and cone function (Pfau *et al.*, 2021; Simunovic *et al.*, 2021). The cyan and red stimuli have been calibrated according to the International Commission on Illumination (CIE) 1951 scotopic luminosity function in healthy individuals, so that, in radiance, the red stimuli, at 0.0 dB, are approximately 20.0 dB brighter than cyan at 0.0 dB. Therefore, in a healthy retina, the cyan minus red difference should be 0.0 dB, and any difference between cyan and red sensitivities can theoretically indicate the underlying dominant photoreceptor function (Table 1.1).

Table 1.1: Scotopic Microperimetry interpretation aid, adapted from Heeren et al. (2019).

Cyan minus red (dB)	Cyan stimuli sensitivity	Red stimuli sensitivity	Interpretation	Maia colour code
0	Normal	Normal	Normal dark-adapted responses	Olive-green
0	Reduced	Reduced	Equally impaired mixed cone/rod and rod responses	Olive-green
<0	Reduced	Normal or mildly reduced	Impaired rod function relative to mixed cone/rod responses	Red
>0	Normal or mildly reduced	Reduced	Uncertain	Cyan

Decibels (dB)

The anatomical distribution of rods has been shown to correspond with the topographical sensitivity detected by cyan stimuli, with peak sensitivity at approximately seven degrees eccentricity and a sudden reduction in sensitivity between one and three degrees centrally, corresponding with the rod-free area (Curcio *et al.*, 1987; Curcio *et al.*, 1990; Pfau *et al.*, 2017). Dark adaptation has been shown to significantly affect the sensitivity to cyan stimuli, with greater sensitivity seen with longer adaptation time. However, this effect was not seen with red stimuli. This is further evidence that cyan stimuli tested under scotopic conditions are driven by rod photoreceptor function, and red stimuli are more cone-driven (Montesano *et al.*, 2021). Montesano *et al.* (2021) and Pfau *et al.* (2017) also found prolonged average response times with scotopic cyan testing compared to scotopic red or mesopic testing. This was attributed to the slower kinetics associated with the rod response pathway, due to retinal summation processing. Although Pfau *et al.* (2017) suggested it could be a result of the lower stimulus intensities, or perhaps a combination of both. Nevertheless, this supports the hypothesis that cyan stimuli in healthy participants preferentially probe rod photoreceptor-driven function. However, it is unclear if these results translate to testing patients with rod-cone degeneration and other retinal degenerations.

1.7 Aims of PhD and Critical Analysis

This research aimed to evaluate different visual function tests for use as outcome measures in clinical trials for rod-cone degeneration. Since patients with rod-cone degeneration report significant visual difficulties in low-light conditions, this work particularly focused on tests under these conditions.

Specifically, the following research themes were addressed:

1. To investigate VA testing approaches, including standard VA letter chart selection, low-contrast VA, LLVA, and the MAC, as potential clinical trial outcome measures (see Papers 2-7 discussed in section 3.1).
2. To evaluate the suitability of mesopic microperimetry sensitivity indices for detecting functional decline in patients with IRD (see Paper 8, discussed in section 3.2).
3. To further explore the feasibility of scotopic microperimetry as an outcome measure in IRD (see Papers 9-11, discussed in section 3.3).

This critical analysis synthesises and evaluates this series of work to develop evidence-based recommendations for the use of visual function outcome measures for use in rod-cone degeneration clinical trials. Ultimately, this seeks to support future clinical trial design and ensure that visual function outcome measures are optimised to measure treatment effects, which are required to evaluate therapeutic efficacy.

Chapter 2: Structure of Critical Analysis and Methods

This chapter describes the structure of this critical analysis, the publications included, and the outcome measure evaluation approaches. Chapter 3 will then synthesise and evaluate the findings from the 11 peer-reviewed publications included in this PhD by prior publication submission, where I (Laura Taylor, née Wood) was a major contributor (Table 2.1). These include five first-authored original research publications, one co-first-authored original research publication, two co-senior-authored original research articles, two first-authored literature reviews and a first-authored published study protocol. All publications have been published in high-quality peer-reviewed national and international journals between 2021 and 2025. The publication references and author contributions for each article are summarised at the beginning of each relevant section in Chapter 3. The critical analysis also discusses other literature where relevant to enable thorough evidence-based recommendations. The visual function outcome measure evidence-based recommendations for use in clinical trials targeting rod-cone degeneration are then discussed in Chapter 4.

Table 2.1: Summary of published works discussed in this critical analysis.

Publication		Research Focus	Key Findings
Protocol Paper			
1	A cross-sectional study to assess the clinical utility of modern visual function assessments in patients with inherited retinal disease (Taylor et al., 2023)	Study protocol for a study investigating visual function tests in inherited retinal disease	Detailed test methodology for each visual function test, the need for test validation is explored
Acuity Publications			
2	Investigating the impact of asymmetric macular sensitivity on visual acuity chart reading in choroideremia (Baffour-Awuah et al., 2024)	Analysing how asymmetric macular function affects VA in choroideremia	Asymmetric sensitivity impacts VA test performance

3	Low luminance visual acuity as a clinical measure and clinical trial outcome measure: a scoping review (Wood et al., 2021)	Review of LLVA and its use as an outcome measure	Identified methodological inconsistencies and areas for standardisation
4	Low luminance visual acuity and low luminance deficit: optimising measurement and analysis (Forte et al., 2025)	Standardising LLVA testing conditions	Small but statistically significant changes in LLVA scores were found in different test conditions
5	Low Luminance Visual Acuity and Low Luminance Deficit in Choroideremia and RPGR-Associated Retinitis Pigmentosa (Wood et al., 2021)	Evaluating LLVA as a marker for disease severity	Changes in LLVA appear to be a marker for the onset of late-stage disease
6	Exploring standard and low luminance visual acuity and the Moorfields Acuity Chart as outcome measures in inherited retinal disease (Taylor et al., 2025)	Comparing standard VA, LLVA, and the Moorfields Acuity Chart	Comparable sensitivity and reliability in the three tests. Similar decline rates in early disease.
7	Low-contrast visual acuity versus low-luminance visual acuity in choroideremia (Wood et al., 2021)	Comparison of low-contrast and low-luminance VA in Choroideremia	LLVA detected greater functional variation than low-contrast VA
Mesopic Microperimetry Publications			
8	Microperimetry as an Outcome Measure in RPGR-associated Retinitis Pigmentosa Clinical Trials (Taylor et al., 2023)	Evaluating mesopic microperimetry for <i>RPGR</i> -associated RP clinical trials	Highlight test-retest variability limitations with pointwise analyses and limitations with mean sensitivity indices
Scotopic Microperimetry Publications			
9	Scotopic microperimetry: evolution, applications, and future directions (Taylor et al., 2022)	Review of Scotopic Microperimetry	Identified testing considerations and directions

			for future research
10	Exploring Scotopic Microperimetry as an Outcome Measure in Choroideremia (Taylor et al., 2024)	Investigating scotopic microperimetry in choroideremia	Reported high false-positive rates and test variability
11	Scotopic Microperimetry: An Analysis of Healthy Controls (Adeyoku et al., 2025)	Assessing scotopic microperimetry in healthy participants	Identified baseline variability and reliability markers

2.1 Visual Function Data Collection

Paper 1

A cross-sectional study to assess the clinical utility of modern visual function assessments in patients with inherited retinal disease: a mixed-methods observational study protocol (Taylor et al., 2023b)

A published protocol for the Visual Function in Inherited Retinal Disease study (VFIRD), outlining the study design, tests and planned analyses.

Freely available at:

<https://bmcoophthalmol.biomedcentral.com/articles/10.1186/s12886-023-02974-6>

Author contributions

LT (main contributor) was responsible for developing the study design and drafting the protocol. The National Institute for Health and Social Care Research Design Service, as well as all co-authors, also provided input into aspects of the study design and statistical analyses.

The data used in the publications discussed in this critical analysis were collected primarily as part of the recruitment process for retinal gene therapy clinical trials (NCT03359551, NCT02407678, NCT03116113) as well as part of the Visual Function in Retinal Degeneration study (ISRCTN24016133, Paper 1). Specific methodology is discussed in each publication, with further details included in Paper 1 (Taylor *et al.*, 2023b). In all studies, patients attended specialist retinal genetics clinics at Oxford Eye Hospital and completed specialist testing, usually alongside their NHS outpatient appointments. Paper 3 (Forte *et al.*, 2025) is the only exception, which included data collected from the Oxford University medical school as a hospital access result of restrictions during the coronavirus pandemic.

2.2 Vision Test Evaluations

Currently, there is no standardised framework for validating and determining the most effective outcome measures (Thompson *et al.*, 2020). Given the diverse

methodologies used in vision testing, it is unlikely that a single framework could effectively address the nuances of each outcome measure and its specific application. The publications included in the PhD by prior publication submission, and this critical analysis aligns with the priorities set out by the Monaciano Consortium over the last 10 years (Thompson *et al.*, 2015; Thompson *et al.*, 2020; Thompson *et al.*, 2025). These include improving outcome measure testing with a focus on test standardisation, reliability, repeatability and understanding clinical and meaningful significance.

2.2.1 Test Standardisation

Standardisation of testing procedures and parameters is fundamental to enable meaningful comparisons across studies and clinical trials. The publications referenced in this critical analysis examine the standardisation of LLVA testing, mesopic microperimetry sensitivity indices, and scotopic microperimetry testing parameters.

2.2.2 Reliability

A test is considered reliable if it produces consistent results when repeated under the same conditions. Henceforth, reliability indices and patient performance are reviewed and reported using descriptive statistics, including medians and interquartile ranges (IQR) for non-parametric data and means, standard deviation (SD) and confidence intervals (CI) for parametric data.

2.2.3 Repeatability

Bland-Altman analyses, coefficients of repeatability (CoR), and 95% limits of agreement (LoA) are also used throughout this critical analysis to evaluate test-retest variability and to determine and redefine clinically significant change that is beyond natural variability (Bland and Altman, 1986; Bland and Altman, 1999). Previous studies have proposed using repeatability coefficients to provide a threshold value for the 'smallest real difference', to distinguish natural measurement variability from true 'clinical' change, following an intervention or disease progression (Beckerman et al

2001; Hamilton et al 2026; Vaz et al 2013). Karuntu et al (2025b) further stated that test-retest repeatability, defined from natural variability between repeated tests and used to determine a CoR, was essential for developing meaningful criteria for clinical trial endpoints.

2.2.4 Significance Definitions

The terminology for clinical significance or clinical meaningfulness is somewhat ambiguous, particularly across clinical trial and clinical practice settings. In clinical trials, as discussed above, clinical significance has been defined as a threshold value distinguishing natural variability from true clinical change (but not necessarily meaningful change), also referred to as the smallest real difference, and derived from repeatability analyses (Beckerman et al 2001; Hamilton et al 2026; Vaz et al 2013). Arguably, this would more accurately be termed Measurement Significance or Repeatability-Based Clinical Significance. Furthermore, clinical significance in everyday practice may refer to the magnitude of an observed change that is large enough to impact patient management, prognosis or visual status. However, for clinical trials, this is difficult to quantify and arguably overlaps with the proposed definition of visual function significance. To align with the publications included in this critical analysis as well as previous relevant literature, the following terminology, defined in Table 2.2, is used throughout this critical analysis.

Table 2.2: Summary of significance definitions proposed and used throughout this work.

Significance Definitions	
Statistical Significance	A quantitative evaluation of whether an observed result is likely due to chance or represents a true underlying relationship, typically determined by a p-value below a chosen threshold (e.g., 0.05).
Clinical Significance	A quantitative evaluation of whether the observed result is beyond that expected due to natural variability or inherent noise levels present in any given test – Repeatability-Based Clinical Significance.
Functional Significance	A qualitative or quantitative measure of how any observed changes in a test impact patients' functioning in everyday life, or measures of quality of life.

To explore these definitions in a clinical context, a recent phase I/II *RPGR* gene therapy trial reported a modest statistically significant improvement in BCVA at Week 26 between treated and control participants (least-squares mean treated–control difference 3.39 letters, 95% CI 0.22–6.56; $P = 0.037$) (Michaelides et al., 2024). However, several studies have reported larger test–retest variability limits (up to 7 ETDRS letters) for VA assessment (Taylor et al., 2021; Taylor et al., 2025). Therefore, despite achieving statistical significance, a change of 3.39 letters remains within the expected measurement variability of the test and is unlikely to represent a true clinically meaningful improvement from an outcome measure perspective. Conversely, if a hypothetical VA test demonstrated near-perfect repeatability with negligible measurement variability, even extremely small changes could exceed the repeatability threshold and therefore represent a ‘real’ detectable change. However, such changes may still lack meaningful functional relevance to patients in everyday life. This distinction highlights the importance of considering functional significance alongside statistical and repeatability-based clinical significance when interpreting clinical trial outcomes, as well as the importance of separating out the three distinct definitions.

Regulatory guidance from the FDA and EMA emphasises that outcome measures used to support therapeutic efficacy should demonstrate relevance to everyday functional vision and patient benefit (Csaky, Richman and Ferris, 2008; Csaky et al.,

2017). Consequently, the value of an outcome measure that achieves both statistical and repeatability-defined clinically significant change must also be interpreted within the broader context of functional relevance and treatment risk–benefit considerations. It is only after considering all three definitions that statements regarding trial success or failure can be made.

Chapter 3: Synthesis of Publications and Critical Review

This chapter synthesises and critically evaluates the eleven publications listed in Table 2.1 and included in this PhD by prior publication submission. It is organised into the three categories of visual function assessment examined: visual acuity, mesopic microperimetry, and scotopic microperimetry.

3.1 Acuity Tests

Paper 2

Investigating the impact of asymmetric macular sensitivity on visual acuity chart reading in choroideremia (Baffour-Awuah et al., 2024)

This primary research paper explores how patients with choroideremia, who have severely constricted vision, read across the acuity chart.

Freely available at: <https://onlinelibrary.wiley.com/doi/10.1111/opo.13356>

Author contributions

LT (joint main contributor) developed the study plan, collected some of the data, mentored KB, and revised and finalised manuscript drafts. KB (joint main contributor) collated the data, completed summary statistics and prepared initial manuscript drafts. AJ completed additional statistical analysis and provided professional oversight along with REM. JKJ also collected some of the data and reviewed the final drafts.

Paper 3

Low luminance visual acuity as a clinical measure and clinical trial outcome measure: a scoping review (Wood et al., 2021b)

This scoping review summarises the development, use and future applications for low luminance visual acuity.

Available at: <https://onlinelibrary.wiley.com/doi/10.1111/opo.12775>

Author contributions

LT (main contributor) was responsible for the development of research questions and the literature search. Data synthesis and drafting of the manuscript. Co-authors provided expert feedback and reviewed the final drafts.

Paper 4

Low luminance visual acuity and low luminance deficit: Optimising measurement and analysis (Forte et al., 2025)

Primary research paper analysing LLVA data from three separate studies to answer research questions identified in Paper 3, including exploring the effects of dark adaptation, luminance levels and normative ranges.

Freely available at: <https://pubmed.ncbi.nlm.nih.gov/40010402/>

Author contributions

This was a collaborative project with JKJ (joint senior contributor). LT (joint senior contributor) developed the research questions, study plan and setup for the Low Luminance Variability Study and mentored RA to undertake the study. For the Visual Function in Retinal Degeneration study, LT designed and set up the study, completed most of the data collection and collated the data. LT supported final manuscript drafting. RA completed data collection for the Low Luminance Visual Acuity Variability study. JKJ developed the research questions and study plan for the Electronic Visual Acuity Study and supported data collection, as well as mentored and supervised GF throughout. GF analysed the data and led the drafting of the manuscript.

Paper 5

Low Luminance Visual Acuity and Low Luminance Deficit in Choroideremia and RPGR-Associated Retinitis Pigmentosa (Wood *et al.*, 2021c)

This primary research paper investigates the performance of LLVA and LLD in patients with two distinct causes of IRD.

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2772299>

Author contributions

LT (main contributor) developed the research question and completed some data collection, data collation and analyses, interpretation, and drafting of the final manuscript. Other co-authors completed data collection (JKJ), gave feedback and reviewed the manuscript before submission (JKJ, AJ, TMWB, REM).

Paper 6

Exploring standard and low luminance visual acuity and the Moorfields Acuity Chart as outcome measures in inherited retinal disease (Taylor, Josan and MacLaren, 2025)

This primary research paper compares and evaluates the performance, sensitivity and repeatability of three acuity tests in a mixed cohort of patients with IRD.

Freely available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12153033/>

Author contributions

LT (main contributor) developed the research question and completed the study setup, participant recruitment, and most of the data collection. As well as collating the data and undertaking summary statistical analyses. Other co-authors assisted with some data collection and supported the linear mixed models and receiver operator curve analysis (AJ). REM provided professional oversight throughout.

Paper 7

Low-contrast visual acuity versus low-luminance visual acuity in choroideremia (Wood *et al.*, 2021a)

Primary research paper: cross-sectional evaluation of two acuity tests in choroideremia compared to controls.

Freely Available at: <https://onlinelibrary.wiley.com/doi/10.1111/cxo.13087>

Author contributions

LT (main contributor) was responsible for data analysis and drafting the final manuscript. JKJ and DH collected and collated the data. JKJ and REM provided support throughout and feedback on manuscript drafts.

3.1.1 Standard Visual Acuity

Paper 2 (Baffour-Awuah *et al.*, 2024) explored ETDRS testing in patients with choroideremia, who typically experience greater nasal macular retinal degeneration, thereby affecting the central temporal visual field. In these patients, when impaired retinal regions fell ahead of the line being read (as observed in the right eye), a statistically significant (cumulative link mixed model: $P = 0.002$) greater number of letter errors were made from the temporal side of the chart, which was attributed to impaired line tracking and letter localisation ability (Figure 3.1). No similar trend was observed in the left eye. A reanalysis of the cumulative linear mixed model, to include a laterality interaction term, right eye versus left eye difference in the N2 versus T2 error scores in CHM patients showed a statistically significant difference ($P=0.0005$), indicating an error score trend difference between right and left eyes.

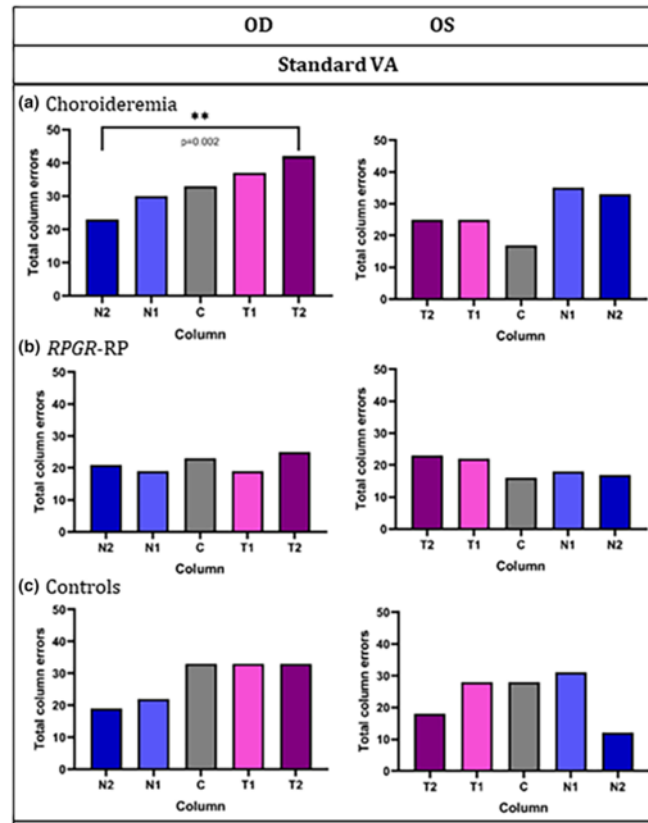


Figure 3.1: The distribution of VA (number of letters) total column errors for (a) choroideremia, (b) *RPGR*-associated RP and (c) control participants. N2, N1, T2 and T1 refer to the second and first columns of letters on the letter chart, away from the central ‘C’ position on the nasal and temporal sides of the chart, respectively. Image from Baffour-Awuah *et al.* (2024)

The study suggested that nasal macular degeneration in the right eye affected the patient's ability to read and track along the line of letters and limited letter localisation (Figure 3.2). The VA test, therefore, becomes a test of letter localisation, in addition to resolution, increasing the variability and reliability of results. However, greater temporal errors could also partly reflect incomplete reading of threshold rows, whereby patients instructed to read from left to right fail to complete letters towards the end of a line. Although, no greater temporal errors were observed in either the control group or *RPGR*-associated RP groups, suggesting this was not a significant factor.

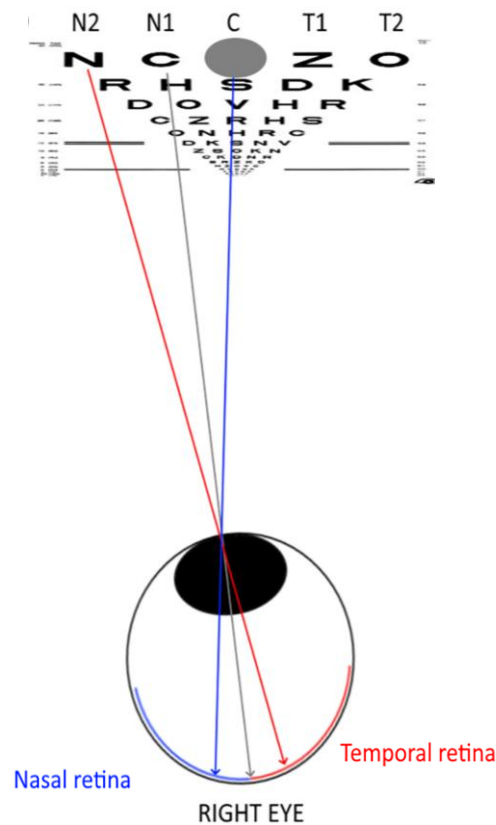


Figure 3.2: A schematic representation of the right eye turning inwards to simulate reading a letter chart from left to right. In choroideremia, subsequent letters are tracked and localised by the degenerated nasal retina. The grey circles represent the temporal visual field scotoma, a result of the nasal retinal degeneration. Image adapted from Baffour-Awuah *et al.* (2024).

To overcome this, Jolly *et al.* (2020) proposed using an electronic VA chart with a crowded single-letter optotype and a forced-choice test paradigm for VA measurement, since this eliminates the need for patients to track along a line of letters during testing. The study noted that some patients subjectively preferred the single crowded optotype setup, which could be due to the brighter screen, forced choice testing or elimination of tracking requirements. However, despite the elimination of tracking issues by using single optotypes, no reduction in test variability was observed. The study gave no explanation why this was the case. The study also reported that participants performed, on average, three letters better with the electronic VA chart

than with the standard ETDRS chart setup; therefore, the charts should not be used interchangeably. The improved performance with the electronic VA chart could be due to the removal of the need to track along the line of letters or a result of the forced choice paradigm.

3.1.2 Low Luminance Visual Acuity

The development, design, usage protocols and questions for further research into LLVA were reported in an LLVA scoping literature review - Paper 3 (Wood *et al.*, 2021b) summarises some of the LLVA research questions that arose from the LLVA literature review. To address some of these research questions, a follow-up study was conducted, see Paper 4 discussed in the next section (Forte *et al.*, 2025).

Table 3.1: Key LLVA Research Questions that arose from Paper 3, the LLVA literature review.

LLVA Research questions
1. What is the impact on LLVA if background luminance testing levels are reduced to below 1.0cd/m ² ?
2. What is the impact of dark adaptation on LLVA results?
3. Does the left eye benefit from being tested second, as it has a longer time to dark adapt under the occluder than the right eye?
4. What upper LLD values are reflective of younger populations (< 55 years)?
Low luminance deficit (LLD); Low luminance visual acuity (LLVA);

3.1.2.1 Sources of Variability: Background Luminance Level

Paper 3 (Wood *et al.*, 2021b) systematically reviewed and scoped the current LLVA literature and summarised testing at different background luminance levels. Using the ETDRS chart and standard testing setup, with the addition of a 2.0 log unit neutral density filter, the background testing luminance was found to be 1.6 cd/m². A previous study suggested that VA reduces significantly as the luminance level falls below 1.0 cd/m² (Rabin, 1994). To explore this further, LLVA testing was performed at a dimmer luminance. Paper 4 (Forte *et al.*, 2025) used additional bulb filters (equivalent to a 0.3 neutral density filter) over the ETDRS lightbox bulbs to reduce luminance levels to 0.85cd/m². A small statistically significant reduction in letter reading performance at

this lower luminance level was reported in both "Eye One" (median difference 1 ETDRS letters [IQR 4], Wilcoxon Signed Rank Test: $p=0.02$) and in "Eye Two" (median difference 1 ETDRS letters [IQR 3], Wilcoxon Signed Rank Test: $p=0.02$). These differences are within previously reported LLVA test-retest variability as indicated by the CoR, discussed in section 4.1.2.5 and Papers 5 and 6 (Wood *et al.*, 2021c; Taylor, Josan and MacLaren, 2025), and so not considered clinically significant using our proposed definitions (Table 2.2). Therefore, small variations in background luminance do not seem to make an appreciable difference, regardless of statistical significance.

The optimum luminance for LLVA testing is yet to be precisely identified. In a meta-analysis of control participants, VA showed a significant decline after luminance dropped below 0.38cd/m^2 (Hichisson *et al.*, 2023). LLVA test sensitivity is discussed later in this chapter. It is unclear if testing at a lower luminance would improve the ability to detect central macular changes in early stages of disease. Paper 4 did not explore this, as the aim was to identify the presence of a significant VA drop after reducing background luminance to below 1.0cd/m^2 . It is presumed that testing at lower luminance levels will likely result in lower VA values, given the trends shown in healthy controls; however, greater floor effects, where a greater number of patients with retinal disease are unable to resolve any letters at all, is a likely unintended consequence of testing under ever-decreasing luminance levels. An increase in the floor effect of any given test would render the test less useful as a potential clinical trial outcome measure and must be weighed against increased test sensitivity and the resulting ability to detect more subtle disease changes.

Given the body of evidence demonstrating VA reductions with decreasing luminance levels, it is reasonable to assume that small variances in luminance levels are unlikely to be clinically significant. However, background luminance should be kept consistent to minimise and avoid the compounding effects of multiple sources of variability. It is therefore recommended to record the test luminance along with LLVA values (Wood *et al.*, 2021b).

3.1.2.2 Sources of Variability: Dark Adaptation

Before the publication of Paper 4, only one study had explored the effects of dark adaptation on LLVA variability in a cohort of patients with geographic atrophy. Sunness *et al.* (1997) reported a 3-letter median (0.06 logMAR) improvement on repeat LLVA after 5 minutes of dark adaptation, in 65 eyes with geographic atrophy, using the conventional ETDRS testing procedure; however, the authors did not perform any null hypothesis testing, and so it was not reported if this change was statistically significant. In their later paper, Sunness *et al.* (2008) reported that despite small improvements in VA after dark adaptation, dark adaptation was not required. It is unknown if these findings translate to other retinal diseases.

Paper 4 explored the effects of dark adaptation in a cohort of participants with no history of significant eye disease, using an alternative method. The rationale for investigating only healthy control participants (with assumed normal dark adaptation) was that these individuals would be more likely to show the best potential for improvement. After 3-minutes of wearing dark-adaptation goggles, participants were asked to reattempt the letters on their threshold line (lowest line previously read), to capture any LLVA improvement that may have arisen from the period of dark adaptation. Three minutes of dark adaptation was selected as this corresponded with the minimal optimal dark-adaptation time for cone photoreceptors, and since LLVA is believed to be a test of primarily cone photoreceptor function (discussed in detail in Paper 3) (Wood *et al.*, 2021b).

Changes in LLVA were assessed before and after dark-adaptation under two lighting levels, 1.6 cd/m² and 0.85 cd/m². Small gains in LLVA were measured after 3-minutes of dark adaptation. At 1.6 cd/m² a median increase of 1 ETDRS letter (IQR 3) was observed, and at 0.85 cd/m² an increase of 1 ETDRS letter (IQR 2) was observed. Paper 4 (Forte *et al.*, 2025) highlighted, despite statistically significant (Wilcoxon Signed Rank Test: $P < 0.001$) results in both cases, that these gains are within the previously reported test-retest variability results (defined at 1.6 cd/m²), so not considered clinically significant (Wood *et al.*, 2021c). Although test-retest variability has not been defined for 0.85 cd/m², it is reasonable to expect it to be similar; however,

variability could increase as luminance decreases due to increased difficulty, or variability could reduce as the patients become unable to resolve as many letters and 'guess' less. Despite this, the study results are significantly limited, since no control group repeated the test procedure without dark adaptation. Any LLVA improvement could have been a result of having a second attempt at reading the threshold line (the last line of letters attempted), akin to a learning effect. Randomisation of letters with the use of different charts on the second attempts, as well as the addition of a control group, who did not undergo dark adaptation before repeat testing, would confirm the hypothesised findings of this publication.

In addition, the study reported in Paper 4 (Forte *et al.*, 2025) investigated the effects of different states of dark adaptation between the first eye tested (by convention, the right eye) and the second eye (left eye) to determine if the left eye routinely benefits from the small amount of additional dark adaptation time whilst occluded. Interestingly, there were no significant differences between LLVA (or LLD) at either 1.6cd/m² (Wilcoxon Signed-Rank tests: $p = 0.66$; $p = 0.49$, respectively) or 0.85 cd/m² (Wilcoxon Signed-Rank tests: $p = 0.62$; $p = 0.53$, respectively). This indicates no benefit (LLVA gain or LLD decline) was found for the second tested eye due to an extended period of occlusion and the resulting informal period of dark adaptation, while the first eye underwent testing. Since the standard ETDRS testing procedure requires participants to read every letter down the chart to their threshold limits, it is reasonable to assume that any benefits of dark adaptation, such as increased retinal sensitivity, are quickly negated by the 'bright' retro-illuminated chart. This occurs because the adaptation of healthy cone cells to light is nearly instantaneous, happening as soon as the tested eye is exposed to the luminance of the chart again. Alternatively, dark adaptation in the left eye may be counteracted by interocular summation mechanisms and affected by the light-adapted state of the contralateral eye (Auerbach, Dorrenhaus and Cavonius, 1992). These effects of monocular versus binocular dark adaptation on LLVA testing remain to be explored.

3.1.2.3 Sources of Variability: Electronic Letter Charts

Paper 3 (Wood *et al.*, 2021b) summarises the use of electronic letter charts to measure LLVA. Electronic charts have many advantages, and their use in clinical practice is increasing. They allow greater testing options and optotype randomisation, reducing opportunities for learning effects, as well as supporting forced-choice paradigms. They facilitate automated scoring and recording of responses, which reduces the scope for recording errors. These benefits could provide opportunities for improved visual function testing in clinical trials. However, Paper 4 (Forte *et al.*, 2025) indicated significant variability in reproducibility between LLVA scores obtained with the Electronic Visual Acuity (EVA) system (M&S Technologies, Niles, IL, USA), compared to LLVA scores obtained using the standard ETDRS chart, in controls as well as patient participants (Figure 3.3: A) Bland-Altman plot comparing ETDRS chart and E-ETDRS LLVA in the healthy volunteer group, the lower limits of agreement (LoA) are -5 (95% CI: -6 to -4), and the upper LoA is 10 (95% CI: 9 to 11). B) Bland-Altman plot comparing the ETDRS chart and E-ETDRS LLVA in the patient group, lower LoA -13 (95% CI: -15 to -11) and the upper LoA 18 (95% CI: 16 to 20). In the Bland-Altman plots, the dashed line denotes the mean difference between the tests, while the dotted lines indicate the LoA. Figure taken from Paper 4 (Forte *et al.*, 2025).). However, in patient participants, the large degree of reproducibility appears to be a result of two outlier patients with particularly low chart LLVA scores compared to EVA LLVA scores. It is unknown if these are true outliers or due to errors in data collection or recording. Overall, inconsistent background luminance levels between the two tests were believed to be a significant source of variability, as the EVA E-ETDRS chart with the 2.0 neutral density filter luminance levels are 2.1cd/m², which is 0.5cd/m² brighter than the standard ETDRS LLVA setup. This could also explain the two outliers, where brighter EVA background luminance may support low vision patients, resulting in improved letter scores compared to standard ETDRS-based LLVA testing. It is hypothesised that matching the background luminance levels should lead to more comparable LLVA results. This could be achieved by increasing the standard ETDRS testing luminance or programming the EVA with an LLVA testing module with reduced screen luminance to 1.6cd/m².

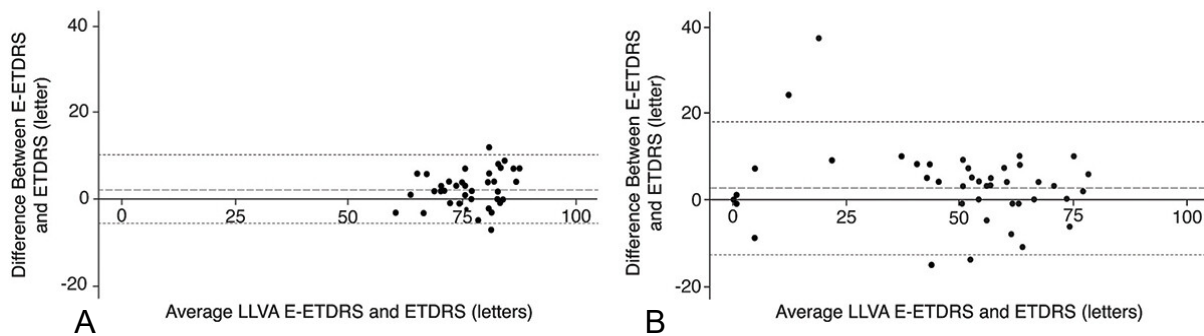


Figure 3.3: A) Bland-Altman plot comparing ETDRS chart and E-ETDRS LLVA in the healthy volunteer group, the lower limits of agreement (LoA) are -5 (95% CI: -6 to -4), and the upper LoA is 10 (95% CI: 9 to 11). B) Bland-Altman plot comparing the ETDRS chart and E-ETDRS LLVA in the patient group, lower LoA -13 (95% CI: -15 to -11) and the upper LoA 18 (95% CI: 16 to 20). In the Bland-Altman plots, the dashed line denotes the mean difference between the tests, while the dotted lines indicate the LoA. Figure taken from Paper 4 (Forte et al., 2025).

Alternatively, the EVA, with its forced choice and better crowding control, may be more accurate and supports patients to read more letters, particularly in low vision patients. Further review of commercially available electronic letter charts other than the EVA system and the opportunities they offer to support vision testing in clinical trials is required. On the other hand, electronic systems are expensive and not universally available; their software, operating systems, and technology are also prone to becoming outdated quickly, limiting their use in multicentre clinical trials.

3.1.2.4 Low Luminance Visual Acuity in Rod-Cone Degenerations

Paper 5 (Wood *et al.*, 2021c) further explored LLVA and LLD in choroideremia and *RPGR*-associated RP. Both standard VA and LLVA were significantly reduced in *RPGR*-associated RP and choroideremia compared to controls (Kruskal-Wallis test with post hoc analyses, $P \leq 0.01$, Table 3.2: VA, LLVA and LLD summary statistics for choroideremia, *RPGR*-associated RP and controls from Paper 5.).

Table 3.2: VA, LLVA and LLD summary statistics for choroideremia, *RPGR*-associated RP and controls from Paper 5. Interquartile range (IQR).

	n	Age (years)	Median standard VA, ETDRS letters (IQR)	Median LLVA, ETDRS letters (IQR)	Median LLD, ETDRS letters (IQR)	Median central retinal sensitivity dB (IQR)
<i>RPGR</i> -associated RP	24	32 (26-51)	70 (57-75)	52 (5-61)	13 (11-26)	1.2 (0.0-4.0)
Choroideremia	43	44 (31-53)	74 (65-82)	58 (38-69)	16 (12-22)	3.0 (0.7-9.5)
Controls	62	36 (30-51)	88 (84-94)	79(73-82)	11 (7-13)	-

Decibels (dB); Interquartile range (IQR); Low luminance deficit (LLD); Low luminance visual acuity (LLVA); Retinitis Pigmentosa (RP); Visual acuity (VA)

Interestingly, while standard VA remained relatively preserved in both disease groups, reduced LLVA (and greater LLD) was clearly evident, along with an associated reduction in microperimetry mean sensitivity (retinal function marker). It was also noticed that 11 patient participants had extremely poor LLVA despite relatively well-preserved standard VA (Figure 3.3b & Figure 3.3c). After removing those with zero LLVA scores, the linear correlation between LLVA and standard VA was found to be highly significant (controls: $\rho = 0.81$, $P < 0.001$; choroideremia: $\rho = 0.84$, $P < 0.001$; and *RGPR*-associated RP: $\rho = 0.70$, $P = 0.001$) (Figure 3.5a). There was no significant difference between the correlation coefficient for both controls and choroideremia (Steiger's z test: $z = 0.44$, $P = 0.65$) and between controls and *RPGR*-associated RP (Steiger's z test: $z = 0.13$, $P = 0.37$). At earlier disease stages (shown by better VAs), standard VA and LLVA deteriorate at a similar rate, so the LLD remains constant.

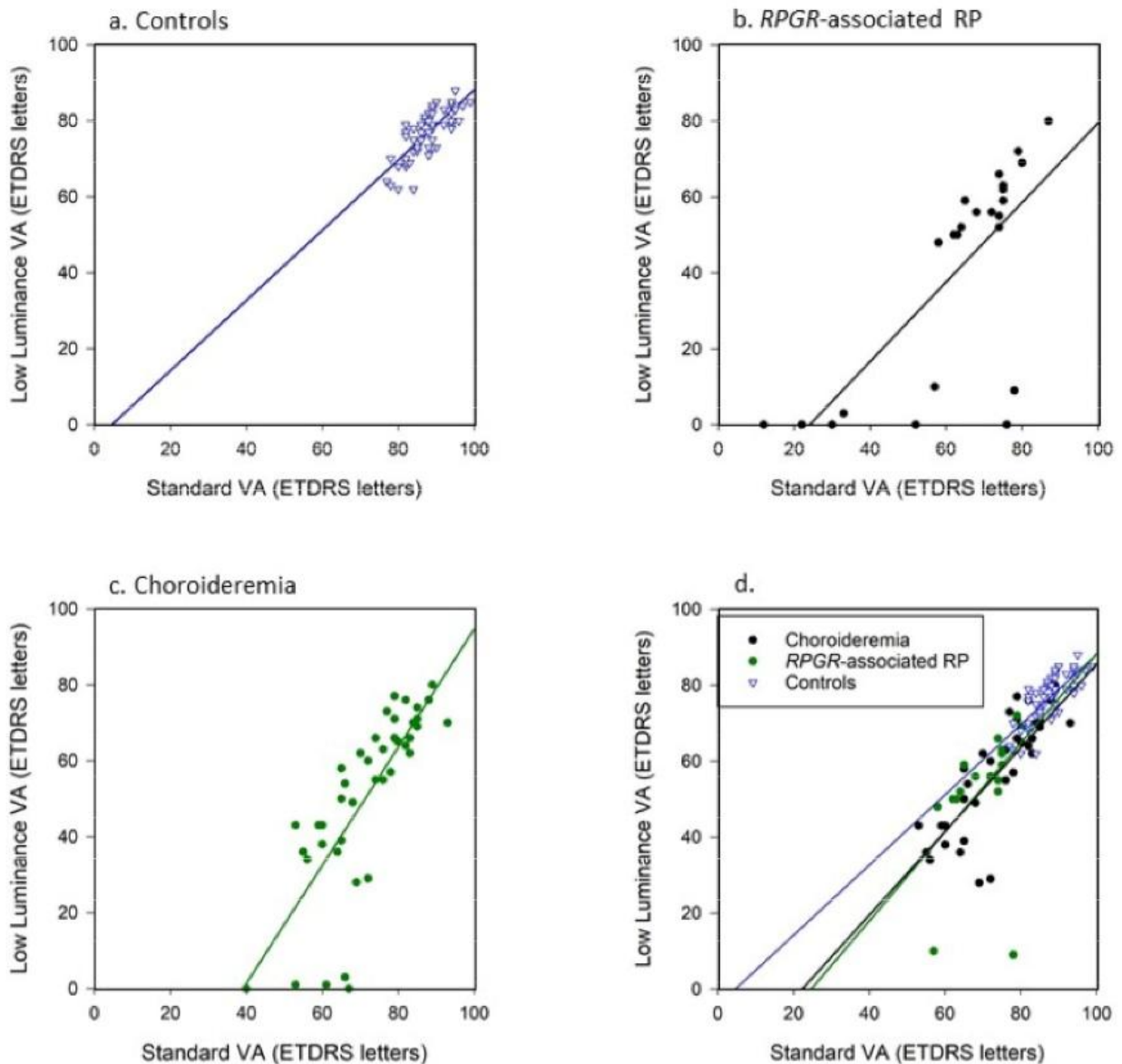


Figure 3.3: Spearman's rank shows significant positive correlations between standard VA and LLVA for a) controls ($Rho = 0.81$, $P < 0.001$). b) *RPGR*-associated RP subjects ($Rho = 0.72$, $P < 0.001$) and c) choroideremia subjects ($Rho = 0.85$, $P < 0.001$). d) A sub-analysis after choroideremia and *RPGR*-associated RP zero (floor effect) LLVA values are removed. Figure from Wood *et al.* (2021c).

The results reported in Paper 5 compare well to those reported in Paper 6, investigating standard VA and LLVA in a mixed cohort of participants with early-moderate stage rod-cone degeneration ($n=35$) and controls ($n=36$) (Taylor, Josan and MacLaren, 2025). Despite (in Paper 6) LLVA showing statistically significantly reduced

LLVA values in the patient group compared to controls, both groups showed a significant correlation (Linear Mixed Model Kenwood-Roger: $P < 0.0001$) between standard VA, LLVA and microperimetry, with no significant difference in the gradient of the slope (Figure 3.5). These findings suggest that LLVA declines consistently with standard VA in early-moderate disease.

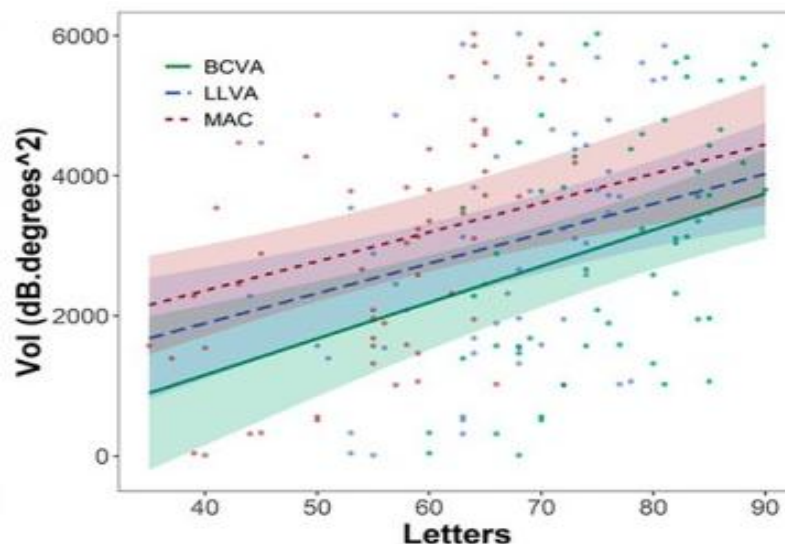


Figure 3.5: A linear mixed model analysis was performed with patient study identity number as the random intercept variable and BCVA letter score as the predictor, along with exam type as an interaction term. The fitted relationships demonstrated comparable relationships between microperimetry central retinal sensitivity, represented by total volumetric hill-of-vision sensitivity (Vol; $\text{dB} \cdot \text{degrees}^2$, discussed further in Section 4.2.1.3), and letter score for standard VA, LLVA and MAC VA.

Figure adapted from Taylor, Josan and MacLaren (2025).

Dissociation from the linear correlation between LLVA and standard VA seemed to occur at later disease stages, as reported in Paper 5. This effect was observed in patients with choroideremia once standard VA fell below approximately 35 ETDRS letters, and in patients with *RPGR*-associated RP once standard VA fell below approximately 50 ETDRS letters (Figure 3.3b and 3.4c). In Paper 5, further investigation revealed that individuals with zero letters on LLVA (i.e., had reached the floor of the testing method) also had no detectable central macular microperimetry sensitivity (using the conventional 10-2 grid, discussed in detail in Chapter 4) (Figure

3.4). It appears that in patients with measurable standard VA at four metres but very poor (only 1-2 ETDRS letters) or unmeasurable LLVA (high LLD), likely marks the beginning of late-stage disease and is highly suggestive that standard VA loss may be imminent. In Paper 5, this led to the proposal that significant reductions in LLVA, over that of standard VA, could be an early indicator of central macular sensitivity reductions. Therefore, LLVA could be used as a pre-screening tool to indicate the presence or absence of detectable central retinal sensitivity. In these IRD disease groups, the dissociation between standard VA and LLVA appears to occur as degeneration encroaches into a specific region in the perifoveal area within the macula, as denoted by the shaded green circle shown in Figure 3.6c. Further investigation using patients with RP, since they typically demonstrate a more symmetrical central seeing island compared to choroideremia, may enable more precise quantitative investigation of any relationship between LLVA and the preserved EZ area.

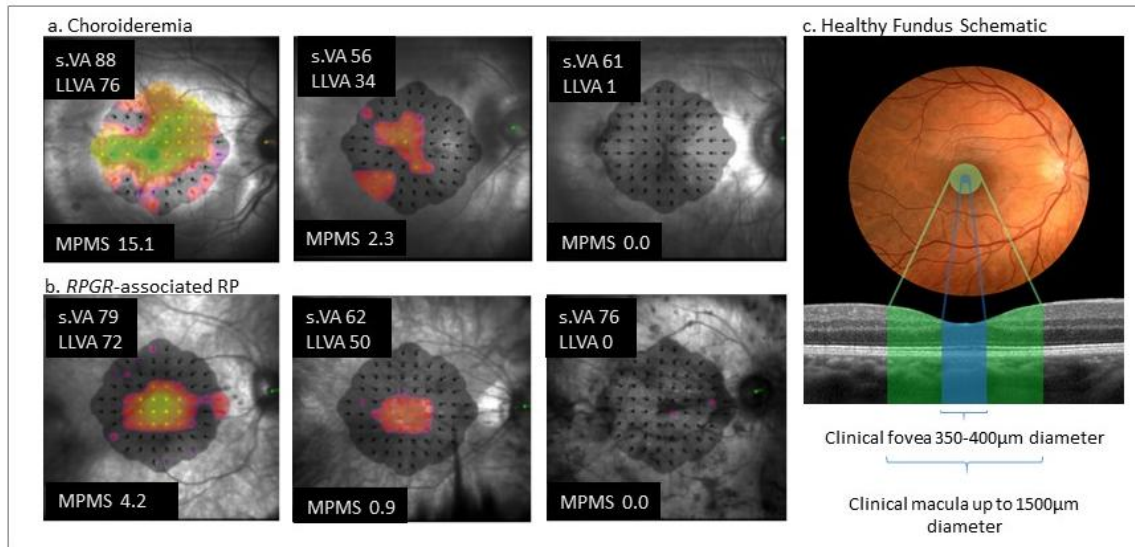


Figure 3.4: a) Choroideremia microperimetry results, standard VA (denoted as s.VA in this figure only) and LLVA for three different subjects with a range of central retinal sensitivity function to represent different disease stages. The brighter the colour of the threshold sensitivity heatmap, the greater the central function. The darker red, purple and black areas indicate reduced or no sensitivity function. b) *RPGR*-associated RP microperimetry results, standard VA and LLVA for three different subjects with a range of central retinal sensitivity function representing different disease stages. c) A schematic diagram to illustrate the clinical definitions of the extent of the foveal and macular areas as specified in Paper 5. MPMS - Microperimetry mean sensitivity. Figure from *Wood et al. (2021c)*.

Paper 3 summarised the uses for LLVA in different diseases (*Wood et al., 2021b*). Since this publication, LLVA has been used in several international clinical trials for IRD and associated rod-cone degenerations. The XOLARIS trial, an *RPGR*-associated RP natural history study, used LLVA as a secondary outcome measure. The study split patients into high standard VA (≥ 74 letters) and low standard VA groups (VA between 34-73 ETDRS letters). They showed that after 24 months, the mean LLVA change from baseline was -1.9 (SD ± 10.09) ETDRS letters and -0.5 (SD ± 4.85) ETDRS letters in each group, respectively (*MacLaren et al., 2025*). These LLVA changes, after two years of natural disease progression, are very small and likely reflective of slow disease progression and preservation of the central retina. LLVA was

also used as a secondary outcome measure in the XIRIUS phase I/II and II/III (split into parts 1 and 2) *RGPR*-associated RP gene therapy trial (von Krusenstiern *et al.*, 2023). The number of participants showing an LLVA change greater than or equal to 15 ETDRS letters was reported. In part one, 3/11 treated eyes and 1/11 fellow eyes showed a gain (of greater than or equal to 15 ETDRS letters) in LLVA at 12 months. The response in the untreated eye was attributed to natural variability (von Krusenstiern *et al.*, 2023). In the XIRIUS part 2 at 12 months, 3/9 high-dose, 1/8 low-dose, and no control participants showed the 15 ETDRS LLVA letter gain (Lam *et al.*, 2024). It is difficult to determine whether these poor responses are a result of early phase clinical trial design, lack of efficacy, poor LLVA test sensitivity or whether a 15-letter gain is unrealistic.

Comparisons of LLVA to standard VA have been explored in other studies, including the following two reports, where I am a co-author, although they are not extensively featured in this thesis. In choroideremia female carrier patients, LLVA was significantly reduced compared to controls in all phenotype classifications (fine, coarse, geographic and male-pattern). In comparison, standard VA was not found to be reduced in the more mild (fine and coarse) phenotypes, with only severe (geographic and male-pattern) phenotypes showing statistically significantly reduced standard VA compared to controls. The authors concluded that LLVA was able to detect changes resulting from mild retinal disease that standard VA could not (Gocuk *et al.*, 2024).

In a retrospective dominance analysis study of fellow untreated eye data from 12 patients with choroideremia (followed up for up to 5 years), who took part in an interventional phase II gene therapy choroideremia clinical trial (REGENERATE [NCT02407678](#)), LLVA ranked sixth and standard VA ranked twelfth in dominant association against a structural disease indicator (changes in blue autofluorescence - BAF). The functional tests with higher ranking associations with BAF were Pelli-Robson contrast sensitivity, quantitative contrast sensitivity (specifically spatial frequency 10), Cambridge colour testing (specifically deutan) and microperimetry, which ranked second after time (Josan *et al.*, 2024).

More recently, in a validation study for a novel patient-reported outcome measure developed specifically for use in patients with IRD, called the Michigan Retinal Degeneration Questionnaire, a significant correlation between LLVA and central vision and contrast sensitivity questionnaire domains was reported (Karuntu, Nguyen and Boon, 2024). This suggests LLVA reflects aspects of functional central vision and contrast sensitivity activities, supporting the hypothesis that LLVA is primarily a cone photoreceptor-driven test.

3.1.2.5 Low Luminance Visual Acuity Repeatability

Paper 5 (Wood *et al.*, 2021c) reported comparable CoR in standard VA and LLVA, in a mixed cohort of patients with choroideremia and *RPGR*-associated RP and healthy controls (Table 3.3). Paper 6 (Taylor, Josan and MacLaren, 2025) also reported comparable CoR (1 letter difference) in a broader mixed cohort of IRD patients and healthy controls (Figure 3.6, section 4.1.3.1), indicating that LLVA shows similar repeatability to standard VA. These results suggest that for a clinically significant change, beyond test-retest variability, a difference greater than approximately seven letters is required.

Table 3.3: Coefficients of repeatability (CoR) for standard VA and LLVA in a mixed cohort of patients with choroideremia and *RPGR*-associated RP (Wood *et al.*, 2021c).

Group	n	Standard VA CoR (LoA), ETDRS letter	Low Luminance VA CoR (LoA), ETDRS letter
Controls	18	7.2 (-8.0-6.6)	6.0 (-6.9-5.1)
Rod-Cone Degeneration	19	7.2 (-5.3-9.7)	6.7 (-6.6-6.8)

Coefficient of repeatability (CoR); Early treatment diabetic retinopathy study (ETDRS); Limits of agreement (LoA); Visual acuity (VA)

3.1.2.6 Low Luminance Deficit Reference Ranges

Paper 3 (Wood *et al.*, 2021b) reviews the use of LLD in the literature. This paper synthesises data from control participants across six different studies to determine an

upper 'normal' LLD limit of 13 ETDRS letters. The review recommends that an LLD of greater than 13 letters should prompt further investigation. However, this data was from age-matched control data reflecting individuals over 55 years of age (Keenan, Cukras and Chew, 2021). This result may not be reflective of younger individuals, which is of particular relevance in IRD studies.

To provide an LLD reference value for younger healthy control populations, data from three different studies (n=73, mean age 27.9, range 18–54 years) were collated and reported in Paper 4 (Forte *et al.*, 2025). From this, the mean LLD was 10 (± 0.8 , 95% CI) ETDRS letters; therefore, a new LLD upper limit of 11 ETDRS letters was proposed. Since this is two letters less than the LLD range identified in Paper 3 (Wood *et al.*, 2021b), the difference may be reflective of natural test-retest variability and so would not be considered a clinically significant difference from the previous findings. Further investigation is required to explore LLVA (and LLD) across a broader range of ages in healthy controls. Significant improvement in LLVA (10.2 ETDRS letters, SD ± 6.9) and subsequent decreases in LLD (-3.0 ETDRS letters, SD ± 5.3) have been reported following cataract extraction. Although the sample size of the study was small (n=25), this suggests that lens opacities are a significant influencing factor in LLVA measurements and must be taken into account (Shen *et al.*, 2022).

LLD is useful as it highlights and quantifies the level of dissociation between standard VA and LLVA, which Paper 5 showed was relevant in IRDs (Wood *et al.*, 2021c). However, there are situations in which the raw LLVA and VA values are useful, as they can reveal the extent of foveal function. Sunness *et al.* (2008) explains that some patients with geographic atrophy may have reduced standard VA due to central atrophy but intact peripheral foveal function, preserving LLVA and resulting in a small LLD, despite clearly reduced central vision. Paper 3 (Wood *et al.*, 2021b) emphasised that LLD should not be interpreted in isolation but in the context of LLVA and VA.

To investigate this further and explore the possible consistent changes in early disease (patients with VA better than or equal to 6/60), standard VA and LLVA data from the

Visual Function in Retinal Degeneration (VFIRD) study (Paper 1) were reanalysed (controls n=36, patients n=35) and a normalised LLD index (LLDi) calculated.

$$LLDi = \text{standard VA} - LLVA / \text{standard VA} = LLD / \text{standard VA}.$$

In this unpublished analysis, the aim was to normalise LLD against VA. Using this normalised index eliminates the possibility of misinterpreting a small LLD in the presence of reduced standard VA as “normal” since values of LLDi closer to one, which would either indicate a large LLD or a reduced standard VA, both of which would raise suspicions. The benefit of this index is that it allows different LLD values to be compared even when standard VA values are different and, in particular, when both standard VA and LLVA have been reduced equally; however, a significant drawback is that LLDi is a unitless, non-intuitive variable. For analysis and graphical plotting, it is a useful metric. The mean LLDi (using the right eye data) was 0.1 (SD ± 0.05 , 95% CI 0.1-0.1) and 0.2 (SD ± 0.2 , 95% CI 0.1-0.2) for controls and patients, respectively. The LLDi showed no significant difference (Paired t-test, $P > 0.05$) between patients and healthy controls, and no significant correlation between LLDi and microperimetry mean sensitivity. This finding provides evidence that LLD and LLDi are of limited value in detecting subtle central macular changes in early-to-moderate IRD. Further investigation is needed to explore the usefulness in later disease (once VA reaches the previously stated threshold of 50 ETDRS letters), where LLVA is seen to drop precipitously, as reported in Paper 4 (Wood *et al.*, 2021c).

Since LLD and LLDi are composite measures (analogous to body mass index), combining data from standard VA and LLVA, hence establishing measures of test-retest variability must be combined appropriately. To the best of our knowledge, this has not been previously explored, but it is likely to be a significant issue if LLD is used as a clinical trial outcome measure. Therefore, LLD or LLDi cannot yet be considered as viable outcome measures until further research is available.

3.1.3 Low Contrast Acuity

Paper 7 (Wood *et al.*, 2021a) explored low contrast acuity in patients with choroideremia compared with healthy controls. Significant floor effects were observed with both 1.25% and 2.5% low contrast testing, with only 62% (18/29) of subjects able to read letters on the 1.25 % low contrast chart and 79% (23/29) able to read letters on the 2.5% low contrast chart. In comparison, 97% (28/29) were able to complete LLVA testing. Those who completed testing showed significantly lower acuity scores than controls (Mann-Whitney U Test: $P < 0.05$), suggesting potential utility. The range of low contrast acuity scores was also smaller than LLVA. As a result of these findings, use of the 1.25% and 2.5% low contrast acuity tests was ruled out as a possible useful outcome measure in choroideremia. However, correlation analyses between standard VA and 2.5% low contrast VA were significant before the floor effects were experienced at around a VA of 70 ETDRS letters. After this, no 2.5% low contrast letters could be resolved, which is comparable (albeit far sooner) to the floor effect phenomena reported in LLVA, discussed in Paper 5 (Wood *et al.*, 2021c). By extrapolating the findings for LLVA, i.e. LLVA possibly being an indicator of retinal function across a larger area of the central macula than standard VA. The even earlier floor effect seen here could suggest that low contrast acuity testing is reflective of an even larger area of central macular function. Alternatively, the significant floor effects may be indicative that low contrast acuity testing performance involves a greater degree of post-receptor processing, which could also provide a greater degree of sensitivity to disease progression.

In a study assessing low contrast acuity in children, it was reported that during normal development, low contrast acuity stabilises after eight years old (Baldev *et al.*, 2023). Therefore, in children with IRD, any degenerative changes that occur before eight years old could impair visual development, including the ability to resolve low contrast. Hence, any reduced acuity in IRD could be reflective of amblyopia as opposed to (or in addition to) progressive retinal disease. This could explain the greater number of patients reaching the floor effects of the test seen with 1.25% and 2.5% low contrast acuity charts in Paper 7. For LLVA, to the best of my knowledge, such early-age natural

history data is not available. Therefore, it is unknown whether early disease changes inhibit low-light visual development.

3.1.4 Moorfields Acuity Chart

Paper 6 (Taylor, Josan and MacLaren, 2025) is the only study that has explored the use of the MAC in IRDs. Here, both a mixed cohort of patients with IRD (n=35) and control participants (n=36) showed reduced MAC VA compared to standard VA. Despite this, the difference between MAC VA scores and standard VA scores between the two groups was comparable. This suggests the MAC chart was unable to detect any additional macular dysfunction. Test sensitivity (true positive rate) and specificity (false positive rate), using Receiver Operator Curve (ROC) analyses, showed high sensitivity and low specificity, with no significant differences in areas under the curve (AUC) ($P>0.05$) for all three test types (Figure 3.5). This suggests high precision between the three acuity tests to differentiate patients from controls in the two well-defined cohorts. The MAC showed no greater sensitivity in detecting disease or visual function change than standard VA. However, these findings could be biased by the patient cohort, many of whom had well-defined vision loss.

A subset of participants completed mesopic microperimetry central retinal sensitivity assessment. After reliability screening, 60 patient tests and 68 healthy control tests were included in a correlation analysis. Standard VA and MAC VA letter scores each correlated significantly with microperimetry retinal sensitivity (Kenward-Roger: $P<0.001$) (Figure 3.5). Using microperimetry as a surrogate marker of disease severity, all three letter charts showed a significant decline in VA with greater disease severity (reduced microperimetry sensitivity). Further analyses using linear mixed-effect models showed that there was, however, no significant difference between the gradients of each slope (Tukey: $p>0.05$), suggesting VA, LLVA and MAC VA decline similarly with disease progression, indicating no greater benefit or ability to detect disease using one chart type over another.

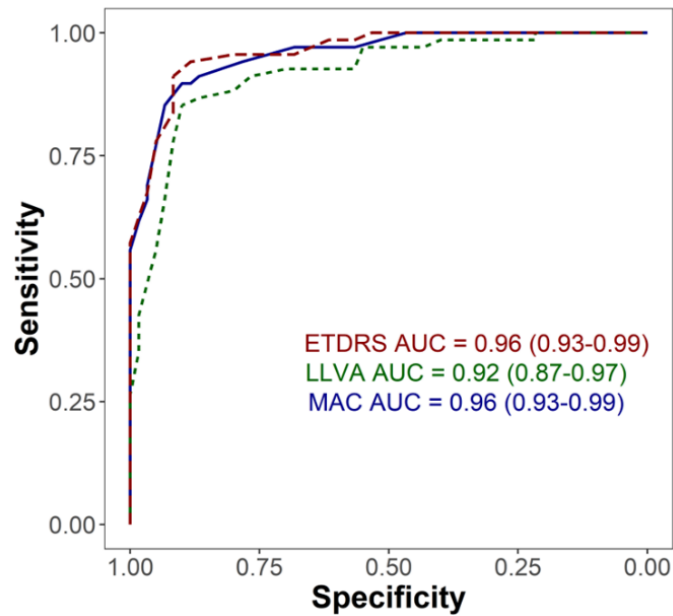


Figure 3.5: Comparable ROC curves for discrimination between IRD versus healthy controls using VA, LLVA and MAC VA with bootstrapped 95% confidence intervals. Area under the curve (AUC). Figure from Taylor, Josan and MacLaren (2025).

3.1.4.1 Moorfields Acuity Chart Test-Retest Variability.

To improve detection of disease progression, test-retest variability should, wherever possible, be minimised. Shah *et al.* (2016) showed no significant difference in test-retest variability (CoR) between standard VA and MAC testing. They reported for healthy controls, CoR for standard VA was 0.09 logMAR (4.5 ETDRS letters) and 0.11 logMAR (5.5 ETDRS letters) for MAC VA. Bland-Altman analyses showed no association between acuity and level of agreement. Shah *et al.* (2016) reported for age-related macular degeneration subjects, standard VA CoR was 0.12 (6 ETDRS letters) and for MAC VA 0.09 (4.5 ETDRS letters). These results compare to those reported in Paper 6 (Taylor, Josan and MacLaren, 2025), where test-retest variability reported via CoR was explored in patients with IRD (n=35) and healthy controls (n=36). Mean biases varied in direction but were small in magnitude (<1.5) across all comparisons, with comparable CoR and similar 95% LoA (Figure 3.6). Overall, test-retest variabilities did not appear to be affected by IRD or acuity test type. This was a surprising result since the MAC was designed to reduce variability arising from differences in stopping rules/end criteria. The small observed improvements in test-

retest variability (around 1-2 letters) contradict previous findings reporting end criteria as a significant source of variability (Shah *et al.*, 2014) and instead suggest that the end criterion is only a minimal source of variability when participants are encouraged to do their best and pushed to achieve their threshold acuity. Trying to 'guess' at, or beyond, their threshold ability and inducing forced errors to achieve a particular stopping rule is often uncomfortable for participants, and the results presented here suggest it may be unnecessary.

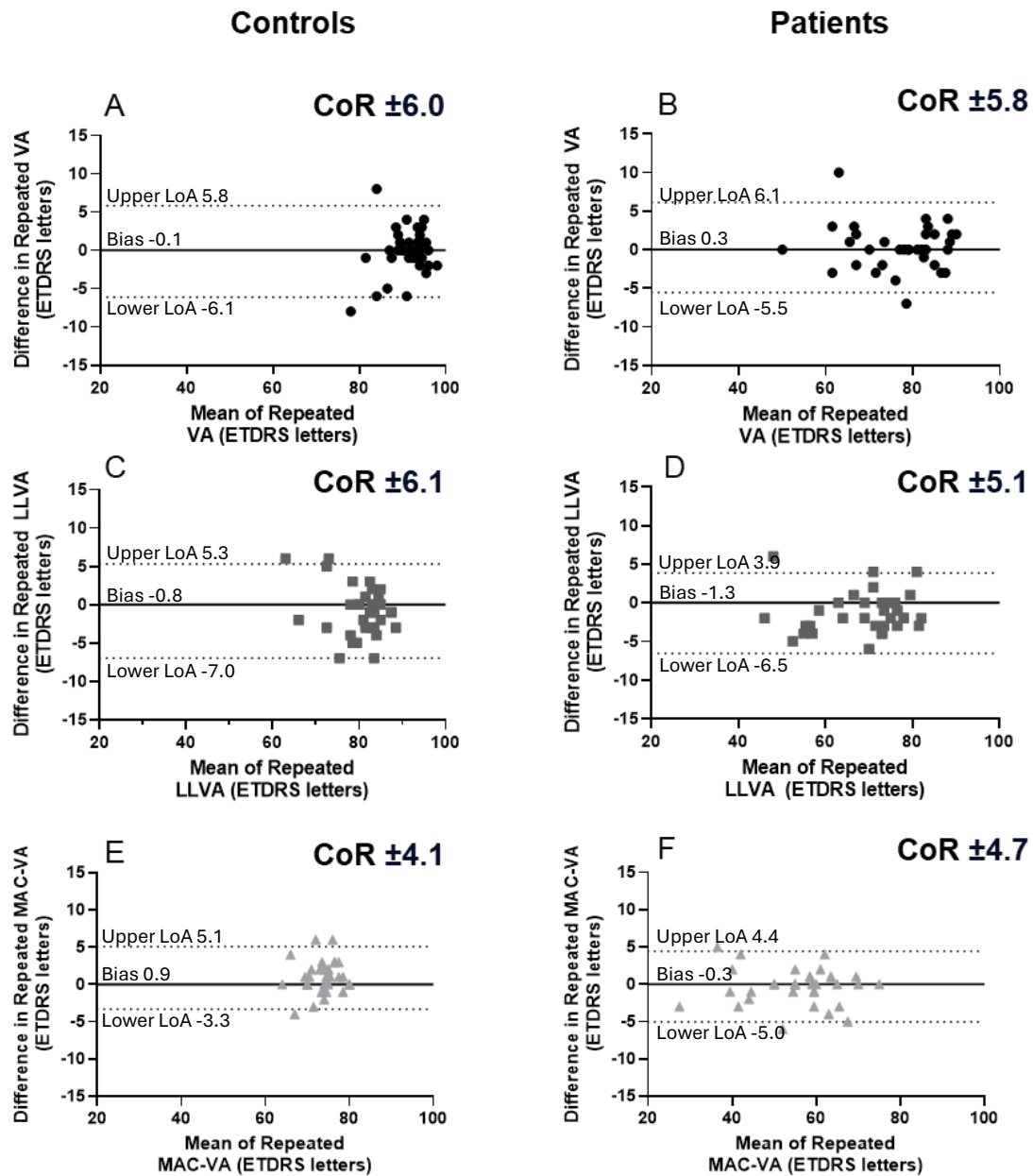


Figure 3.6: Comparable Bland-Altman repeatability plots for standard VA, LLVA and MAC VA for both controls (A, C, E) and patient participants (B, D, F). Coefficient of repeatability (CoR). 95% Limits of agreement (LoA). Image from Taylor, Josan and MacLaren (2025).

Interestingly, the test-retest variability analysis used by Shah *et al.* (2016) used data collected from chart one and then repeated the data from chart two. The use of different charts minimises any potential learning effects due to chart memorisation. However, when using the ETDRS VA testing procedures, chart one is typically used for the right eye and chart two for the left, although they were designed to have equal difficulty and be comparable (Ferris *et al.*, 1982). In Paper 6, participants reread the same chart to determine repeatability (Taylor *et al.*, 2023b), which could have resulted in a learning effect arising from letter memorisation. However, the comparable results between the two approaches suggest this was unlikely. This also provides further evidence of comparable difficulty between chart one and chart two.

3.1.4.2 Moorfields Acuity Chart Limitations

Paper 6 (Taylor, Josan and MacLaren, 2025) reported that maintaining a consistent testing background luminance between tests was difficult. This was because the test relied on standard room lighting, which was variable and difficult to control. The impact of variable light levels is unclear and remains to be explored. A further limitation is the vanishing optotype effect, used in the MAC, breaks down with extrafoveal viewing, as the detection and recognition thresholds differ due to limited neural sampling density and limited recognition acuity (Shah, Dakin and Anderson, 2012). The same has been reported with advancing disease, where the MAC letters no longer behave like vanishing optotypes, the detection and resolution limits begin to separate, limiting the charts use in later-stage patients (Shah *et al.*, 2016).

3.2 Mesopic Microperimetry

This section critically analyses the following publication on mesopic microperimetry.

Paper 8

Microperimetry as an Outcome Measure in RPGR-associated Retinitis Pigmentosa Clinical Trials (Taylor et al., 2023a)

This primary research paper evaluates microperimetry performance, repeatability and sensitivity indices in patients with *RPGR*-associated RP.

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2785690>

Author contributions

LT (main contributor) was responsible for developing the research question and study setup, some data collection, and data collation. In addition to undertaking descriptive and repeatability analyses, interpretation and drafting of results. AJ supported statistical data analyses and provided input into the interpretation. JKJ and REM provided professional oversight.

3.2.1 Microperimetry Sensitivity Index and Optimum Endpoint

Paper 8 (Taylor *et al.*, 2023a) explored which microperimetry sensitivity indices are most suitable for use as an outcome measure and clinical trial endpoint. It analysed repeat and longitudinal test data to evaluate standard sensitivity output indices, pointwise and mean sensitivity, and the use of alternative volume sensitivity indices.

3.2.1.1 Pointwise Sensitivities

The suggested FDA regulatory endpoint in microperimetry has been a change of 7 dB or more in five or more prespecified test points (Csaky *et al.*, 2017). This was based on studies of static perimetry in glaucoma rather than microperimetry in IRDs and was used as an endpoint in recent gene therapy clinical trials for *RPGR*-associated RP (Lam *et al.*, 2024; Yang *et al.*, 2025). Paper 8 (Taylor *et al.*, 2023a) aimed to explore the appropriateness of this endpoint using a Bland-Altman analysis to evaluate test-retest variability in a cohort of patients with *RPGR*-associated RP who had completed

triplicate microperimetry testing at two separate visits. Figure 3.7 shows the pointwise variabilities after accounting for paired data and suggests a change greater than ± 9.0 dB (rounded for convenience) is required to be clinically significant (as defined in Table 2.2).

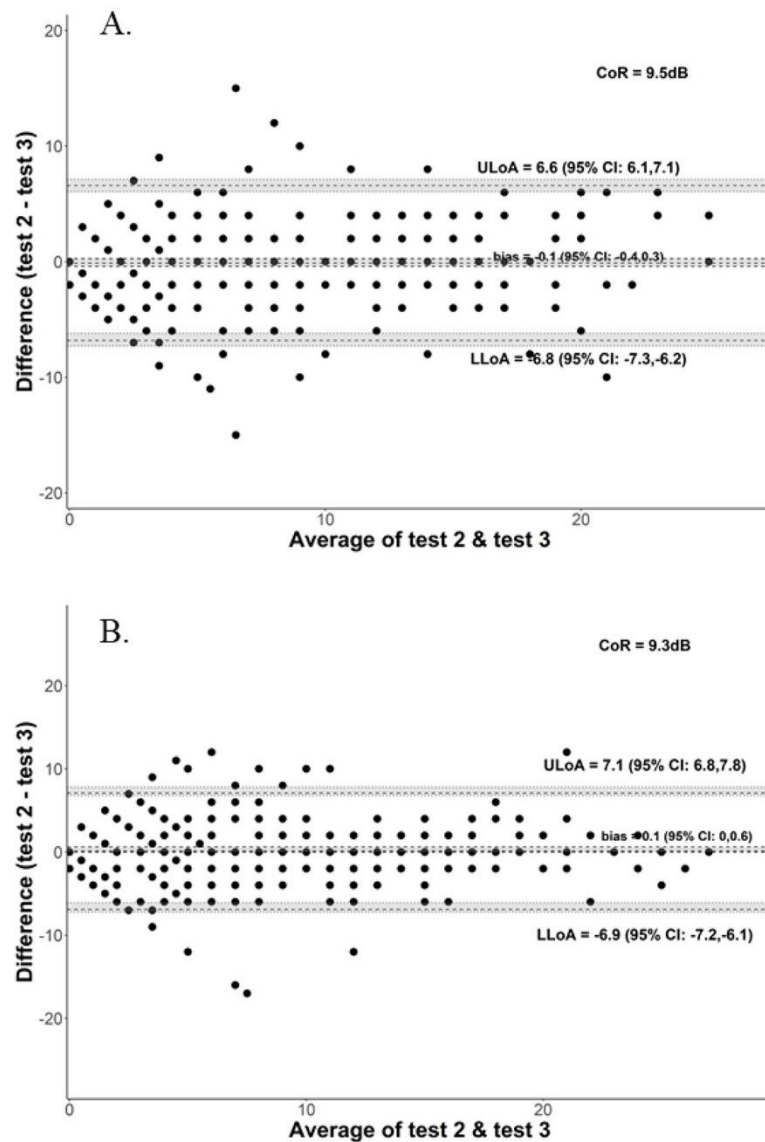


Figure 3.7: Bland-Altman plots evaluating test-retest variability of individual microperimetry loci (pointwise thresholds) for participants with *RGPR*-associated RP for the right (A) and left eyes (B). The pointwise CoR for the right and left eyes were 9.5 dB and 9.3 dB, respectively. LLoA denotes the lower limit of agreement; ULoA denotes the upper limit of agreement. Figure from Taylor *et al.* (2023a).

Karuntu *et al.* (2025b) reported comparable intra and inter-pointwise test-retest variability for a mixed cohort of patients with RP with poor VA (≥ 0.4 logMAR, CoR 8.6 dB [95% LoA -7.7 to 6.2 dB] and CoR 10.2dB [95% LoA -9.8 to 8.3 dB], respectively) and moderate VA (< 0.4 logMAR, CoR 7.3 dB [95% LoA -5.3 to 5.2] and CoR 7.7 dB [95% LoA -5.3 to 5.9 dB], respectively). In addition, the pointwise sensitivity CoR values compare well to previously reported overall values for choroideremia (± 8.7 dB), where it was considered that later-stage patients show greater variability (Dimopoulos, Tseng and MacDonald, 2016). However, it is unclear if the study compensated for paired data.

The pointwise CoR (defined in Figure 3.7) was then used to determine the probability of meeting the FDA regulatory endpoint of a 5-point 7 dB change by chance or natural variability. The summing of probabilities by applying a binomial cumulative distribution function results in an overall 25% probability of obtaining five or more points (out of the 68 tested) with a greater than ± 9.0 dB change from baseline due to variability alone. The probability of achieving five points with the less stringent criteria of ± 7.0 dB would be expected to be even greater than 25%. However, if only the central 16 points were considered, then the probability of obtaining five points with a change of greater than ± 9.0 dB reduces to a more reasonable 0.1%. For 36 central points, the probability of achieving five points with a change of greater than ± 9.0 dB would be approximately 3%.

Paper 8 (Taylor *et al.*, 2023a) then used natural history longitudinal data from patients with *RPGR*-related RP to investigate how many patients, due to natural variability alone, would hypothetically meet the FDA regulatory recommended microperimetry endpoint (≥ 7 dB in at least 5 loci, discussed above) in the absence of any intervention. Two untreated patients with *RGPR*-associated RP (out of 14, who completed testing on both right and left eyes) met this endpoint simply due to natural variation, which aligns with the reported probabilities reported above. This included one patient who showed significant changes within the stricter central 16 test point criteria.

On the other hand, in a natural history study of patients with *USH2A*-associated RP (RUSH2A study), only 5% of participants (due to disease progression) showed a decline meeting the FDA regulatory significant sensitivity change criteria (≥ 7 dB in at least 5 loci) after 4 years (Maguire *et al.*, 2024). This may suggest the proposed FDA criteria is relatively insensitive to retinal sensitivity decline over time or reflects the slow progressive nature of *USH2A*-associated RP.

Overall, despite the advances in stimulus placement tracking capabilities of microperimetry compared to conventional static perimetry, the use of pointwise sensitivity changes as an outcome measure or trial endpoint is not recommended due to a high degree of natural variability that still remains. This results in a subsequent inability to detect clinically meaningful change over time that is required in novel therapies for IRDs, and particularly, in slow degenerative conditions such as RP.

3.2.1.2 Mean Sensitivities

The mean sensitivity is the average of all individual pointwise thresholds. This averaging reduces the impact of any potential highly variable outlier points, although at the cost of limiting the ability to detect small focal sensitivity changes. The use of mean sensitivity is, however, hampered in the device by the arbitrary assignment of -1.0 dB to any unseen points (loci with no detectable sensitivity) and any negative mean sensitivity is expressed as zero dB. In patients with a large number of unseen points, this results in a negatively inflated frequency distribution, artificially lowering the mean sensitivity result, driving the overall mean sensitivity towards zero. This is particularly problematic in rod-cone degenerations, where many patients in mid-to-late disease stages may have extensive parafoveal ring-like scotoma. As the disease progresses and the area of central retinal sensitivity further constricts, the number of non-seen loci around the parafoveal testing area increases (Figure 3.10).

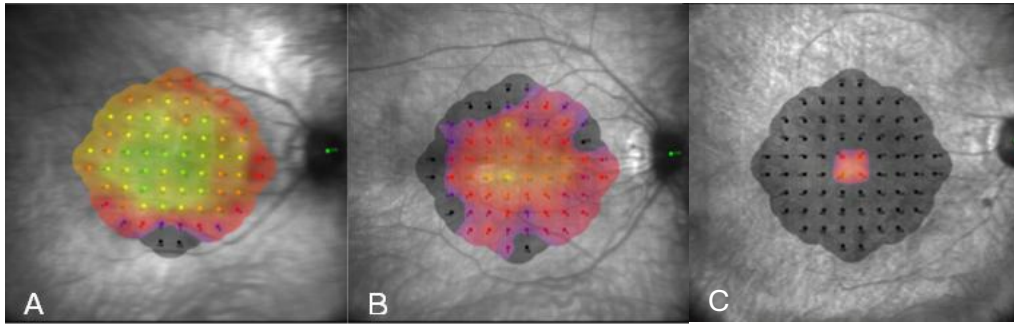


Figure 3.8: Is a representative example of microperimetry sensitivity heatmaps from three patients with *RPGR*-associated RP at different stages of disease, illustrating the concentric parafoveal disease progression. A) A relatively early-stage patient with a mean sensitivity of 18.0 dB. B) moderate-stage patient with a mean sensitivity of 5.6 dB. C) A later-stage patient with a mean sensitivity of 0.0 dB.

Paper 8 evaluated 884 pointwise loci from the longitudinal data of patients with *RPGR*-associated RP, 32% and 40% (right and left eyes, respectively) of stimuli were unseen and assigned -1.0 dB, resulting in a highly positively skewed distribution (Figure 3.9). In these instances where more than half of the number of test loci (>34) have no detectable sensitivity and are assigned -1.0 dB, the overall mean sensitivity becomes very low (<1.0 dB). Once the number of unseen loci extends to 50 or more points (out of a total of 68 points), the mean sensitivity in these patients is driven down to 0.0 dB (Figure 3.9).

This highlights a significant limitation with the default mean sensitivity calculation reported by the device, as it creates an artificial floor effect due to the averaging of many negative threshold values. Although the mean sensitivity metric has the advantage of damping the effects of large variation outlier pointwise changes, its use as a sensitivity index is far from ideal. Using mean sensitivity in later-stage patients as an endpoint in a clinical trial severely limits the potential to show any therapeutic changes due to the above-reported issues.

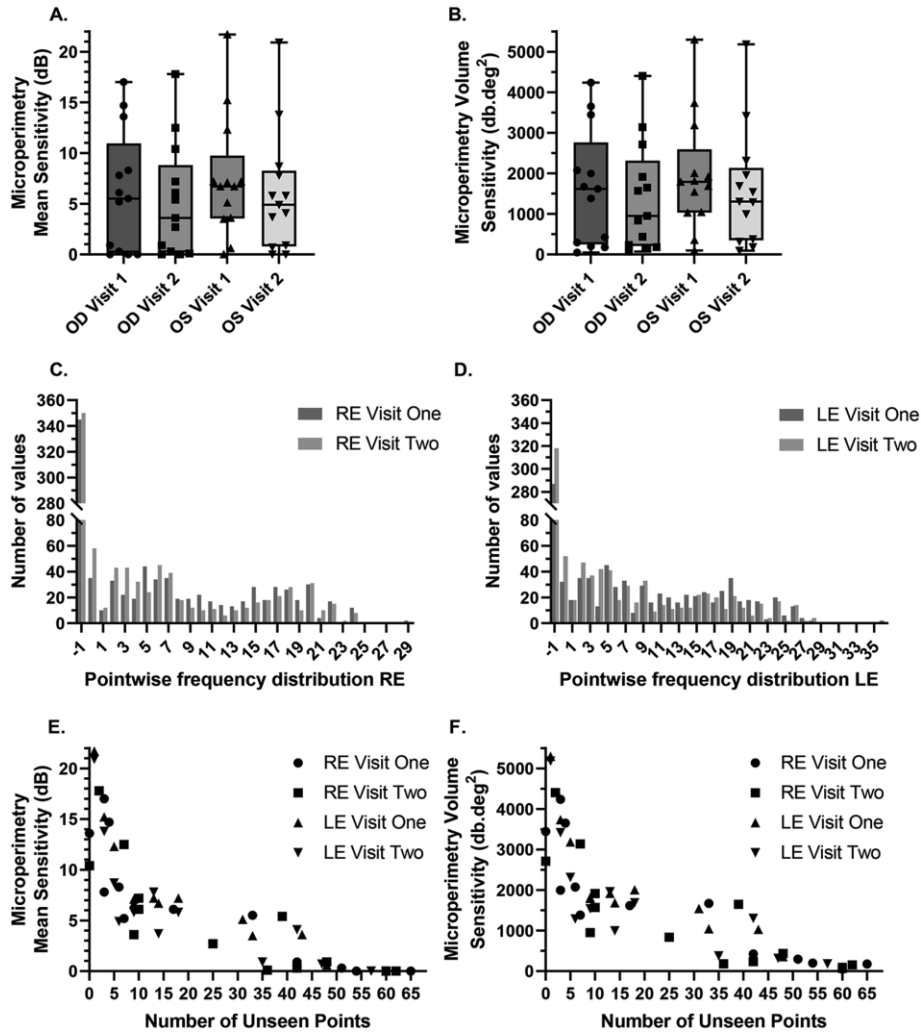


Figure 3.9: (A) The median mean sensitivity for the right eyes visit one was 5.7 dB (IQR, 0.2–9.2 dB), and for visit two it was 3.2 dB (IQR, 0.3–8.5 dB). Left eye median mean sensitivity for visit one was 6.9 dB (IQR, 2.8–9.2 dB), for visit two it was 4.3 dB (IQR, 0.5–9.3 dB). (B) The volume sensitivity data for visits one and two for each eye. The median volume sensitivity for the right eye was 1497 dB*deg² (IQR, 272–2460 dB*deg²) for test one and 894 dB*deg² (IQR, 223–2218 dB*deg²) for test two. Left eye median volume sensitivity was 1748 dB*deg² (IQR, 861–2435 dB*deg²) for visit one and 1143 dB*deg² (IQR, 285–2322 dB*deg²) for visit two. (C and D) Frequency distribution plots for microperimetry pointwise sensitivity values, from visits one and two, show a non-normal, positively skewed distribution, with –1.0 dB containing the highest number of values for the right and left eyes, respectively. (E and F) Scatter plots show the relationship between the number of unseen points and sensitivity, for mean sensitivity and volume sensitivity, respectively. Right eye (RE); left eye (LE). Figure from Paper 8 (Taylor *et al.*, 2023a).

Paper 8 (Taylor *et al.*, 2023a) also explored mean sensitivity CoR and found 0.7 dB [95% LoA -0.6 to 0.7 dB] and 1.3 dB [95% LoA -1.4 to 1.2 dB] in the right and left eyes, respectively. The mean sensitivity variability was much smaller than that reported for pointwise sensitivity, reported above (Figure 3.9). These results are also comparable with previously reported mean sensitivity CoR for choroideremia (± 1.3 dB and 1.5 dB [95% LoA -1.62 to 1.24]) and *USH2A*-associated RP (2.0 dB) (Dimopoulos, Tseng and MacDonald, 2016; Jolly *et al.*, 2017; Maguire *et al.*, 2024) but were smaller than inter-visit reported in a mixed cohort of patients with RP (2.4 dB [95% LoA -4.7 to 6.1 dB] and 1.6 dB [95% LoA -1.8 to 1.0 dB], low and moderate VA groups, respectively)(Karuntu *et al.*, 2025b). However, Dimopoulos, Tseng and MacDonald (2016) used a smaller 37-point radial testing grid, and Maguire *et al.* (2024) used a custom 38-point radial grid, as opposed to the 68-point rectilinear grid.

In the *RUSH2A* natural history study, mean sensitivity CoR (± 2.2 dB) was used to define a clinically significant change. At two years, 14% of participants showed a decline greater than this CoR, 1% showed an increase. At four years, 33% showed a decrease greater than the CoR, which was greater than the 5% of participants who showed a decrease using the FDA pointwise criteria (reported above) at four years (Maguire *et al.*, 2024). However, the FDA endpoint criteria was proposed to report a sensitivity gain, not a decline, but arguably, this demonstrates the insensitivity and unsuitability of this criterion as an endpoint. In addition, these analyses were limited by the use of the radial testing grid, which resulted in spatially weighted averages. Small grids with fewer test points take less time to complete, are less fatiguing, and so this may reduce variability. However, it is a trade-off resulting in less spatial sensitivity information.

In the *RPGR*-associated RP gene therapy trials (discussed in section 2.2.3), only the mean sensitivity indices showed statistically significant therapeutic changes at month 12 in the part 2 cohort (Lam *et al.*, 2024). None of the pointwise analyses showed any significant results, including crucially the 7 dB gain in 5 or more of the central 16 points, forming the primary endpoint. Limiting the analyses to specific pointwise changes

within the central area limits the ability to detect overall sensitivity changes across the entire test grid (Figure 3.10).

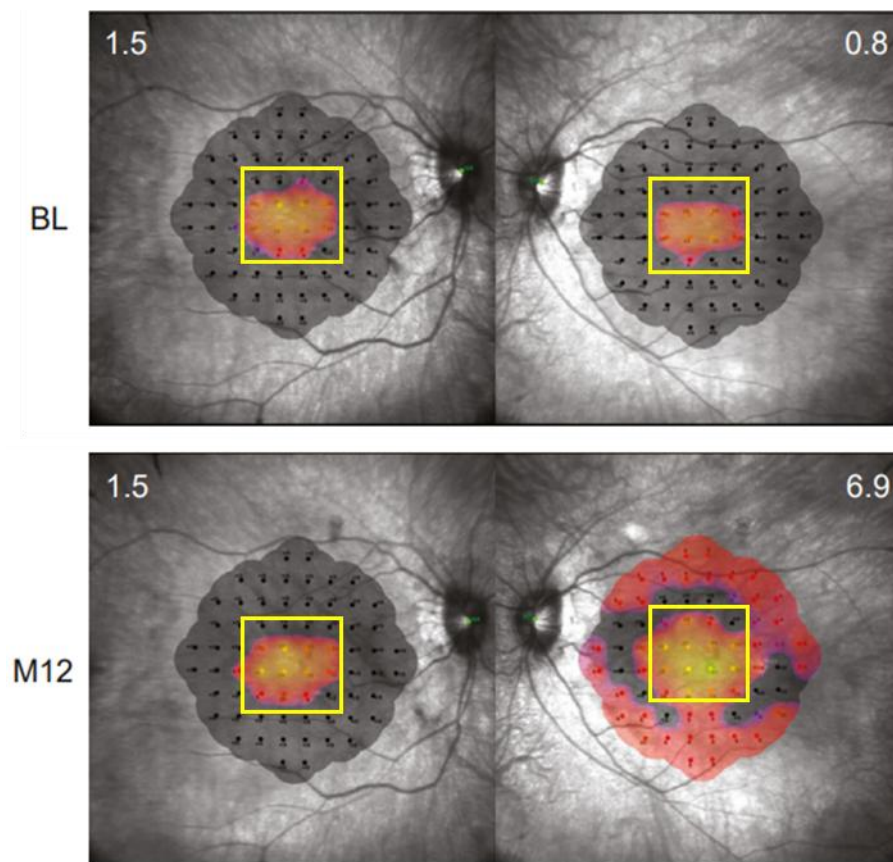


Figure 3.10: Is an adapted figure from Lam *et al.* (2024), indicating the microperimetry heat map images and mean sensitivities (noted in the top right corner in dB) showing the changes from the baseline of a representative study participant receiving gene therapy to the left eye. The yellow box encompasses the central 16 loci. The image illustrates the limitations of only focusing on the central 16 loci.

Although large changes are seen within the central 16 loci, further sensitivity changes are seen outside of this, which were ignored by the regulatory agencies mandated primary endpoint

An alternative endpoint, previously suggested by the FDA as clinically significant, is a between-group mean difference of at least a 7.0 dB mean sensitivity change for the entire field. This was previously used in standard automated perimetry glaucoma clinical trials (Weinreb and Kaufman, 2009). However, 7.0 dB still appears to be

arbitrary, and its translation to microperimetry is not evidence-based. Figure 3.10 shows a representative example of a participant with a 6.1 dB improvement, despite this large increase in sensitivity compared to the untreated eye showing no change, this participant would fall short of the proposed 7.0 dB mean sensitivity required change.

Data reporting the mean sensitivity rate of decline for rod-cone degenerations are limited. A co-authored study by Poli *et al.* (2023) explored the rate of total microperimetry sensitivity decline between 2 and 5 years in choroideremia patients and showed no significant declining trends. However, this is limited by a very small sample size in a very slowly progressing disease. In another co-authored study, a statistically significant average yearly decline of -0.7 dB and -0.8 dB mean sensitivity was reported by Gocuk *et al.* (2025) in female carriers of choroideremia. In *USH2A-associated* RP, the yearly rate of mean sensitivity change was 1.0 dB since this was less than the reported CoR (± 2.0 dB), this would suggest a testing interval of greater than 2 years is required to detect any potential clinically significant change (Maguire *et al.*, 2024). These slow rates of change are likely reflective of the slow degenerative nature of the disease; also, these analyses assume the degeneration declines in a linear trend, which is unlikely, although maybe a reasonable approximation over short time periods.

In another co-authored study of 50 patients with *RPGR-associated* RP, the annual rate of decline in the mean sensitivity of the central 16 loci (using the 10-2 68-point MAIA microperimeter grid) was 10.8% (95% CI: 6.6, 14.8) in cone-rod degenerative phenotypes versus 5.1% (95% CI: 2.9, 7.1) in rod-cone degenerative phenotypes (Josan *et al.*, 2025). This highlights how central mean sensitivity analyses can reveal significantly different rates of functional decline between *RPGR-associated* RP phenotypes and supports the use of microperimetry central mean sensitivity analyses.

Overall, microperimetry mean sensitivity indices appear to be a more suitable surrogate measure of disease progression than prespecified individual pointwise

retinal sensitivities; it is more repeatable and reflects the full testing area, which has shown treatment gains. However, it is limited by averaging floor effects in very late-stage patients and reduced resolution, potentially masking large focal gains.

3.2.1.3 Volume Sensitivity

Volume sensitivity analyses were explored in Paper 8 (Taylor et al., 2023a) using the volumetric programme developed by Josan et al. (2021) (Figure 3.13). Since this software was developed specifically for microperimetry, the assigned units were decibels-degree² (dB.deg²) rather than decibels-steradians, reflecting the relatively flat area assessed by microperimetry. This is considered acceptable given the small retinal area being tested and provides a more interpretable unit of measure. Volume sensitivity is a weighted linear transformation of the underlying pointwise sensitivity data. It incorporates both retinal sensitivity and spatial extent when summarising overall retinal function. A practical advantage is that volumetric analyses are not affected by weighted-averaging issues that can arise when summarising sensitivities from unequally spaced testing grids, such as the commonly used 37-point radial microperimetry grids (Josan et al., 2021).

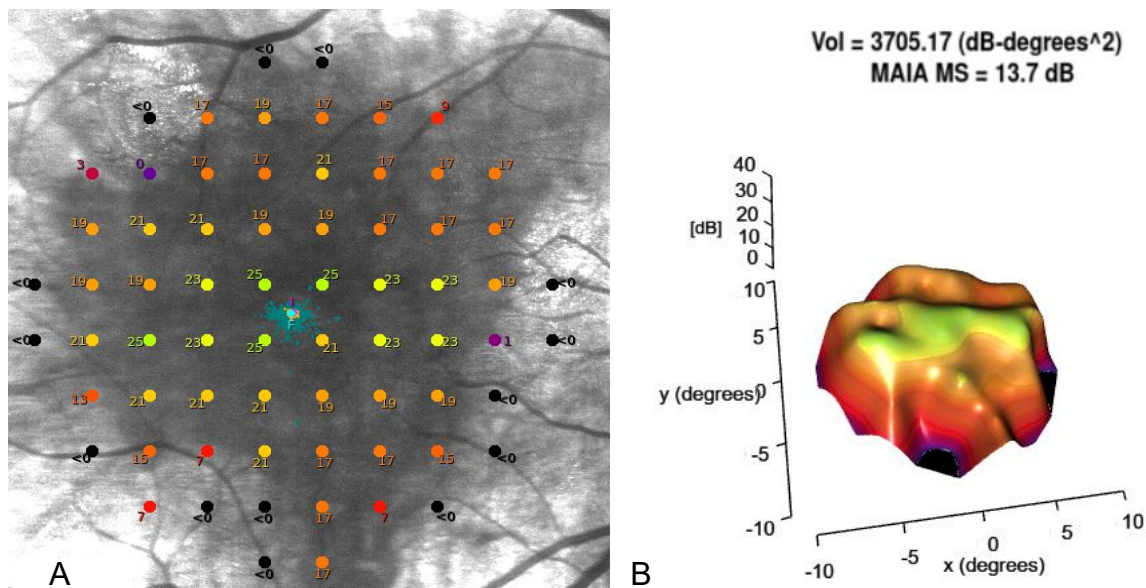


Figure 3.11: A) Details the standard pointwise sensitivity output of the MAIA from a representative patient with choroideremia (collected as part of the VFIRD study). B) Shows the corresponding volume sensitivity output for the same patient using Josan et al. (2021) programme.

In Paper 8 (Taylor et al., 2023a), volumetric analyses (Figure 3.13) demonstrated measurable volume sensitivity values across all examinations, with greater dispersion of values in the lower sensitivity ranges than observed with mean sensitivity (Figures 3.11e and 3.11f). This appeared to provide a more clinically interpretable representation of residual visual function in patients with advanced disease. However, this effect is likely influenced by the volumetric methodology itself. The software converts any 'just seen' loci (0.0 dB) to an arbitrarily small value of 0.1 dB and converts non-seen loci, assigned -1.0 dB by the MAIA device, to 0.0 dB. Consequently, volumetric measures only return a value of zero when all loci are truly not seen. If equivalent data manipulations were applied before calculating mean sensitivity, a similar reduction in floor effects may be observed. Therefore, alternative approaches to calculating mean sensitivity could potentially address this limitation and warrant further investigation. Current clinical trial practice uses the standard mean sensitivity output provided by the microperimetry device, and alternative approaches would require further validation before widespread adoption.

The rate of volumetric change across different IRDs remains largely unexplored. Furthermore, volume sensitivity is limited by the use of comparatively large numerical values, which may be less familiar to clinicians and examiners than conventional sensitivity metrics.

Paper 8 (Taylor *et al.*, 2023a) also explored the volume sensitivity repeatability, with CoR. This was 145 dB.deg² (95% LoA: -133.3 to 150.0 dB.deg²) and 324 dB.deg² (95% LoA: -349 to 287 dB.deg²) for the right and left eyes, respectively. The increased CoR in the left eye was attributed to fatigue effects but is more comparable with more recent inter-visit CoR of 387 dB.deg² (95% LoA -44.2 to 227 dB.deg²) reported in a mixed cohort of patients with RP and moderate BCVA groups. Here, the greater variability was attributed to a greater time between repeat visits (14 days as opposed to 1 day in Paper 8) (Karuntu *et al.*, 2025b).

3.2.1.4 Alternative Sensitivity Indices

Alternative sensitivity indices include those used in static automated perimetry, such as pattern deviation, total deviation, mean deviation, pattern standard deviation, mean defect, loss variance and short-term fluctuation as well as cluster analyses (Flammer *et al.*, 1985; Heijl, Lindgren and Olsson, 1987; Pfau *et al.*, 2018). However, these do not appear to have been applied in IRD microperimetry analyses. This could be due to these alternative sensitivity indices relying heavily on pointwise thresholds, and as shown above, these are prone to relatively high levels of test-retest variability. Small undetected changes in retinal positioning can lead to a slightly adjacent location of the macular area being tested, leading to greater variation in results. These limitations also apply to standard static perimetry; however, since the macula has less neuronal summation than the peripheral retina, there may be greater variations in pointwise sensitivity. Overall, these standard perimetry indices were designed to detect defects or patterns in larger visual fields, since microperimetry assesses only the macular area, and focal areas of loss are easily seen.

Another consideration is amblyopia; in *RPGR*-associated RP patients, visual function may be limited by underlying amblyopia, due to dysfunctional photoreceptors in early childhood (Menghini *et al.*, 2021). This could result in a ceiling effect in their maximum possible threshold sensitivity despite the appearance of preserved retinal structure. Since total deviation analyses rely on comparisons to normative databases, this may limit the ability to detect subtle variations in defects if the entire patient population has reduced sensitivity. In addition, given the heterogeneity and rarity of each IRD, obtaining a ‘normative’ patient database required for these alternative metrics is unlikely.

It is unclear whether probability analyses would be suitable as outcome measures in clinical trials. The level of change required to be clinically significant remains to be defined. In addition, probability analyses are limited by the heterogeneity of visual function loss in IRDs, unlike in glaucoma, where visual field loss patterns follow a relatively consistent trend in keeping with the organisation of the retinal nerve fibre bundles. Patients with rod-cone degenerations (due to IRDs) typically present with ring scotomas on microperimetry testing, rather than isolated focal defects, suggesting limited applicability to pattern deviation analyses. Furthermore, probability analyses are not included as standard MAIA outputs; they require post-hoc analyses, limiting their widespread use (Cassels *et al.*, 2019). Automated software, which includes volumetric analyses as well as sensitivity change analyses over time, comparable to Glaucoma change probability, would be hugely beneficial and has previously been recommended by Yang and Dunbar (2021). This would negate post-hoc analyses, which are impractical to undertake in a clinical setting and could lead to greater method variation between trials.

3.2.2 Mesopic Microperimetry Learning and Fatigue Effects

In *RPGR*-associated RP clinical trials, testing in triplicate has been performed at baseline and other key follow-up visits (i.e. final trial visit), using only the last of the triplicate test results for analyses to minimise learning effects (Buckley *et al.*, 2020). The duration of any learning effects is unknown, and it is unclear whether a learning test is necessary at every follow-up visit, particularly where a substantial amount of

time (e.g., 6 or 12 months) has passed since initial testing. In Paper 8 (Taylor *et al.*, 2023a), for the longitudinal data collection, there was no formal learning test undertaken, yet left eye testing (which was performed after the right eye) did not show any significant improvements in test performance (reliability and sensitivity), suggesting no further learning effects occurred. Furthermore, there is little exploration in the literature focused on the type of learning test that should be performed. Undertaking a short suprathreshold practice test enables patients or participants to become familiar with the testing procedure, yet it does not provide precise training on determining a threshold stimulus-response (i.e. recognising a stimulus at the limits of detection). Arguably, to optimise training from a practice test, it should be a short threshold exam to provide a realistic experience, but still avoid inducing fatigue effects.

Due to the complexities of statistical analysis when collecting data from both (paired) eyes, many studies have only used data from one eye in subsequent analysis. However, Paper 8 (Taylor *et al.*, 2023a) showed both the CoR, for mean sensitivity and volume sensitivity, with greater variability in the left eye than the right eye. Since it is conventional to test the right eye first, this suggests the increased variability seen in the left eyes could be a result of fatigue effects. To the best of our knowledge, this is the only study that has demonstrated fatigue effects, and further research is needed to fully understand the impacts and determine which effects (fatigue or learning) are more significant. In a clinical trial, it is preferable to minimise any sources of variability. Therefore, it is now recommended to complete testing on the study eye first.

3.3 Scotopic Microperimetry

This section synthesises and evaluates the publications focused on scotopic microperimetry.

Paper 9

Scotopic microperimetry: evolution, applications, and future directions (Taylor et al., 2022)

This scoping literature review summarises the development of scotopic microperimetry, uses and testing parameters, to inform considerations for future research.

Freely available at:

<https://onlinelibrary.wiley.com/doi/10.1080/08164622.2021.2023477>

Author contributions

This was a collaborative project. LT (main contributor) completed the literature search and review of the NIDEK microperimetry section, as well as tying together the colour fundus perimetry and MAIA microperimetry sections, redrafted, and wrote the discussion. JKJ searched and drafted the MAIA microperimetry section, AJ drafted the two-colour perimetry section. MP and MS provided expert feedback and reviewed the manuscript before submission.

Paper 10

Exploring scotopic microperimetry as an outcome measure in choroideremia (Taylor et al., 2024)

Primary research paper, exploring the performance, repeatability and sensitivity of scotopic microperimetry in patients with choroideremia.

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2800850>

Author contributions

LT (main contributor) was responsible for developing the research question and study setup, participant recruitment, and patient participant data collection. In addition to

data collation, descriptive analyses, interpretation, and drafting of the manuscript. Other co-authors contributed with healthy control data collection (DA), linear mixed modelling and repeatability analyses (AJ). JKJ and REM contributed professional oversight.

Paper 11

Scotopic Microperimetry: An Analysis of Healthy Controls (Adeyoku et al., 2025)

Primary research paper, exploring the performance and repeatability of scotopic microperimetry in healthy controls

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2803104>

Author contributions

This primary research publication was part of the Visual Function in Retinal Degeneration study that LT (joint senior contributor) designed and set up. LT provided expert input and supervisory support throughout. DA (main contributor) completed data collection, collation, and initial analyses of the results and manuscript drafts. JS completed additional statistical analyses and completed the final draft of the manuscript. REM provided professional oversight throughout.

The evolution, parameters and uses of scotopic microperimetry are discussed in Paper 9 (Taylor *et al.*, 2022), which recommends the need for a standardised testing grid, such as the rectilinear grid, to avoid spatially weighted averages of stimulus thresholds. It summarises the use of scotopic microperimetry testing in different retinal diseases, including age-related macular degeneration. Since this review, scotopic microperimetry (using only red stimuli) has been used as an exploratory outcome measure in a phase I/II clinical trial for *RPGR*-associated RP (Michaelides *et al.*, 2024). A significant least-squares mean difference between treated (n=15) and controls (n=7) was 1.06 dB (95% CI 0.05-2.07, P=0.041). These changes, whilst statistically significant, are minimal and highly likely to be within test-retest variability (discussed in section 3.3.4), indicating no clinical significance. Additionally, there are no details

provided about the type of microperimeter used, testing parameters, or the reliability indices.

Early studies used the S-MAIA prototype software with a limited dynamic range (0.0-20.0 dB), which resulted in ceiling effects in healthy controls (Pfau *et al.*, 2017). The latest software increased the dynamic range to 0.0-36.0 dB, with the lower range extended by 10.0 dB and the upper range increased by 6.0 dB (Pfau *et al.*, 2020). Paper 11 (Adeyoku *et al.*, 2025) explored scotopic microperimetry in healthy controls with this extended dynamic range. The new upper limit of effective dynamic range for this healthy cohort was 32.0 dB for cyan stimuli and 34.0 dB for red stimuli, indicating a sufficiently updated range. This subsequent section evaluates the testing parameters and performance of scotopic microperimetry, evaluating it as a potential outcome measure in IRD.

3.3.1 Dark Adaptation Time Before Scotopic Microperimetry Testing

The state of retinal dark-adaption is a critical consideration in scotopic testing to ensure accurate and repeatable results with optimal sensitivity to subtle disease-related changes. Dark-adaptation times of between 20-45 minutes have been used in different scotopic microperimetry studies and are summarised in Paper 9 (Taylor *et al.*, 2022). Twenty minutes is feasible in a busy clinical setting and corresponds with the recommended standard for scotopic electrophysiological testing (Robson *et al.*, 2018; Jolly *et al.*, 2023). Paper 9 further discusses how standardising dark-adaptation is challenging, as it is dependent on the level of light exposure before testing, as well as age and severity of eye disease, both of which can considerably prolong the dark-adaptation rate (Jackson, Owsley and McGwin, 1999; Nigalye *et al.*, 2022). To achieve absolute threshold results in retinal disease where rod function is impaired, extensive dark adaptation times (greater than eight hours) may be required to ensure individuals are fully dark-adapted to detect subtle changes in rod photoreceptor sensitivity (Roman *et al.*, 2022). However, the everyday generalisability and functional significance of a therapeutic response that requires over eight hours of dark adaptation to be detected is limited. Paper 9 (Taylor *et al.*, 2022) concludes that dark-adaptation time should be decided on the balance of priorities, between accuracy of a uniform adaptation state, associated increased response accuracy, practicalities and

ultimately, the purpose and relevance of the assessment. For clinical trial outcomes, twenty minutes appears to be sufficient for healthy individuals and patients with early-moderate disease, with longer times (within practical reason) advised for more severe disease.

To investigate whether the left eye (typically tested second) benefits from longer dark adaptation time while the right eye undergoes testing, Paper 11 (Adeyoku *et al.*, 2025) compared interocular symmetry for both cyan and red stimuli in healthy controls. No significant difference in mean volumetric retinal sensitivity was found between the right and left eyes (paired *t* test: cyan, $P = 0.452$; red, $P = 0.569$). Indicating no beneficial effects (of longer dark adaptation time or learning effects) to the left eye, when the right eye is tested first. Although not strictly assessed, this also suggests there are no statistically or clinically significant confounding effects from an interocular light/dark adaptation summation mechanism, whereby the covered (non-testing) contralateral eye may light adapt to the level of the uncovered testing eye (Auerbach, Dorrenhaus and Cavonius, 1992).

Another important consideration is the brightness of the fixation target, which has been suggested to impact central dark adaptation and the detected central retinal sensitivity (Pfau *et al.*, 2017). This may be more significant in later-stage patients who require a brighter fixation target during scotopic testing.

3.3.2 Scotopic Microperimetry Reliability

Papers 10 and 11 evaluated the reliability of scotopic microperimetry performance, using both the standard reliability indices summarised in section 2.2.2, including 'fixation stability, fixation losses, and a novel rod-free area detection analysis discussed below.

3.3.2.1 Fixation stability

Patient fixation is tracked throughout microperimetry (scotopic and mesopic) testing; the results are used to infer the overall stability of the patient's fixation. In Paper 10 (Taylor *et al.*, 2024), participants with choroideremia had low BCEA scores (median [IQR] BCEA95 for cyan and red was 1.2 (0.6–3.4) and 1.0 (0.5–1.8), respectively). These were lower than those reported in Paper 11 (Adeyoku *et al.*, 2025) for scotopic microperimetry from healthy controls (mean [SD] BCEA95 for cyan and red was 2.9 ± 5.9 and 2.00 ± 1.7), where a reliability cutoff of >5 degree² was recommended. This suggests that fixation stability was not a limiting reliability factor. Since the fixation target intensity is adjustable in scotopic testing (but not for mesopic), this could lead to better visibility and more stable fixation. This could explain the better fixation in the choroideremia group. However, as discussed above, the increased brightness could then impact central dark adaptation and subsequent central sensitivity, as well as test variability (Pfau *et al.*, 2017). The S-MAIA has fundus tracking abilities to improve testing accuracy and limit poor reliability due to poor fixation. However, the tracking capabilities are limited by the 25Hz refresh rate of the scanning laser ophthalmoscope. Therefore, good fixation is still critical to avoid undetected stimulus placement error, which may occur from rapid saccadic eye movements that are beyond the device's detection capabilities (Josani *et al.*, 2023).

3.3.2.2 Fixation losses

Like in mesopic microperimetry, fixation losses are determined via a 10 dB suprathreshold stimulus presented to the blind spot at an arbitrary rate of approximately one per minute (Josani *et al.*, 2023). This is a false positive type test, although it differs from traditional false positive catch trials used in standard perimetry, where audible cues suggest stimulus presentation when there is not. In Paper 10 (Taylor *et al.*, 2024) 60% of tests from participants with choroideremia and healthy controls had 0% fixation losses. Despite this, fixation losses (greater than 30%) were the leading cause of test exclusions, forming 4/7 (57%) of excluded choroideremia participants and 4/4 (100%) of excluded healthy controls. In Paper 11 (Adeyoku *et al.*, 2025), which included a reanalysis of this healthy control data, reported 18% (n=14) of tests were also excluded due to 'fixation losses'; however, this was using the stricter

cut-off of 20% fixation losses. There appears to be no consistent or standardised fixation loss cutoff in the current literature.

3.3.2.3 Rod-Free Area Detection

Paper 10 (Taylor *et al.*, 2024) proposed the identification of the central rod-free area as a novel indicator of reliability. Due to decreased rod density towards the fovea, described by Curcio *et al.* (1990), an absent (or much decreased) cyan foveal response would be expected in this central region. The absence of detection of the rod-free area can serve as a proxy for false positive responses, particularly in those with good fixation. In light of the limited reliability markers in microperimetry testing, this was deemed to be a useful addition. In Paper 10, the lack of rod-free area detection was determined by central cyan responses greater than 8.0 dB, determined by the upper quartile of all responses. This resulted in the second-highest cause for test exclusion, making up 6/11 exclusions (three participants with choroideremia and three healthy controls). Some participants showed both no rod-free area detection and high fixation losses (discussed in the above section) (Taylor *et al.*, 2024). In Paper 11, an absence of rod-free area detection was found in 18% of all eyes, and 14% of eyes after exclusion of those with greater than 20% 'fixation losses' (Adeyoku *et al.*, 2025).

While this approach is readily available to the practitioner with no modification to the testing protocols, it creates bias toward greater cyan test exclusions. Furthermore, the standard 37-point radial grid (manufacturer's recommendation for scotopic testing) only contains a single foveal test locus. This reliability indicator would have greater validity if multiple central loci were tested to ensure consistency of responses. In addition, it may be limited by greater cyan variability within this region, since Pfau *et al.* (2017) reported greater cyan CoR from central test points within the rod free area (7.5 dB) compared with 3-7 degrees eccentricity (CoRs up to 4.3 dB). Interestingly, this difference in variability across different regions was not seen with mesopic or scotopic red testing.

3.3.2.4 Overall Scotopic Microperimetry Test Reliability Performance

After reviewing the reliability indices reported above, Paper 10 (Taylor *et al.*, 2024) reported that 27% of tests (n=11), from a cohort of patients with choroideremia, were excluded before sensitivity analyses due to demonstrating poor reliability. In both Paper 10 (Taylor *et al.*, 2024) and Paper 11 (Adeyoju *et al.*, 2025) false positive responses (including both fixation losses and lack of rod-free area detection [paper10 only]) were the leading source of exclusion. Both papers reported mostly stable fixation. This potentially indicates participants' difficulty with detecting threshold stimuli in scotopic conditions and performing the test, despite stable fixation.

3.3.3 Scotopic Microperimetry Sensitivity Indices

3.3.3.1 Pointwise and Mean Sensitivity

Scotopic microperimetry uses the same sensitivity indices as mesopic microperimetry, pointwise and mean sensitivity. Paper 10 (Taylor *et al.*, 2024) demonstrated the same limitations with pointwise and mean sensitivity explored in mesopic microperimetry in patients with rod-cone degeneration, Paper 8 (Taylor *et al.*, 2023a). When many cyan and red loci show no detectable sensitivity (floor effects), there is a negatively inflated sensitivity distribution (Figure 3.14), which affects the averaging of mean sensitivity and results in much lower mean sensitivity values, creating an *artificial* floor effect.

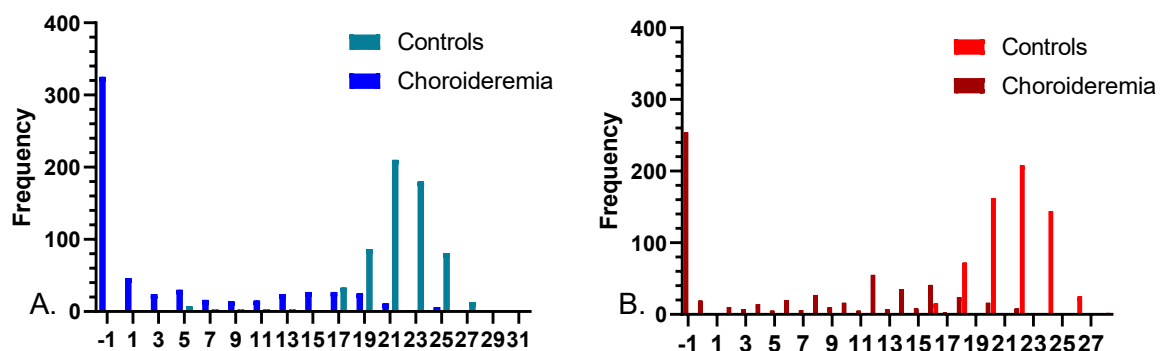


Figure 3.12: Frequency distribution plots of pointwise sensitivities for cyan (A) and red (B) stimuli from a cohort of patients with choroideremia (37 test points x 16 participants).

The default scotopic microperimetry grid (and the grid used in Paper 10) is the radial 37-point grid. Its radial design causes test loci to be unequally spaced, resulting in spatially weighted mean sensitivity outputs. Figure 3.15 illustrates the radial grid and how it overlies three representative patients with choroideremia; many of the test points fall onto areas of degenerated retina. An alternative rectilinear grid with a central point may enable more accurate detection of the rod-free zone and adjacent areas. However, careful consideration between testing time and the number of points is required; the 68-point 10-2 rectilinear grid used in mesopic microperimetry is likely to be too tiring, given that the scotopic testing using the S-MAIA is typically performed twice (once for each stimulus colour).

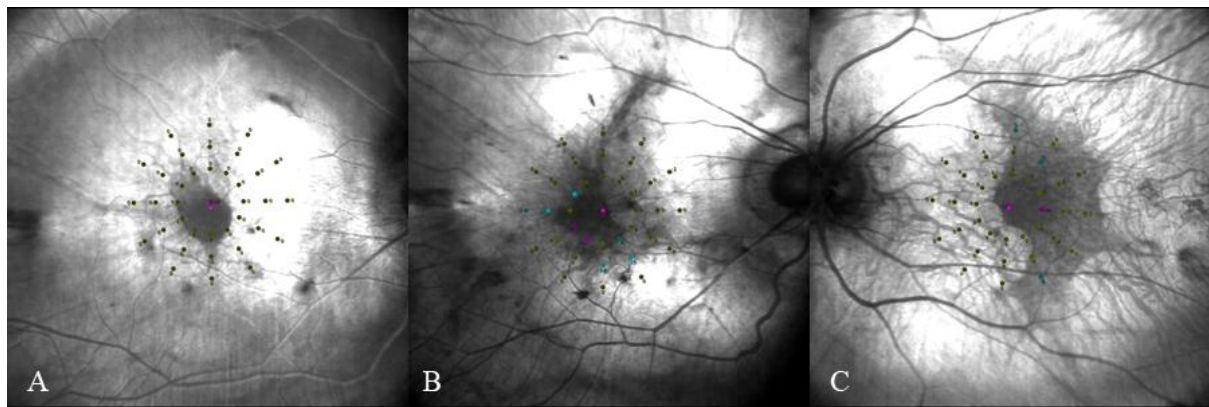


Figure 3.13: Scotopic cyan minus scotopic red difference plots overlaid over fundus autofluorescence images from three participants with choroideremia using ImageJ (Version 1.53T and plugin: align image by line ROI V3 0.4). A) Details how the central island is mostly unassessed, as it is within the central ring of testing points. B) Details another patient's residual island that falls outside the testing grid. C) Shows a patient with a larger preserved temporal island.

Despite the limitations with the standard grid employed in scotopic microperimetry, Paper 10 (Taylor *et al.*, 2024) showed, in a cohort of patients with choroideremia, statistically and clinically significant (defined in section 3.3.4) reductions in cyan and red scotopic mean sensitivities (median mean sensitivities 2.3 dB (IQR, 0.4–6.3) and 4.9 dB (IQR, 1.3–10.0, respectively), compared to healthy controls (median mean

sensitivities of 20.6 dB (IQR, 19.6–20.9) and 21.8 dB (IQR, 20.4–22.4), respectively (Mann-Whitney U Test, $P < 0.002$).

3.3.3.2 Volume Sensitivity

Paper 10 (Taylor *et al.*, 2024) advocates for the use of hill of vision volumetric spatial sensitivity indices, termed volume sensitivity, using an app modified for scotopic microperimetry (ocular.shinyapps.io/scotopicMAIA) (Figure 3.14). All participants with choroideremia showed measurable volume results (median volume sensitivity 462 dB.deg² (IQR, 178–998) for cyan and 878 dB.deg² (IQR, 366–1502) for red). Two participants with choroideremia had very low cyan volume sensitivity (0.9 dB.deg² and 0.5 dB.deg²), the standard S-MAIA means sensitivity for both these participants was 0.0 dB. This demonstrates detectable sensitivity with volumetric indices that was not detectable with mean sensitivity indices. The overall results showed that median volume sensitivity with both cyan and red stimuli was significantly reduced compared to healthy controls ($P \leq 0.001$, Mann–Whitney U test).

Paper 11 reported a normative range of volume results in a cohort of ‘young’ healthy participants (median age 24, IQR 9.5 years): the mean ($\pm$ SD) cyan volumetric sensitivity was 2868 ± 157 dB.deg² and 2892 ± 205 dB.deg² in the right and left eyes, respectively. The mean red volumetric sensitivity was 3077 ± 176 dB.deg² and 3126 ± 207 dB.deg² in the right and left eyes, respectively. Further analysis showed no significant difference between right and left eyes (paired *t*-test: cyan, $P = 0.452$; red, $P = 0.569$), indicating interocular symmetry. In addition, no significant differences were found between male and female volume sensitivities (*t* test: cyan, $P = 0.149$; red, $P = 0.073$), and no significant correlation between volume sensitivities and age was found (Spearman's correlation: cyan, $\rho = -0.007$, $P = 0.971$; red, $\rho = -0.002$, $P = 0.993$), although the age range of healthy controls was too small to make any definitive conclusions.

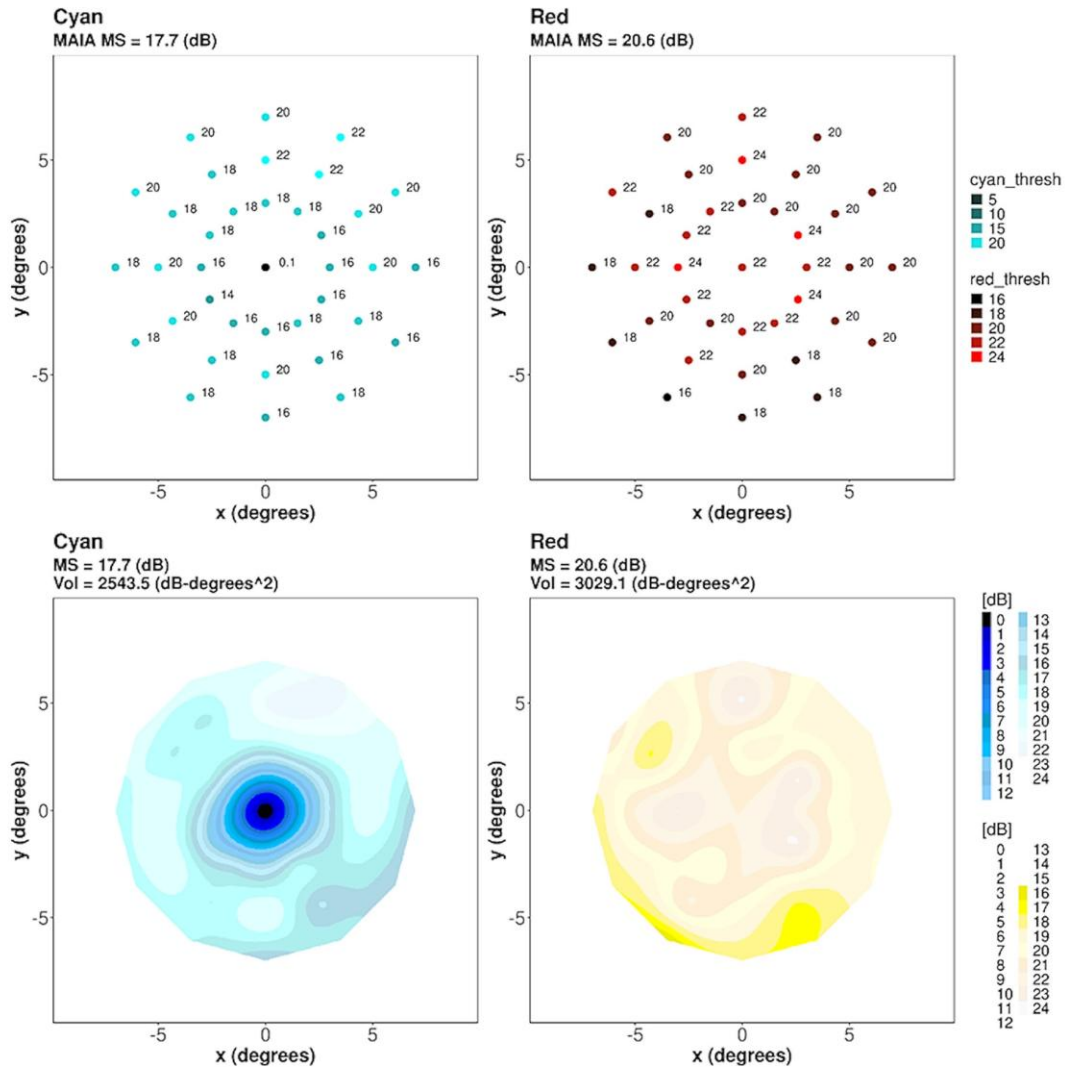


Figure 3.14: Custom volumetric program output where retinal sensitivity values and locations are transformed to a three-dimensional plot—the X–Y plane retains the position of the test points on the grid, and the Z axis is scotopic threshold sensitivity, forming a hill of vision via interpolation. The volume under this hill of vision measures the total amount of sensitivity in dB.deg².

3.3.4 Scotopic Microperimetry Repeatability

Paper 10 also explores the repeatability of scotopic microperimetry testing. Only 10 participants with choroideremia, completed reliable scotopic microperimetry testing (passed screening) and completed intrasession repeat testing. Of the included patients, high pointwise and mean sensitivity test-retest variabilities were found (Table 3.4)(Taylor *et al.*, 2024). These pointwise CoR were higher than those previously

published by Jolly *et al.* (2023), who reported a CoR of 7.2 dB (95% LoA -7.1-7.2 dB) for cyan and 11.7 dB (95% LoA -10.7-12.7 dB) for red. However, it is unclear if that study accounted for nested data, which would result in underestimations of pointwise CoR. Furthermore, the CoRs found in Paper 10 (Table 3.4) are higher (i.e. show more variability) than those previously reported for choroideremia mesopic microperimetry, 4.7 dB and 1.5 dB for pointwise and mean sensitivity, respectively (Dimopoulos, Tseng and MacDonald, 2016; Jolly *et al.*, 2017). Repeatability for mesopic microperimetry is explored in section 4.2.

Table 3.4: Descriptive and coefficient of repeatability (CoR) summary of pointwise, mean, and volume cyan and red sensitivities for 10 Participants with Choroideremia. Interquartile range (IQR).

	Cyan Stimuli	Red Stimuli
Pointwise Sensitivity		
Pointwise CoR, dB, (95% LoA)	15.4 (-13.9-12)	12.4 (-9-8.7)
Mean Sensitivity		
Median Mean Sensitivity Test 1, dB (IQR)	2.6 (1.5-6.3)	4.9 (1.4-10.6)
Median Mean Sensitivity Test 2, dB (IQR)	2.1 (0.6-6.3)	5.2 (1.3-10.3)
Significance (Wilcoxon signed rank test)	P>0.05	P>0.05
Mean Sensitivity CoR, dB, (95% LoA)	3.3 (-3.8-3.1)	1.4 (-1.6-1.4)
Volume Sensitivity		
Median Volume Sensitivity Test 1, dB*degrees ² (IQR)	525 (278-525)	878 (357-1669)
Median Volume Sensitivity Test 2, dB*degrees ² (IQR)	427 (221-1050)	715 (265-1596)
Significance (Wilcoxon signed rank test)	P>0.05	P=0.04*
Volume Sensitivity CoR, dB.deg ² , (95% LoA)	527 (-601-481)	217 (-251-187)
Coefficient of repeatability (CoR); Decibels (dB); Interquartile range (IQR); Limits of agreements (LoA)		

Paper 11 (Adeyoku *et al.*, 2025) investigated cyan and red scotopic microperimetry intersession repeatability in 16 healthy control individuals, on the right eye only, using the updated S-MAIA with the extended dynamic range (0.0-36.0 dB). The second test was undertaken within four weeks of the initial test. Although only 12 completed testing, with less than 20% fixation losses and were included for analysis. The CoRs were considerably lower in controls (Table 3.5) compared to choroideremia (Table 3.4). Despite this, these results were considerably greater than the intersession pointwise CoR reported for cyan (5.3 dB, 95% LoA -4.7 to 5.7 dB) and red (4.1 dB, 95% LoA -3.6-4.4 dB), as previously reported by Pfau *et al.* (2017), in healthy controls.

Table 3.5: Descriptive and coefficient of repeatability (CoR) summary for pointwise, mean and volume cyan and red sensitivity for 12 healthy control participants.

	Cyan	Red
Pointwise Sensitivity		
Pointwise CoR,dB, (95% LoA)	±7.2 (-5.2-5.2)	7.9 (-6.1-5.8)
Mean Sensitivity		
Test 1 Mean (dB)	20.1	21.4
Test 2 Mean (dB)	20.9	22.1
Mean Sensitivity CoR. dB, (95% LoA)	±1.4 (-1.4-1.4)	2.1 (-2.3 – 1.9)
Volume Sensitivity		
Test 1 Mean (dB.deg2)	2883	3144
Test 2 Mean (dB.deg2)	3012	3257
Volume Sensitivity CoR, dB.deg ² , (95% LoA)	197 (-214-197)	349 (-385-349)
Coefficient of repeatability (CoR); Decibels (dB); Degrees (deg); Limits of agreement (LoA)		

Greater CoR results mean that a greater post-interventional difference is required for results to be considered clinically significant and beyond natural variability. This is likely to be particularly problematic with scotopic cyan tests performed in clinical trials for rod-cone degenerations. Where reduced rod-isolated sensitivities are expected to

be overshadowed by the very large between-test variability. As a result, any possible treatment efficacy signal is likely to be lost within the ‘noise’ of natural variability.

The larger cyan CoR in patients is likely due to greater rod degeneration, impacting cyan reliability. Alternatively, considering cyan is rod-driven (detailed in 1.6.2), rod-mediated vision has a lower resolution compared to cones, and repeatability is usually worse at lower vision levels (Karuntu *et al.*, 2025b). It may be unfair to expect the same repeatability as seen in cone-driven tests.

3.3.5 Scotopic Microperimetry Learning and Fatigue Effects

In Paper 11 (Adeyoku *et al.*, 2025), healthy control participants showed no obvious trend in reliability or sensitivity metrics to suggest any learning or fatigue effects. The study did not undertake any formal training tests apart from performing mesopic microperimetry before scotopic testing. In another study, participants were given ‘a brief instruction and practice of the trigger function’, but no dedicated training test was provided, and again, no major learning effects were reported (Pfau *et al.*, 2017). All participants in Paper 10 (Taylor *et al.*, 2024) were familiar with the principles of microperimetry before testing, as mesopic microperimetry was completed first. However, no formal scotopic learning test was undertaken.

Jolly *et al.* (2023) explored scotopic microperimetry in patients with rod-cone degeneration after conducting a ‘training test under mesopic conditions’ before triplicate scotopic testing. Despite undertaking the training, a learning effect was still found in the main results, with greater Bland Altman LoA reported between tests 1 and 2 than between tests 2 and 3 for both cyan and red stimuli, in both patients and controls. This suggests the learning test was insufficient, although it is unclear how reliability metrics changed between the different tests.

In another study by Montesano *et al.* (2021) exploring scotopic microperimetry in healthy controls, a significantly greater cyan sensitivity on the second test was

reported, despite performing a fast scotopic exam before the first test. This implies that an additional learning effect occurred after the fast scotopic training test. The same study also showed greater cyan variability after 10 minutes of dark adaptation. Since this was performed first, the higher variability could be explained by the testing order and be more reflective of a learning effect rather than dark adaptation-related variability. However, the authors also suggested it could be due to cyan sensitivities being further from the machine's ceiling effects (as the data was collected on an older device with the limited 0.0-20.0 dB dynamic range), limiting detectable learning effects. Red stimuli showed no statistically significant sensitivity increase, with repeat testing, suggesting no learning effect, but could also be masked by limited dynamic range.

Overall, these results tentatively suggest that these various learning tests did not provide adequate training. Although participants are aware of the physical concept of the test, training that mirrors the exact testing conditions and activities (threshold stimuli detection) may be required. It is also unknown if separate training tests are required for scotopic cyan and red stimuli, or whether one set of learning tests undertaken by one eye is sufficient for testing both eyes. Furthermore, given the tiring nature of scotopic microperimetry, there may be a trade-off between learning and fatigue effects when performing multiple tests.

3.3.6 Rod versus Cone Function with Scotopic Microperimetry Testing

The comparison between cyan and red responses is flawed since the central rod-free area is still accounted for in the mean difference calculation, which skews mean sensitivity outputs towards a negative cyan-red difference. Many of the studies that are reviewed in Paper 9 (Taylor et al., 2022) reported early reduced cyan dysfunction and interpreted this as early rod dysfunction. It is unclear if these studies accounted for the expected reduced cyan sensitivity in the rod-free area, contributing to a greater cyan minus red difference. Furthermore, in patients with complete rod dysfunction in a given region, a bright cyan stimulus may elicit a low-threshold response from cones, as often seen by non-zero cyan threshold values measured in the rod-free area (Papers 10 & 11). Therefore, in patients with rod-cone degeneration, ambiguity arises when interpreting the underlying physiological function. Furthermore, the interpretation

of positive cyan–red differences is also unclear due to scotopic cone and rod overlapping red stimuli spectral sensitivities and may not necessarily indicate greater cone dysfunction (Perlman, 2007, Figure 18). This potential confounding interpretation, as well as the high degree of variability, severely limits scotopic microperimetry as an outcome measure.

Paper 10 (Taylor *et al.*, 2024) demonstrates how cyan minus red analyses suffer from the cumulative effects of cyan and red variability; as a result, a pointwise difference of >13.00 dB is required to identify a clinically meaningful difference, representing a further significant drawback with scotopic microperimetry. Currently, the standard S-MAIA output employs an arbitrary threshold of ± 4.0 dB to highlight potentially dysfunctional cyan versus red relative pointwise differences. Only values above this are highlighted as dysfunction. Paper 10 also explored global cyan minus red variability differences and recommended a difference of ± 2.7 dB for mean sensitivity and ± 414 dB*deg² for volume sensitivity to be considered clinically significant beyond natural variability. Overall, this research concludes that cyan minus red analyses are not recommended and are of limited value in understanding underlying photoreceptor dysfunction in rod-cone degeneration.

Chapter 4 Visual Function Recommendations for Clinical Trial Outcome Measures and Critical Reflection

This chapter presents recommendations for the selection of visual function outcome measures in rod–cone degenerations caused by inherited retinal disease (IRD).

Visual function test recommendations are based primarily on which tests remain measurable at different disease stages (Figure 4.1). When floor effects or unreliable test performance are encountered due to a level of visual function loss, alternative assessments are required. Therefore, this critical analysis recommends selecting outcome measures according to both clinical phenotype and disease stage. In very early disease, scotopic microperimetry may be appropriate in patients with preserved central vision reporting nyctalopia, although further validation is required. As disease progresses, floor effects and poor reliability appear to reduce the utility of scotopic assessments (see section 3.3.3). Mesopic microperimetry remains informative until later disease stages, when central vision becomes increasingly compromised, VA loss may be detected, and low-vision-specific assessments may be required (Figure 4.1). Luminance testing conditions appear to influence a vision test's dynamic range across the spectrum of disease. Consequently, in rod-cone degenerations as rod-mediated responses diminish (detected under scotopic testing conditions), optimum testing should transition to mesopic and later photopic assessments, as cone-mediated visual function becomes the predominant measurable outcome.

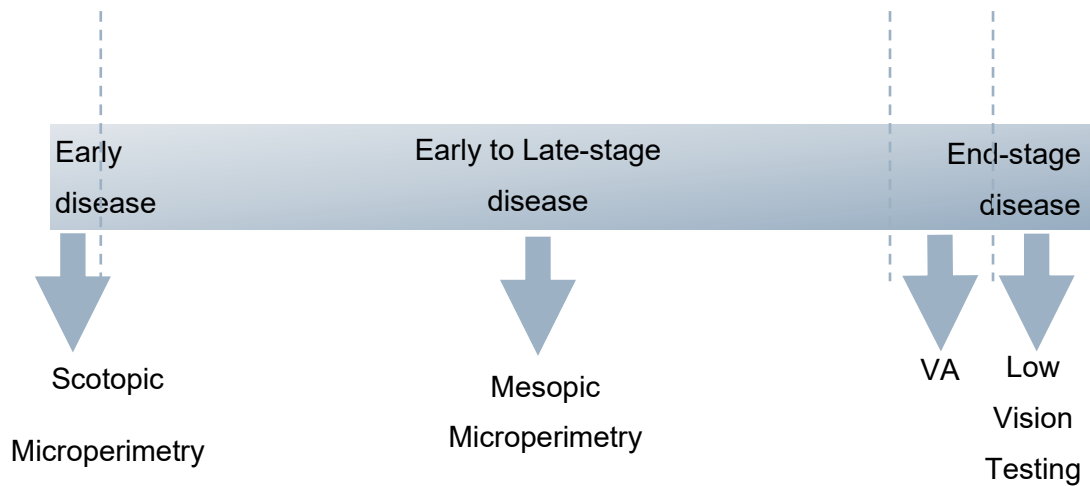


Figure 4.1: Visual function outcome measure recommendations for patients with rod-cone degeneration based on disease severity.

4.1 Early-Stage Disease: Scotopic Microperimetry

Most visual function tests assess cone function. Scotopic microperimetry using cyan stimuli, discussed in section 3.3, is the only subjective test available to spatially assess rod-driven sensitivity. However, poor reliability performance, indicated by high false-positive responses, is a significant test limitation in many patients with choroideremia who typically have preserved central vision (Taylor *et al.*, 2024). Paper 11 (Adeyoku *et al.*, 2025) validates scotopic microperimetry testing in healthy controls. Further investigation is required in patients with very mild phenotypes, such as female carriers or those with late-onset (adulthood onset) RP, with preserved VA and preserved mesopic microperimetry, to determine whether scotopic microperimetry has the potential to detect very early rod changes.

To improve testing reliability, section 3.3.5 of this critical analysis argues that a threshold learning test (of short-to-moderate length to avoid fatigue reported in Paper 8) is recommended, as well as moderate dark adaptation time, of at least 20 minutes but possibly longer in patients with IRD, due to impaired adaptation. Although further validation is required, a rectilinear test grid is recommended with a uniform spread of

loci to avoid spatially weighted assessment (as discussed in section 3.3.3) and a central locus with several test repetitions for a more robust assessment of the rod-free area (discussed in section 3.3.2.3).

4.2 Early to Late-Stage Disease: Mesopic Microperimetry

This critical analysis highlights mesopic microperimetry as a leading outcome measure in IRDs. Its large dynamic range and relative ease of use are suitable for measuring the central retinal sensitivity function in most patients with early to later stage rod-cone degeneration (Taylor *et al.*, 2023a). Although a standardised protocol has been published, this critical analysis highlights additional recommendations to further improve testing rigour. The radial testing grid should be avoided unless employing a volumetric post-hoc analysis (Taylor *et al.*, 2023a). Although the 10-2 grid works well and typically overlies the region of interest in IRD gene therapy trials, it is limited in very early-stage patients due to the relatively narrow testing radius (~10 degrees). A larger testing grid with greater spacing is not possible with the second-generation MAIA. However, this could be achieved with the newly released MAIA3, which assesses up to a 60-degree field of vision (Ansari *et al.*, 2025). The 10-2 grid is also limited in much later-stage rod-cone degeneration patients, where a smaller, centrally concentrated grid should be considered. This critical analysis argues that a short threshold training test should be completed before each test, irrespective of prior test experience. In a co-authored paper by Josan *et al.* (2023), it was recommended that test results should be considered unreliable if BCEA95 is $> 4 \text{ deg}^2$ and BCEA95 $> 30 \text{ deg}^2$, for patients and controls, respectively. Pointwise and mean sensitivity analyses should be avoided due to high test-retest variability and limitations from averaging effects and floor effects (Taylor *et al.*, 2023a). In agreement with Karuntu *et al.* (2025b), this critical analysis demonstrates that volume sensitivity analyses should be used to measure the overall hill of vision and analyse any sensitivity changes.

4.3 Late-Stage Disease: Visual Acuity

4.3.1 Low Luminance Visual Acuity

Section 3.1.2 highlights that LLVA should be tested using the 2.0 neutral density filter and the ETDRS chart (Wood *et al.*, 2021b). Dark adaptation before testing is not considered necessary (Forte *et al.*, 2025). In patients with rod-cone degeneration, in earlier stages of disease, VA and LLVA decline at a similar rate (Taylor, Josan and MacLaren, 2025). During late-stage disease, when degeneration encroaches on the fovea, LLVA appears to drop suddenly and significantly (it becomes unmeasurable) as microperimetry retinal sensitivity also deteriorates. This suggests LLVA performance is dependent on a larger area of central retinal sensitivity than standard VA (Wood *et al.*, 2021c). Therefore, this critical analysis concludes that LLVA is not beneficial as an outcome measure for any stage of disease. However, it could be a useful screening tool for the presence or absence of detectable central retinal sensitivity measured with microperimetry and the onset of end-stage disease.

LLD is a useful tool to compare VA relative to LLVA, as significantly low LLVA compared to VA indicates impaired central function but preserved foveola function (Wood *et al.*, 2021c). However, as an outcome measure, it is unlikely to be sensitive to measure the therapeutic gains required for regulatory approval and is limited by the combined variability of VA and LLVA measures. LLD may make a useful safety signal measure.

4.3.2 Standard Visual Acuity

Visual acuity is considered the 'gold standard' visual function measure; it is readily available and easy to understand by patients and clinicians. It is a useful test to measure central foveal function, but it is insensitive to eccentric macular and retinal changes when foveal function is preserved, as experienced in patients with rod-cone degeneration. Using alternative VA tests, explored in this critical analysis, did not show any improvement in sensitivity or test-retest variability. Although longitudinal investigations to assess how sensitive alternative acuity tests are to subtle disease progression are still required. VA is recommended to be included as a standard test for all stages of disease, in addition to other tests, as it is a useful safety indicator for

clinicians. However, clinically significant VA changes are likely only possible in late-stage disease. The ETDRS testing procedure provides a highly standardised methodology that works well in clinical trials, but may be limited in patients with very constricted asymmetrical central retinal sensitivity, as shown in Paper 2 (Baffour-Awuah *et al.*, 2024).

4.4 End-Stage Disease

Tests for end-stage disease were beyond the scope of this research. However, the Harmonisation of Outcomes and Vision Endpoints in Vision Restoration Trials (HOVER) consortium has published recommendations for testing patients with very severe visual impairment (Ayton *et al.*, 2020).

4.5 Identifying Disease Stage

There is no formal categorisation classifying disease stage; it is a sliding scale from early to end-stage disease. Retinal imaging characteristics have previously been used to aid classification, such as smooth versus mottled appearance of retinal regions in fundus autofluorescence, marking early and more advanced disease in choroideremia (Stevanovic *et al.*, 2020; Poli *et al.*, 2023). In female *CHM* carriers, disease severity classifications have been based on RPE and retinal imaging appearances – fine, coarse, geographic and male pattern (Gocuk *et al.*, 2024). In RP, phenotype classification has been proposed using a composite scoring system and combining results from VA, Goldman visual fields, and ellipsoid zone imaging (Iftikhar *et al.*, 2019). However, Goldman visual fields are highly variable and not routinely used in clinical trials. As a proposition, the tests reviewed in this critical analysis could be used to determine disease stages in rod-cone specific diseases (Table 4.1).

Table 4.1: Categorising rod-cone degeneration disease severity based on visual function test performance.

Disease Stage	Marker
Early	Detectable scotopic cyan sensitivity and/or preserved mesopic microperimetry, good VA
Moderate	Absent scotopic cyan sensitivity, reduced mesopic microperimetry, good VA
Late	Absent mesopic microperimetry sensitivity or undetectable or significantly reduced LLVA
End	Significantly reduced VA

Visual acuity (VA); Low luminance visual acuity (LLVA)

4.6 Limitations of Critical Analysis

Inherited retinal diseases are rare; therefore, the sample sizes used in the studies included in this critical analysis and informing these recommendations are small compared to studies for acquired conditions such as age-related macular degeneration (Terheyden *et al.*, 2020). For larger sample sizes, costly international studies are required, such as the RUSH2A study (Maguire *et al.*, 2024). Despite this, this work has provided significant contributions to the literature and influenced clinical trial design.

All *RPGR*-associated RP participants reported here were assumed to present with a rod-cone degeneration. However, it is now known that 30% of patients present with a cone-rod presentation, where central vision deteriorates sooner (Cehajic-Kapetanovic *et al.*, 2022). Patients with cone-rod degeneration typically present with more severe vision loss and show a faster rate of disease progression (Josan *et al.*, 2025). However, the overall outcome measures recommendations for this group of patients remain unchanged.

Repeatability assessments reported in both the LLVA and microperimetry publications were all undertaken using data from a single site, Oxford Eye Hospital or the University of Oxford. Ideally, data from different research centres would ensure more

generalisable repeatability assessments. Intrasession and intersession repeatabilities were analysed, but these were not the primary focus of the studies, and so approaches varied based on resources and participant availability between the different publications. The CoRs reported in this critical analysis should only be used as a guide. ideally, clinical trials should report their own test-retest repeatabilities specific to their patient population.

The findings of this critical analysis suggest that outcome measures should be selected according to clinical phenotype and stage of disease. However, regulatory authorities have historically favoured established standardised outcome measures and endpoints, such as BCVA, to facilitate comparison between studies and treatments and ideally also provide evidence of meaningful patient benefit. Consequently, there is a balance between selecting outcome measures to maximise sensitivity and selecting those that maintain consistency for regulatory review and acceptance. Future clinical trial design may approach this challenge, with secondary endpoints potentially providing a mechanism for incorporating disease-specific assessments alongside standardised outcome measures.

Other visual function tests are available but were not considered in this research; they include standard automated perimetry, patient-reported outcome measures, near vision and reading, colour vision and functional vision tests.

4.7 Future Research Directions

4.7.1 Further Vision Test Standardisation and Validation

The need for validation and standardisation of outcome measures persists (Thompson *et al.*, 2025). Table 4.2 summarises the scope for further research highlighted in this critical analysis. There are opportunities to modernise VA assessment, particularly with the move away from fluorescent light bulbs used in ETDRS chart testing; a switch to LED charts is soon required. However, this may be an opportunity to move to computerised VA testing and enable greater potential for alternative VA testing, such

as low contrast testing, without the need for additional equipment. Low contrast acuity may have potential as an earlier measure of retinal change, which was not explored in Paper 7 (Wood *et al.*, 2021a). Further research is needed to validate low contrast VA testing under mesopic conditions, as advocated by Cedrun-Sanchez *et al.* (2025). As well as to establish repeatability and structure-function correlations and associations with microperimetry.

Scotopic microperimetry may be a useful measure in mild IRD or IRD carrier patients, who typically have very well-preserved central vision. The effect of pupil dilation on scotopic microperimetry testing across all ages has not been explored, but due to the Maxwellian view design, it is unlikely to have a significant impact on sensitivity. The impact of tropicamide on accommodation is another factor that has yet to be explored. However, due to the in-built focusing abilities of the S-MAIA, this is unlikely to have a significant effect. There is considerable scope to improve inbuilt test reliability indices and to use these to develop a greater understanding of the components of a reliable test; these recommendations apply to mesopic as well as scotopic microperimetry. In addition, the impact of age and cataracts on scotopic testing sensitivities is unknown but is likely to affect scotopic cyan sensitivity more than red stimuli, as also seen in full-field stimulus testing (Karuntu *et al.*, 2025c).

The possible impact of fatigue effects, highlighted in Paper 8 (Taylor *et al.*, 2023a), demonstrates a need to consider the order of testing during clinical trials to minimise fatigue effects. Further research is needed to explore the optimum order of testing and whether the primary endpoint and study eye should be tested first or early in the testing procedures.

Functional vision tests that more directly reflect everyday visual tasks, and patient-reported outcome measures have not been explored here (Table 4.2). The FDA and EMA have indicated a preference for functional tests as they should more closely demonstrate a direct patient benefit. Further investigation is needed to evaluate, standardise and validate different functional vision tests. In addition to exploring

associations between functional vision tests and patient-reported outcome measures. These analyses could help identify the level of change required in clinical tests to reflect meaningful improvements in patients' everyday functioning.

Longitudinal studies assessing visual function test capabilities to detect change over time and rates of natural disease progression are required for all visual function tests. This would, in turn, support evidence-based endpoint selection (see section 4.7.2).

Table 4.2: Summarises the visual test development and validation progress for each of the vision tests for use in rod-cone degeneration, discussed in this work. Green indicates substantial validation, yellow indicates some work, but further development and validation are needed. Red indicates an absence of research.

Visual Function Test	Test Standardisation	Statistical Significance	Clinical Significance	Functional Significance
Standard VA	Green	Green	Green	Red
LLVA	Green	Green	Green	Red
Low Contrast VA	Green	Red	Red	Red
MAC VA	Yellow	Green	Green	Red
Mesopic Microperimetry	Green	Green	Green	Red
Scotopic Microperimetry	Yellow	Yellow	Yellow	Red

The COMET (Core Outcome Measures in Effectiveness Trials) initiative aims to standardise outcome measures into ‘core outcome sets’ which represent the minimum that should be measured and reported in all clinical trials for a specific condition, as well as clinical care and other research (Kirkham *et al.*, 2016; Kirkham *et al.*, 2017). This makes it easier to compare studies. To the best of my knowledge, there is no core outcome set for rod-cone degeneration or IRD.

4.7.2 Endpoint Selection & Analysis

Once suitable outcome measures are defined, clinical trial endpoints must be decided. An endpoint is a measurable and relevant outcome used to determine whether the intervention is successful. Endpoints should be selected using evidence-based knowledge of clinically significant changes (and ideally, functionally significant changes) (Thompson *et al.*, 2025) and in line with the significant terminology proposed

in Table 2.2. In patients at different disease stages, a single criterion may be inappropriate. Instead, individual or cohort measures of clinical significance may aid the detection of meaningful treatment effects. Csaky, Richman and Ferris (2008) suggested that larger changes should be considered for later-stage patients and smaller changes in earlier-stage patients with more preserved visual function, who are likely to experience a ceiling effect in potential gain. Several recent IRD clinical trials have failed to meet their predefined endpoint, and it has been suggested that this is due to the endpoint goals being too high and unable to capture slowing vision loss and improved visual function stability (Thompson *et al.*, 2025).

At an endpoint consortium in 2017, the FDA suggested that at least a 15 ETDRS letter change (equivalent to doubling or halving of the visual angle and double reported repeated test-retest repeatability) per responder is required to be clinically significant, and outweigh the risks of treatment, to be worthy of regulatory approval. An EMA representative said in some circumstances, a 10-letter responder change was sufficient (Csaky *et al.*, 2017). However, it is unclear what evidence this is based on. Indeed, there is little scope for early to moderate IRD patients who typically have near-normal visual acuity to increase by 15 letters.

Outcome measure and endpoint selection should be aligned with the therapeutic objective, whether that is demonstrating functional gain, preserving existing function or slowing the rate of deterioration. Historically, ophthalmic clinical trials have often focused on demonstrating visual function gain, such as a 15-letter improvement in standard VA (Csaky *et al.*, 2017). However, for progressive IRD, a more realistic therapeutic objective may be the preservation of visual function. In this context, early intervention may maximise the amount of retinal tissue available for preservation, although visual function tests may be more susceptible to ceiling effects, limiting the ability to detect measurable gains. Conversely, later intervention may permit larger observable improvements but is accompanied by greater floor effects, poorer reliability and limited repairable retinal function.

Furthermore, debate persists regarding the exact method of endpoint analyses (specifically, VA but also microperimetry sensitivities) and whether between-group mean differences are used as opposed to dichotomising continuous variables by reporting the number of individual responders meeting a required change. Beck *et al.* (2007) recommended reporting comparisons of the overall change as the primary outcome and categorising binary outcomes as the secondary outcome to avoid loss of data and statistical power. Either way, functionally significant changes for many visual function outcome measures remain to be determined.

4.8 Conclusion

This critical analysis synthesised a series of publications evaluating visual function tests to inform recommendations for outcome measures in rod–cone degeneration clinical trials. The publications were split into three research themes, as outlined in Chapter 1 (Section 1.7) and included the evaluation of different VA assessments, mesopic microperimetry and scotopic microperimetry. The first aim, to investigate alternative VA testing approaches as potential outcome measures, was achieved by evaluating standard VA, low-contrast VA, LLVA, and MAC VA. Although initial findings appeared favourable to LLVA, subsequent work demonstrated that alternative VA tests showed no significant advantage over standard VA in patients with preserved central vision. The second aim, to evaluate mesopic microperimetry sensitivity indices, was achieved by comparing pointwise, mean, and volumetric sensitivity measures, supporting mesopic microperimetry as a promising outcome measure. The third aim, to explore the feasibility of scotopic microperimetry, highlighted substantial floor effects, poor reliability and challenges relating to photoreceptor specificity, limiting its suitability as an outcome measure in established rod-cone disease. Collectively, these investigations have enabled the development of the outcome measure recommendations presented in this chapter and summarised in Table 4.3.

Table 4.3 Summarises the conclusions of this critical analysis and informs future rod–cone degeneration clinical trial design

Category	Clinical Trial Recommendations and Final Conclusions
Outcome Measure Selection	Outcome measure selection should be based on disease stage, phenotype and therapeutic objective.
VA Testing	Standard VA is a key measure of central cone function but is of limited value in detecting significant visual function changes beyond this unless in very late rod-cone disease stages. Alternative acuity tests did not demonstrate additional advantages on outcome measures – further research investigating low contrast acuity tests is required. LLVA using the 2.0 neutral density filter may be useful as a screening tool for the presence of central retinal sensitivity, and thus clinical trial eligibility, but further research to quantify the exact extent of central function being evaluated through this test is required. Small changes in testing conditions, such as background luminance and dark adaptation time, did not appear to have a clinically significant impact on LLVA results.
Mesopic Microperimetry	Is emerging as a useful functional outcome measure for a broad range of rod-cone degeneration, particularly using the 10-2 rectilinear grid. A threshold learning test before testing, as well as careful consideration of sensitivity indices, are recommended.
Scotopic Microperimetry	Is limited in established rod-cone degeneration due to significant floor effects, patient difficulty in performing the tests indicated by poor reliability and sensitivity variability, and cyan versus red functional ambiguity. Further investigation is required to explore utility in very early stages of disease in those with well-preserved central function.

Overall Clinical Meaningfulness	Consider functional relevance to patients alongside statistical significance and repeatability-based clinical significance.
Disease Stage	In the absence of formal disease classifications, the tests explored in this critical analysis could be used to define and determine rod-cone disease stage.

When selecting outcome measures and endpoints for clinical trials, this work suggests distinguishing between detecting statistically significant change and true repeatability-based clinical change. In addition, to demonstrating functionally meaningful benefit. The ideal outcome measure should be capable of all of this to enable a case to demonstrate therapeutic benefit that outweighs treatment risk. Ultimately, to satisfy regulatory authority (FDA and EMA) approval requirements.

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Appendix I: Co-Author Declaration for Published Work

Paper 1

Protocol paper 1

Publication Title: A cross-sectional study to assess the clinical utility of modern visual function assessments in patients with inherited retinal disease

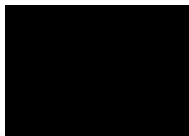
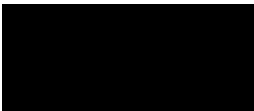
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We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, design and drafting of the final manuscript, and we agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Amandeep S Josan	Manuscript review and feedback		03/09/2025
Irene Stratton	Statistical input into study design		
Jasleen K Jolly	Qualitative input into study design and manuscript review		
Robert E MacLaren	Senior oversight and support		11/9/25

Paper 2

Original Research Paper 1

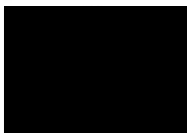
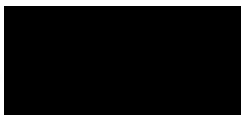
Publication Title: Investigating the impact of asymmetric macular sensitivity on visual acuity chart reading in choroideremia

Authors: Baffour-Awuah, K. A., Taylor, L. J., Josan, A. S., Jolly, J. K., & MacLaren, R. E.

Journal: Ophthalmic & physiological optics. 2024

Freely available at: <https://onlinelibrary.wiley.com/doi/10.1111/opo.13356>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the joint primary researcher responsible for study conception, some data collection, supporting analyses, interpretation, and drafting of the final manuscript. I agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Kwame Baffour-Awuah	Data analyses, manuscript drafting		
Amandeep S Josan	Statistical analyses support and review of the manuscript		03/09/2025
Jasleen K Jolly	Data collection and manuscript review		
Robert E MacLaren	Senior oversight and support		11/9/25

Paper 3

Literature Review 1

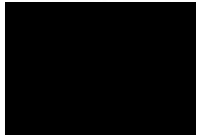
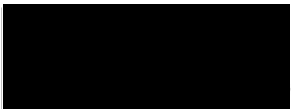
Publication Title: Low luminance visual acuity as a clinical measure and clinical trial outcome measure: a scoping review.

Authors: Wood, L. J., Jolly, J. K., Buckley, T. M., Josan, A. S. and MacLaren, R. E.

Journal: Ophthalmic and Physiological Optics, 2021

Available at: <https://onlinelibrary.wiley.com/doi/10.1111/opo.12775>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, literature searching and synthesis, critical analysis and manuscript drafting, and we agree that this work forms part of Laura's PhD submission.

Co-Author Name Contribution		Signature	Date
Jasleen K Jolly	Manuscript review and feedback		
Thomas M Buckley	Manuscript review and feedback		18/10/25
Amandeep S Josan	Manuscript review and feedback		03/09/2025
Robert E MacLaren	Senior oversight and support		1/9/25

Paper 4

Original Research Paper 2

Publication Title: Low luminance visual acuity and low luminance deficit: optimising measurement and analysis.

Authors: Forte, G., Ahmed, R., MacLaren, R.E., Jolly, J.K., * Taylor, L.J.*

*Joint senior author

Journal: Clinical & Experimental Optometry. 2025

Freely available at: <https://pubmed.ncbi.nlm.nih.gov/40010402/>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the co-senior researcher in this collaborative project. Laura was responsible for study conception, undertaking and supporting data collection, analyses and interpretation and supporting the drafting of the final manuscript, and we agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Giovanni Forte	Data analyses, manuscript drafting		
Rafee Ahmed	Data collection		
Robert E MacLaren	Manuscript review		1/9/25
Jasleen Jolly	Data collection, senior oversight and support		

Paper 5

Original Research Paper 3

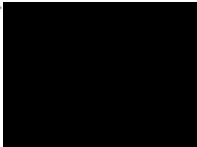
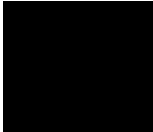
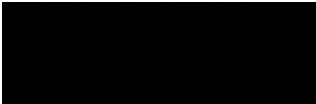
Publication Title: Low Luminance Visual Acuity and Low Luminance Deficit in Choroideremia and RPGR-Associated Retinitis Pigmentosa.

Authors: Wood, L. J., Jolly, J. K., Josan, A. S., Buckley, T. M. W.
and MacLaren, R. E.

Journal: Translational vision science & technology. 2021

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2772299>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, data collection, analyses, interpretation and drafting of the final manuscript, and we agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Jasleen K Jolly	Data collection, manuscript review and feedback		
Amandeep S Josan	Manuscript review and feedback		03/09/2025
Thomas M Buckley	Manuscript review and feedback		13/10/25
Robert E MacLaren	Senior oversight and support		11/9/25

Paper 6

Original Research Paper 4

Publication Title: Exploring standard and low luminance visual acuity and the Moorfields Acuity Chart as outcome measures in inherited retinal disease

Authors: Taylor, L.J., Josan, A.S and MacLaren R.E.

Journal: Ophthalmic & Physiological Optics (In Review). 2024

Freely available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12153033/>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, data collection, analyses, interpretation and drafting of the final manuscript, and we agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Amandeep S Josan	Manuscript review, statistical support and feedback		03/09/2025
Robert E MacLaren	Senior oversight and support		1/9/25

Paper 7

Original Research Paper 5

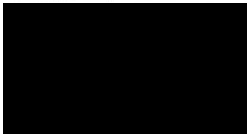
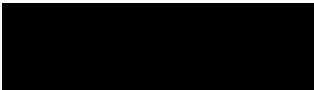
Publication Title: Low-contrast visual acuity versus low-luminance visual acuity in choroideremia.

Authors: Wood, L. J., Jolly, J. K., Andrews, C. D., Wilson, I. R., Hickey, D., Cehajic-Kapetanovic, J. and Maclaren, R. E.

Journal: Clinical & Experimental Optometry, 2021

Freely Available at: <https://onlinelibrary.wiley.com/doi/10.1111/cxo.13087>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, data analyses and drafting the final manuscript. We agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Jasleen K Jolly	Data collection, Manuscript review.		
Colm Andrews	Statistical Analysis Support		12/9/25
Iain Wilson	Statistical Analysis Support		
Doron Hickey	Data collection		
Jasmina Cehajic-Kapetanovic	Senior oversight and support		15/9/25
Robert E MacLaren	Senior oversight and support		1/9/25

Paper 8

Original Research Paper 6

Publication Title: Microperimetry as an Outcome Measure in RPGR-associated Retinitis Pigmentosa Clinical Trials

Authors: Taylor, L. J., Josan, A. S., Jolly, J. K., and MacLaren, R. E.

Journal: Translational Vision Science & Technology. 2024

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2785690>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, some data collection, analyses and interpretation and drafting of the final manuscript. We agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Amandeep S Josan	Statistical analyses support and review of the manuscript		03/09/2025
Jasleen Jolly	Some data collection and manuscript review		
Robert E MacLaren	Senior oversight and support		1/9/25

Paper 9

Literature Review 2

Publication Title: Scotopic microperimetry: evolution, applications, and future directions

Authors: Taylor, L. J., Josan, A. S., Pfau, M., Simunovic, M. P., and Jolly, J. K.

Journal: Clinical & Experimental Optometry. 2022 *invited review

Freely available at:

<https://onlinelibrary.wiley.com/doi/10.1080/08164622.2021.2023477>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for literature searching and synthesis of the NIDEK microperimetry section, critical analyses, synthesis of additional review sections and final manuscript drafting. We agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Amandeep S Josan	Colour perimetry literature review, manuscript review and feedback		03/09/2025
Matthew P Simunovic	Manuscript review and feedback		
Maximillian Pfau	Manuscript review and feedback		
Jasleen K Jolly	MAIA literature review, senior oversight and support		

Paper 10

Original Research Paper 7

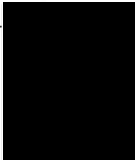
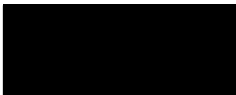
Publication Title: Exploring scotopic microperimetry as an outcome measure in choroideremia

Authors: Taylor, L.J., Josan, A.S., Adeyojo, D., Jolly, J.K. and MacLaren R.E

Journal: Translational Vision Science & Technology. 2024

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2800850>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the primary researcher responsible for study conception, data collection, analyses and interpretation and drafting of the final manuscript. We agree that this work forms part of Laura’s PhD submission.

Co-Author Name Contribution		Signature	Date
Amandeep S Josan	Statistical analyses support and review of the manuscript		03/09/2025
Daniel Adeyojo	Data collection		
Jasleen Jolly	Manuscript review		
Robert E MacLaren	Senior oversight and support		1/9/25

Paper 11

Original Research Paper 8

Publication Title: Scotopic Microperimetry: An Analysis of Healthy Controls.

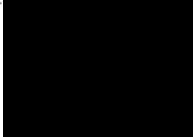
Authors: Adeyoju, D.A.O.,# Josan, A.S.,# Taylor, L.J.* and MacLaren, R.E*

#joint first author, *joint senior author

Journal: Translational Vision Science & Technology (In Review)

Freely available at: <https://tvst.arvojournals.org/article.aspx?articleid=2803104>

We, the undersigned co-authors, confirm that we contributed to this publication and acknowledge that **Laura Taylor** was the joint senior researcher responsible for study conception, supporting data collection, analyses and interpretation and drafting of the final manuscript. We agree that this work forms part of Laura's PhD submission.

Co-Author Name	Contribution	Signature	Date
Daniel AO Adeyoju	Data collection and manuscript drafting		
Amandeep S Josan	Statistical analyses support and manuscript drafting		03/09/2025
Robert E MacLaren	Senior oversight and support		09/25

Appendix II: Link to preprint manuscripts

Access to the preprint manuscripts included in this critical analysis via a private Zenodo link:

https://zenodo.org/records/18199887?token=eyJhbGciOiJIUzUxMiJ9.eyJpZCI6IjNkMWQ3MDdkLTIIYTktNDQ4Zi05OTFhLWQ5ZDFiNTY5ZmZjOSIsImRhdGEiOnt9LCJyYW5kb20iOiIzNTkzYWYxZjkyYmFIMDBiNGMzN2E2MTdkMDZhZTQ2ZSJ9.ySImAFTB2H-6dmZxrFibCUNbggyTieSfxj-UX_gDcvqiE-XRilt4TUty_xJ3XohWChPxVeLmXR2xTONWctGPGw