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Assessing structure - Function relationships in non-neovascular age-related macular degeneration

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ABSTRACT

Age-related macular degeneration (AMD), a neurodegenerative disease, is the leading cause of visual impairment in industrialized countries. Challenges in defining structural/functional relationships at various stages of disease especially with non-neovascular AMD, have slowed therapeutic development. Development of such sensitive and specific markers associated with AMD progression could provide the basis necessary for future regulatory outcome variables that will be useful in assessing new, innovative AMD therapies. Advanced imaging technologies such as high-resolution optical coherence tomography, fundus autofluorescence and near infrared imaging; and functional tests including rod-mediated dark adaptation, microperimetry, fluorescence lifetime imaging ophthalmoscopy and others will be important in the evaluation of these structure/function correlations. Development of more advanced methods to study structure such as high-resolution OCT and *en face* OCT offer further opportunities to better correlate structure and function in clinical trials, and to better define useful biomarkers of visual outcome endpoints. Dark adaptation, although correlated with AMD stage, is difficult to incorporate as endpoint in clinical trials because dark adaptation changes slowly and the technique is time consuming. Microperimetry has become a useful outcome variable in many clinical trials and new methodology may improve its utility in structure-function correlation. These and other newer techniques will require further prospective studies to determine their clinical utility in early AMD detection, prediction of disease progression from intermediate to late stages, and the ability to monitor the advancement of non-neovascular AMD.

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1. Introduction

Age-related macular degeneration (AMD), a neurodegenerative disease of the retina, ranks first in industrialized countries and third worldwide as a major cause of visual impairment (Wong et al., 2014) Although there are FDA approved therapies for both late forms of AMD, neovascular AMD (Rosenfeld et al., 2006) and geographic atrophy (GA) (Heier et al., 2023; Khanani et al. 2023) and oral nutritional supplements ((2013)[Age-Related Eye Disease Study 2]) for intermediate AMD (defined predominantly by the presence of bilateral large drusen), efforts to develop therapeutic strategies for the various stages of AMD are limited by the lack of valid outcome measurements and functional correlates to structural biomarkers associated with changes in disease and visual function. A group of researchers with the Ryan Initiative for Macular Research (RIMR) Group explored this challenge of defining structural/functional outcome variables and correlations that may provide sensitive measures of disease progression at the various severity levels of AMD with a focus on non-neovascular AMD. Developing such sensitive and specific measures associated with AMD progression could provide the basis necessary for future regulatory outcome variables that will be useful in assessing new, innovative AMD therapies and align with the group's mission to refine the current AMD classification in a more granular manner.

The advanced imaging technologies and techniques discussed by the group included high-resolution optical coherence tomography (OCT) for detailed identification and analyses of retinal layers: outer nuclear layer (ONL) thickness, interdigitation zone (IZ) thickness, and ellipsoid zone (EZ) dimensions, as well as *en face* OCT and OCT-angiography (OCT-A) metrics. These measures would add to other clinically pertinent structural features that include drusen size and volume, intraretinal hyperreflective foci [HRF](hyperpigmentation), reticular pseudodrusen (RPD) [also known as subretinal drusenoid deposits (SDD)], choroidal hypertransmission defects, measurements of choriocapillaris flow deficits and others. Newer technologies for detection of early disease include fluorescence lifetime imaging ophthalmoscopy (FLIO) that could potentially provide insight on metabolic changes occurring in the retina/retinal pigment epithelium (RPE) complex and may provide further insight beyond the current modalities of color fundus photographs (CFP), fluorescein angiography (FA), fundus autofluorescence (FAF) imaging, optoretinography (ORG), and OCT-A.

Functional tests assessed include best-corrected visual acuity (BCVA), rod-mediated dark adaptation (RMDA), mesopic and scotopic microperimetry, contrast sensitivity, and low luminance visual acuity. An important future step will be the Integration of these diagnostic tools into a more granular staging system that rigorously captures the progression and variability of AMD. However, despite significant advancements, the validation of these biomarkers, along with the functional testing necessary for demonstrating the correlation with outcomes and their predictive value and sensitivity in clinical trials, remains incomplete. This RIMR group explored methods to enhance the precision of disease monitoring to allow more efficient assessment of new treatment strategies and to ease the path to regulatory approval.

2. Structural changes from intermediate age-related macular degeneration to geographic atrophy

***Natural Course of Lesions Prior to GA*:** Before addressing the correlation of structure with function, the group considered what is known about the structural changes that occur as eyes progress from intermediate AMD to GA. Historically, using color fundus photographs, the Age-Related Eye Disease Study (AREDS) investigators conducted a retrospective evaluation of the retinal 'precursors' that were present prior to the development of GA (Klein et al., 2008). In two AREDS clinical sites, eyes without GA at study baseline that developed GA at or after the 4-year visit were selected for evaluation. Fundus photographs from previous visits were evaluated to identify lesions that preexisted in the sites of GA development. Drusen were found in 100 % of eyes at the site of later GA, drusen ≥125 μm in diameter in 96 % of eyes, confluent drusen in 94 %, hyperpigmentation in 96 %, drusen ≥250 μm in diameter in 83 %, hypopigmentation in 82 % and refractile deposits in 23 %. Time from lesion appearance to onset of GA varied by lesion type, ranging from 5.9 years for confluent drusen to 2.5 years for hypopigmentation or refractive deposits. The typical sequence of disease progression was first, appearance of large drusen followed by hyperpigmentation associated with regression of drusen, then hypopigmentation and finally, GA, with or without the appearance of refractive deposits. GA was preceded by regression of drusen in 75 %. Lesions that preceded regression of drusen included pigment epithelial detachments, vitelliform deposits, vitelliform lesions, and RPD. This pathway to GA was confirmed retrospectively using OCT findings from a single center (Spaide, 2024).

3. Imaging of retinal structure

3.1. High resolution optical coherence tomography (OCT)

High-resolution optical coherence tomography (OCT) achieves axial resolution to 2 μm (Hartl et al., 2001), while maintaining transverse resolution at ~14 μm. This level of resolution is possible because lateral and axial (depth) optical resolutions are independent. While lateral resolution depends on numerous factors that can lead to enlargement of the effective retinal spot size, axial resolution depends largely on the center wavelength and spectral bandwidth of the light source (Izatt and Choma, 2008) Thus, high resolution OCT has been made possible by improving imaging resolution through light source selection and appropriate adaptations (Fujimoto et al., 1998) As demonstrated in Fig. 1, integration of three superluminescent diodes into a single fiber, yielding a spectral bandwidth of 137 nm (full width at half maximum), results in higher resolution images, using a new device not yet cleared for medical use (Spaide et al, 2021).

3.2. Why high resolution matters

In their research on AMD specimens, Shirley and John Sarks described two types of deposits: basal laminar deposits (BLamD) and basal linear deposits (BLinD) (Sarks et al., 2007) BLamD, located between the retinal pigment epithelium (RPE) basement membrane and plasma membrane, consist of basement membrane proteins and long-spacing collagen. In contrast, BLinD are situated between the RPE basement membrane and the inner collagenous layer of Bruch's membrane.

The Sarks identified two distinct pathways for AMD progression associated with these deposits. One pathway involves excess production and decreased clearing of membranous debris, which increases the risk of macular neovascularization (MNV). The other pathway results in formation of drusen and pigmentary changes, which are associated with a higher risk of geographic atrophy (GA) (Sarks et al., 1994).

To differentiate these two risk groups higher resolution OCT is necessary. In normal subjects, BLamD thickness is approximately 0.3 μm, but increases significantly in AMD: 5.5 μm in early-intermediate stages, 4.1 μm in GA, and 5.3 μm in cases of MNV. Current commercial SD-OCT systems, with resolution of 5–7 μm, cannot detect early BLamD (Chen et al., 2023), but Hi-Res OCT can capture and measure these changes, which may be critical for accurate staging of AMD risk groups. This technology could also significantly improve our ability to monitor disease progression and adapt treatment strategies effectively.

3.3. En face OCT

En face OCT represents a significant advancement in retinal imaging. If a dense volume dataset is available for en face reconstruction from the cross-sectional OCT B-scans, then en face OCT can delineate patterns of

abnormal hyper- and hyporeflectivity in a given segmentation plane.

The Classification of Atrophy Meetings (CAM) group developed a more granular OCT classification of atrophy that provides the opportunity to capture earlier stages of atrophic AMD, increasing the number of potential biomarker predictors of vision loss and atrophy. They defined two important OCT correlates of GA, i.e. incomplete RPE and outer retinal atrophy (iRORA) and complete RPE and outer retinal atrophy, cRORA. iRORA is associated with <250 μm choroidal hypertransmission, and cRORA is defined as >250 μm of choroidal hypertransmission. Importantly, choroidal hypertransmission is associated with overlying photoreceptor loss (Cheng et al., 2024) (see Fig. 2).

The quantitative accuracy of cRORA measurement can be enhanced using en face OCT to more precisely capture the lateral dimensions of hypertransmission defects (HTD), and importantly, the progression of HTD may represent a more reliable method to track the progression of atrophy from early AMD to late-stage GA (Fig. 3). En face OCT can also be used to better define novel phenotypes of AMD that can be more precisely studied for genetic risk, anatomic pathologies and visual function outcome, such as the tri-zonal distribution of lesions seen in Fig. 3 (Csaky et al., 2017).

3.4. Fundus autofluorescence imaging (FAF)

Fundus autofluorescence (FAF) imaging using blue light autofluorescence has emerged as the preferred modality for imaging GA and is approved by the FDA as a validated imaging approach for assessing progression of GA (Csaky et al., 2017). FAF provides high contrast grey scale images where atrophy presents as dark (hypo-autofluorescent) lesions with distinct boundaries (Holz et al., 2017). These images allow for a more reproducible assessment of GA area and growth than the traditional assessment using color fundus photography resulting in a better outcome variable (end-point) than color fundus photographs for clinical trials (Holz et al., 2017). A major limitation of FAF is the inability to determine if lesions of geographic atrophy has involved the foveal center.

3.5. Near InfraRed (NIR)

Near infrared imaging (NIR) is an important technology that complements FAF. NIR scanning laser ophthalmoscopy (SLO) uses wavelengths of light at the higher end of the visible spectrum (750–840 nm), which results in minimal patient discomfort and shorter imaging time. At these wavelengths there is less absorption or interference by the lens, other media opacities and macular pigment. Thus, compared with FAF imaging, NIR images allow discernment of foveal involvement by atrophic lesions (Lindner et al., 2015). Another important clinical advantage of NIR imaging is its utility in detecting reticular pseudodrusen with high sensitivity (Buitendijk et al., 2016; Wu et al., 2016). Because NIR reflectance imaging was originally designed to serve as a guidance modality for OCT, it is available on most OCT/SLO devices. NIR images complement FAF imaging and improve the outcome assessment in studies evaluating GA (Abdelfattah et al., 2020)

4. Tests to measure functional and structural biomarkers and their changes to enhance classification of AMD across disease stages

Table 1 provides a list of various tests used to capture functional and structural biomarkers and changes that may be important for detecting each stage of AMD severity. These tests have been used to better characterize the heterogeneity of AMD and to monitor its progression. The most useful of these tests are discussed below.

4.1. Dark adaptation

Dark adaptation (DA) is important in assessing functional changes in rods for both early and intermediate stages of AMD (Wu et al., 2016; Owsley et al., 2001; Flamendorf et al., 2015; Guymer et.al., 2021; Wolf-Schnurrbusch et al., 2011). Rod function is reduced before other visual functions are impaired and before symptoms become noticeable. Owsley et al. demonstrated that rod-mediated dark adaptation (RMDA) is the first functional biomarker associated with incident early AMD (Owsley et al., 2016)

RMDA measured with rod-intercept time (RIT) is a predictive biomarker for early AMD (Owsley et al., 2016). A longitudinal study at the National Eye Institute showed that prolongation of the RIT correlated with increasing severity of AMD. Eyes with RPD demonstrated particularly high rates of RIT prolongation (Figs. 4 and 5). (Chen et al., 2019) This longitudinal study showed that a decline in dark adaptation (DA) function also correlated with patient-reported functional deficits. (Yazdanie et al., 2017). RIT prolongation, as a measure of worsening DA, may be a useful functional outcome measure in AMD clinical studies. However, because DA changes progress slowly over time, and because the testing is time-intensive, practical considerations limit its utility in randomized clinical trials.

Optimization of Test Target Location for RMDA. Owsley et al. emphasized the importance of test target location in RMDA testing efficiency (Owsley et al., 2023). Along with Flynn et al. (Flynn et al., 2018), they demonstrated that RMDA is slower nearer to the fovea (5° vs. 12°) where AMD-related changes such as drusen and RPD/SDDs are

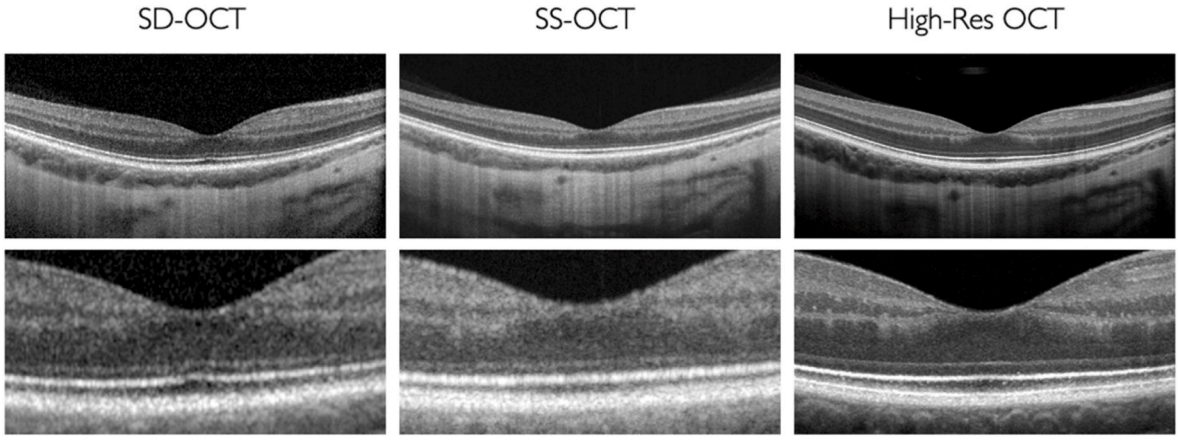


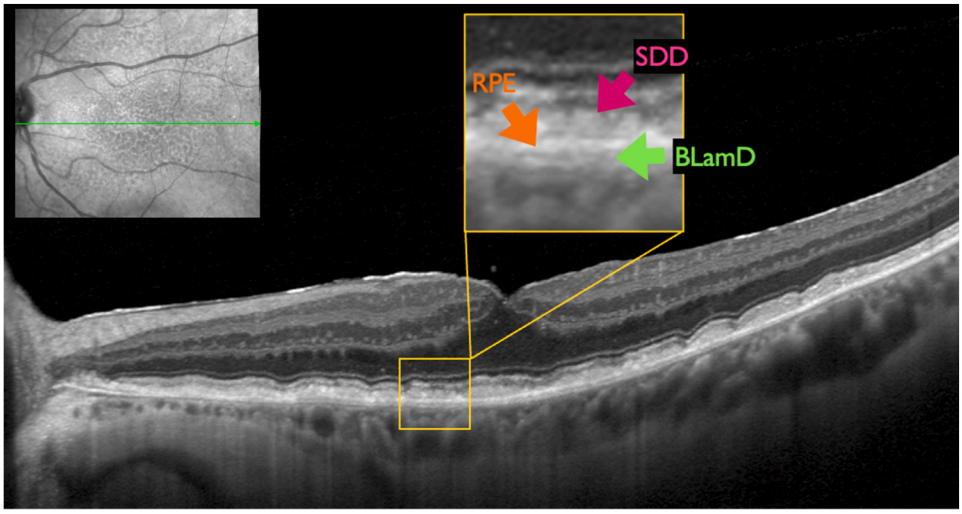
Fig. 1. Comparative imaging of the same subject (normal retina) using Spectral Domain OCT (SD-OCT), Swept Source OCT (SS-OCT), and High-Resolution OCT. SD-OCT (left) provides 6.9 μm axial and 14 μm lateral resolution. SS-OCT (middle) provides 6.3 μm axial and 20 μm lateral resolution, and High-Res OCT (right), 3 μm axial and 14 μm lateral resolution. (Images courtesy of Dr. Giovanni Staurenghi.)

prevalent. Strategic placement of test targets may enhance the sensitivity of RMDA assessments, potentially allowing for more accurate staging and monitoring of AMD progression (Figs. 6 and 7).

Rod-Mediated Dark Adaptation and Structural Biomarkers in 3D OCT Imaging: A recent study from the baseline data of the ALSTAR2 study of early and intermediate AMD evaluated the relationship between RMDA and the status of the outer retinal bands on OCT (Heier et al., 2023). This analysis showed a significant correlation between OCT features and RMDA outcomes, specifically that variations in dark adaptation times are linked to the structural integrity of the retina. Longer RIT was associated with less preserved EZ across the Early Treatment Diabetic Retinopathy Study (ETDRS) grid with the exception of the central subfield Interestingly, no correlation between EZ area and RIT was found in the central subfield. However, in the inner and outer rings of the ETDRS grid, slower RMDA was correlated with less preserved EZ area, but only in eyes with iAMD. These findings suggest that RMDA could be particularly useful in identifying and monitoring structural damage in the eyes with iAMD before RIT can be used as a validated biomarker for progression of AMD (Fasih-Ahmad et al., 2024).

4.2. Microperimetry

Microperimetry is a psychophysical test that marries localization of retinal images with visual function. In combination with eye-tracking, it can create a map of retinal sensitivity that is more accurately correlated with the retinal location/changes as well as allowing for testing smaller area of the retina than traditional perimetry. This method identifies regions of partial or complete loss of sensitivity (scotomas) and links them to underlying retinal pathologies including loss of photoreceptors, RPE and/or choriocapillaris (Yang and Dunbar, 2021;Wu et al., 2024; Messenio et al., 2022; Sato et al., 2015). Wu et al. showed that microperimetry might be more effective than LLVA or BCVA in detecting retinal function deficits in AMD, particularly in early stages of disease (Wu et al., 2021). In AMD, recognizable patterns of functional loss can be associated with specific structural changes. The technique allows precise assessment of visual function in regions affected by drusen, focal hyperpigmentation, SDD, borders of GA, central areas of GA, and after macular neovascularization treatment, offering insights into disease progression and response to treatment. Montesano et al. demonstrated the utility of microperimetry in elucidating structure-function relationships relevant to AMD. Mesopic microperimetry findings correlated



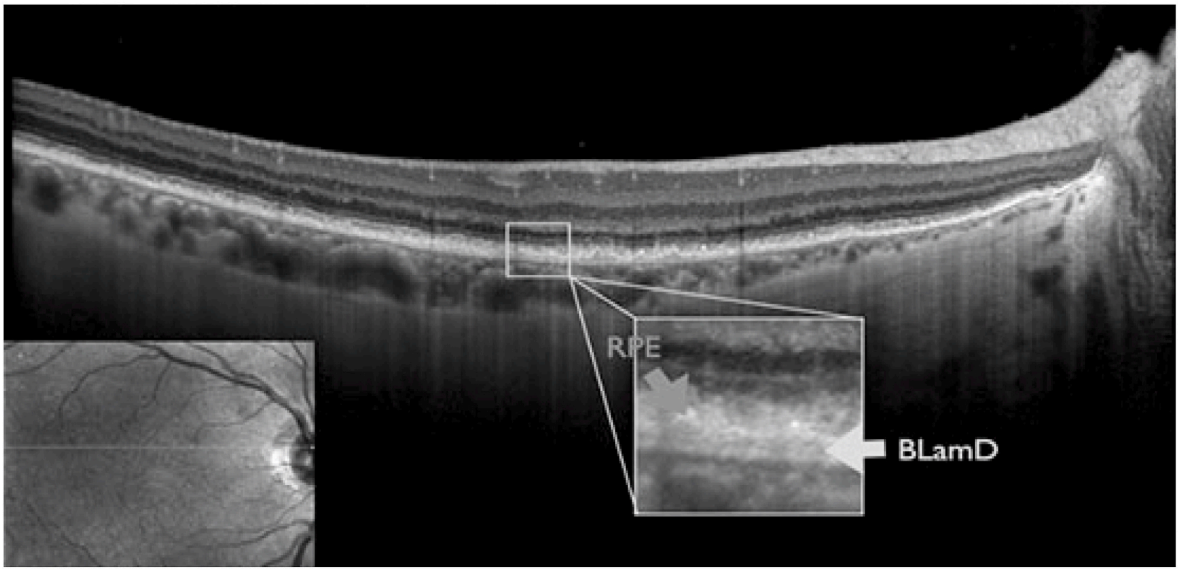


Fig. 2. a and b. High-Resolution OCT facilitates precise detection and measurement of retinal pathology.
RPE = retinal pigment epithelium, SDD = subretinal drusenoid deposits BLamD = basal laminar deposits (Courtesy of Dr. Giovanni Staurenghi.).

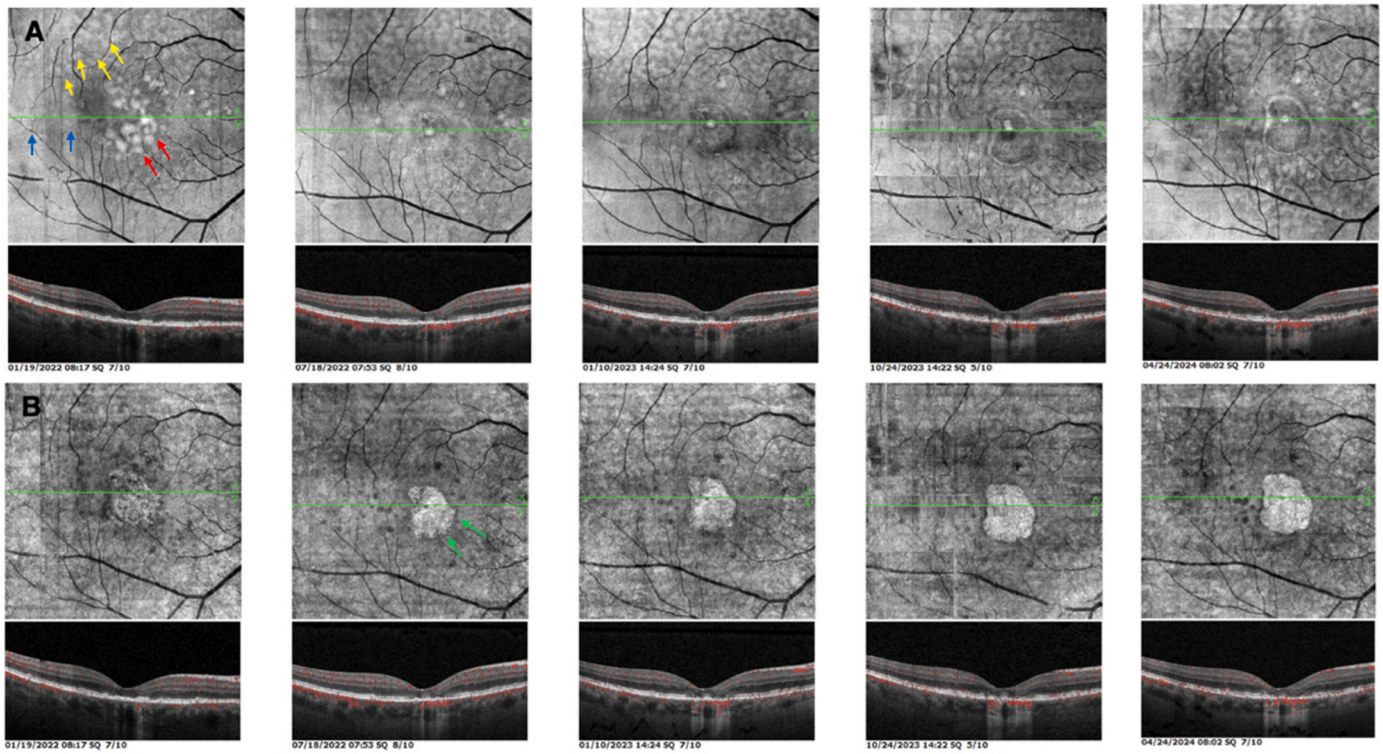


Fig. 3. Sequential tracked en face OCT scans (Row A) with corresponding OCT B scans (Row B) with dates noted, illustrating the progression of drusen, subretinal drusenoid deposits (SDD) and atrophy in the setting of age-related macular degeneration (AMD). A. Sequential tracked en face OCT scans segmented at the level of the outer retina illustrate the progression of **soft (large) drusen (red arrows)** to complete RPE and outer retina atrophy (cRORA) over 23 months. Note that drusen predominate in the central fovea, while **dot SDD (blue arrows)** and **ribbon SDD (yellow arrows)** are distributed in parafoveal and perifoveal areas and beyond, respectively, consistent with a trizonal disease pattern. A progressive increase in the number of dot and ribbon SDD, especially in the nasal and inferonasal macula, is observed over time. **B.** Sequential tracked en face OCT B-scans segmented at the level of the choriocapillaris demonstrate the progressive increase in size of the fovea-involving hypertransmission defect **(hyperTD), i.e. cRORA (green arrows)**, over 23 months. (Courtesy of Dr. David Sarraf). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

better than scotopic testing with macular drusen. (Montesano et al., 2020). Midena et al. also reported that microperimetry sensitivity over large drusen was decreased and demonstrated decreased sensitivity in areas of pigment abnormalities (Midena and Pilotto, 2017).

The primary technical advantage of microperimetry is the ability to correlate structure and function through eye tracking and fundus registration, allowing point-specific visual sensitivity measurements to be mapped to precise retinal locations. Some of the disadvantages include the considerable time required for testing, the need for sustained patient attention and fixation, and the necessity of trained technicians. (Csaky et al., 2019) (Wong et al., 2017).

Correlating microperimetry results to visual function in AMD patients is challenging for several reasons. Retinal sensitivity varies across macular regions, and not all areas contribute equally to everyday tasks, making generalization difficult (Wong et al., 2017; Sampson et al., 2021). There is often a discrepancy between objective microperimetry measures and patients' subjective visual experiences, as subtle changes in retinal sensitivity may not translate into noticeable functional impairments. (Forshaw et al., 2021).

Microperimetry has been commonly utilized in the evaluation of visual function in eyes with GA. In randomized clinical trials and natural history studies of patients with GA, there has been an observed discordance between the progression of GA and BCVA. (Holz et al., 2018) (Holekamp et al., 2020), The location and pattern of progression of GA lesions within the macula contribute to this discordance. GA typically expands in the perifoveal area and around the foveola, leaving BCVA relatively unaffected. Ultimately, when the fovea is involved, BCVA *is* affected, and fixation changes to a preferred retinal locus (PRL). (Costela et al., 2020; Sunness et al., 2000) Many standard measures of visual function used in clinical trials including BCVA, reading speed, and patient-reported outcomes, are insensitive to changes in overall GA area (Csaky et al., 2017; Hanout et al., 2015; Pfau et al., 2020; Heier et al., 2020).

Among psychophysical functional vision tests, microperimetry is the only measure that combines retinal light sensitivity (in decibels) with topographic retinal information, creating a map of retinal sensitivity. This combination allows reasonable correlation of visual function with GA growth (Heier et al., 2020; Csaky et al., 2019). This method also identifies regions of partial or complete loss of sensitivity and links them to underlying retinal pathologies, such as the loss of photoreceptors, RPE, and choriocapillaris (Csaky et al., 2019; Yang and Dunbar, 2021; Hanout et al., 2015; Wu et al., 2024). Because microperimetry systems integrate eye tracking and image co-registration, this test enables in-depth longitudinal analysis of changes in retinal function within specific areas of GA expansion and enables testing even if the fixation site changes or there is no foveal fixation (Csaky et al., 2019; Cideciyan et al., 2012).

Despite the advantages of topographic quantitative functional information from microperimetry mapping, use of microperimetry in GA clinical trials presents several challenges. The standard 10-2 microperimetry grid consists of 68 points, usually spaced 1–2° (~290–580 μm) apart (Charng et al., 2020). GA lesions expand at their borders, with an estimated median lateral spread rate from 100 to 300 μm per year (Fukuyama et al., 2022; Shen et al., 2020). Consequently, the standard grid is often too sparse to detect losses of retinal sensitivity in areas near the GA margin, during a clinical trial lasting less than 2 years. Testing should involve the creation of customized grids.

Fixation stability and the location of GA pathology within the

Table 1
Potential technologies to establish structural and functional changes in 3 categories of AMD severity.

Early AMD	Intermediate AMD	Late AMD (GA)
Dark adaptation- Highly relevant in early detection but constrained by limited availability and need for optimized instruments to reduce testing time and improve accessibility; DA changes very slowly with AMD progression	**Dark adaptation** - Provides insights into rod function	**N/A**
Microperimetry. Assesses retinal function. Scotopic microperimetry examines the eye under low-light conditions and is important for early functional evaluation	**Mesopic and scotopic Microperimetry-** Key in measuring retinal sensitivity under various lighting conditions.	**N/A**
Low luminance VA- Important for assessing vision under diminished lighting, reflecting early functional loss	**Low luminance VA-** Important for assessing vision under diminished lighting, reflecting early functional loss	**N/A**
OCT- A cornerstone in early AMD detection and monitoring	**OCT**- Offers detailed visualization of basal laminar deposits, drusen, RPD, and HRF. **En face OCT and OCT-A** Utilized for detailed observation of hypertransmission and HRF	**OCT**- Offers detailed visualization of basal laminar deposits, drusen, RPD, and HRF, other features of geographic atrophy and neovascular AMD. **En face OCT and OCT-A** Utilized for detailed observation of hypertransmission and HRF
FAF: may be important to reflect the health of the RPE at this early stage of AMD	**FAF-**Useful in tracking changes in autofluorescence, which indicates alterations in retinal health, specifically the RPE.	**FAF-** Remains a valuable tool for monitoring the progression of advanced AMD
Near Infrared reflectance- Essential for visualizing subretinal structures like RPD which may occur early in AMD	**Near Infrared reflectance-** Essential for visualizing subretinal structures like RPD and HRF, pivotal in AMD progression	**Near Infrared reflectance-**Provides deeper imaging capabilities compared with FAF and color fundus photography
FLIO- Although not currently available, it holds promise for future diagnostic-pognostic landscapes.	**FLIO-** Although not currently available, it holds promise for future diagnostic landscapes.	**N/A**
Choriocapillaris integrity (OCT-A) Although not discussed in this paper, in exploratory phases still but recognized for its potential to provide meaningful insights into choriocapillaris health	**Choriocapillaris integrity (OCT-A)** Although not discussed in this paper, in exploratory phases still but recognized for its potential to provide meaningful insights into choriocapillaris health	**OCT-A** May be important in defining neovascular AMD
		Fixation Stability- potential correlation with VA but not currently validated. **VA tests**-lacks sensitivity and specificity, and poorly correlated with structural changes
	Optoretinography used for decades for recording optical signals that reflect retinal function. Proposed as a diagnostic measure to detect and quantify functional losses in intermediate AMD. Further investigation is needed.	**Optoretinography-** used for decades for recording optical signals that reflect retinal function. Proposed as a diagnostic measure to detect and quantify functional losses in late AMD. further investigations needed.
Artificial Intelligence (AI) may eventually be important for all severities of AMD		

Definition of AMD Severity: Early: Eyes with at least medium size drusen (63 to <125 μm), Intermediate: large drusen (≥125 μm), hypo/hyperpigmentary changes of RPE, Late: Presence of macular neovascularization or GA.
FAF: Fundus autofluorescence, OCT: Optical Coherence Tomography, OCT-A: Optical Coherence Tomography-Angiography, FLIO: Fluorescein Lifetime Imaging Ophthalmoscopy, GA: Geographic atrophy.

microperimetry grid are important considerations. The relationship between GA expansion and the onset of microperimetry-detected loss of retinal sensitivity can be diluted by pre-existing scotomatous points in the GA region at baseline. Additionally, the lack of change in retinal sensitivity in areas away from the GA border complicates this relationship. Most points on the grid are located in these latter regions, making it challenging to demonstrate a treatment effect, even if one is present (Chang et al., 2024). Microperimetry points near the boundary of GA lose sensitivity more quickly than areas that are distant from and unaffected by the GA lesion (Meleth et al., 2011). This observation was supported by a recent post hoc analysis from the Chroma and Spectri GA clinical trials, demonstrating that peri-lesional retinal sensitivity measured with microperimetry was more effective than standard pattern microperimetry in tracking functional decline (Chang et al., 2024). Furthermore, residual photoreceptor function approximately 200 μm inside the GA border, as defined by FAF imaging, can be detected using microperimetry (Pfau et al., 2019). Focusing the analysis of retinal sensitivity on microperimetry points at the highest risk for change, particularly those near the active GA lesion margins, delivers results that more accurately reflect functional deficits reflected by GA expansion. This approach may also reduce the time necessary to demonstrate potential functional treatment benefits over the course of a 2-year clinical trial (Chang et al., 2024).

Finally, current limitations of microperimetry, including coarse stimulus spacing and large stimulus size, may be addressed through high-resolution imaging-based testing modalities. Adaptive optics (AO)-enhanced retinal imaging enables microperimetric testing of small retinal regions, down to the level of individual cones. AO-assisted microperimetry has been instrumental in examining retinal light sensitivity within iRORA lesions in intermediate AMD, potentially facilitating testing of novel treatments (Ameln et al., 2024).

4.3. Visual acuity and low luminance visual acuity and others

The gold standard for assessing therapeutic efficacy in ocular diseases has been BCVA, especially for diseases in which the advanced stages of the disease affect the area around or involving the central macula. With gradual loss of the RPE and photoreceptors in GA, there is a continuous and irreversible decrease in BCVA over the course of the disease. However, BCVA does not correlate with the total area of GA lesions, as GA often spares the fovea until late in the course of the disease.

Interestingly, Sunness et al. demonstrated in 2008 that patients with geographic atrophy experienced a 3-fold increased risk of visual acuity loss of 3 or more lines if the baseline visual acuity was 20/50 or worse or

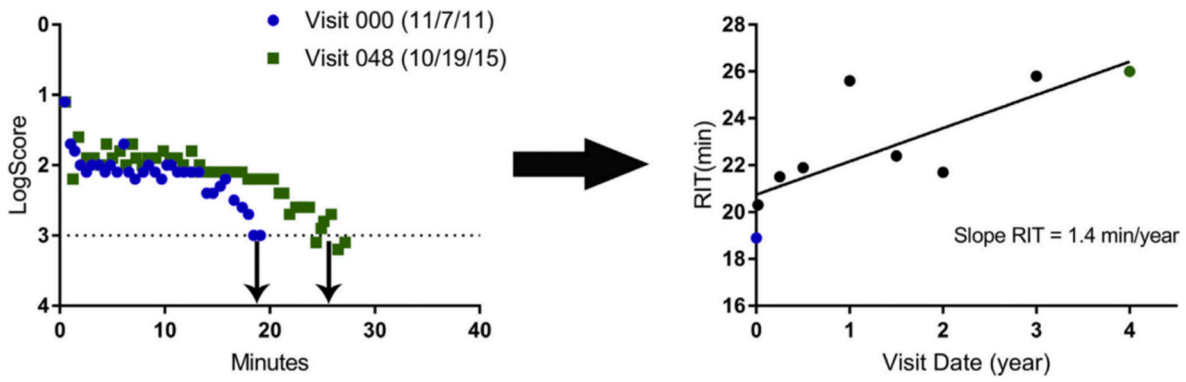


Fig. 4. Left: Representative dark adaptation raw data from a single subject with intermediate AMD at baseline and 4 years. All rod intercept time (RIT) values measured at study visits over 4 years are plotted, and the slope of the linear regression performed defines "slope RIT" Right: This measure (slope of rod intercept time (RIT)) is used to represent change in RIT over time for a given study eye (Chen et al., 2019).

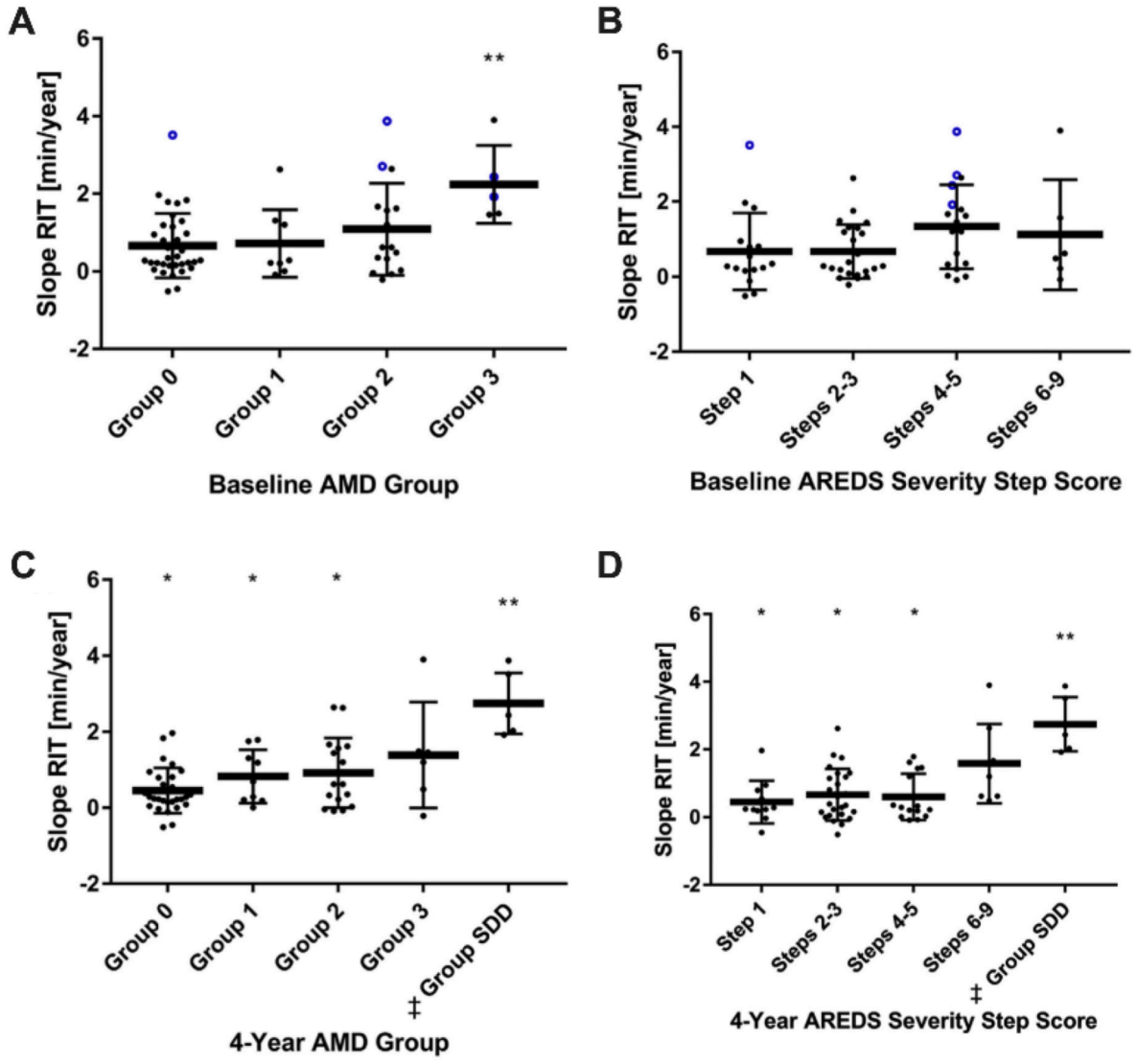


Fig. 5. Slope of rod intercept time (RIT) increases with more advanced AMD. A and C: AMD groups are defined: Group 1: no large drusen or late AMD, Group 2: Large drusen in both eyes with no late AMD, Group 3: large drusen in one eye and late AMD in fellow eye. B and D: AREDS AMD Severity Scale [Detailed Score]), from no AMD to those with large drusen or non-central GA. Most severely affected dark adaptation rod intercept time occurs when Reticular Pseudodrusen (RPD) are present (labeled SDD or subretinal drusenoid deposits in figures C and D) (Chen et al., 2019).

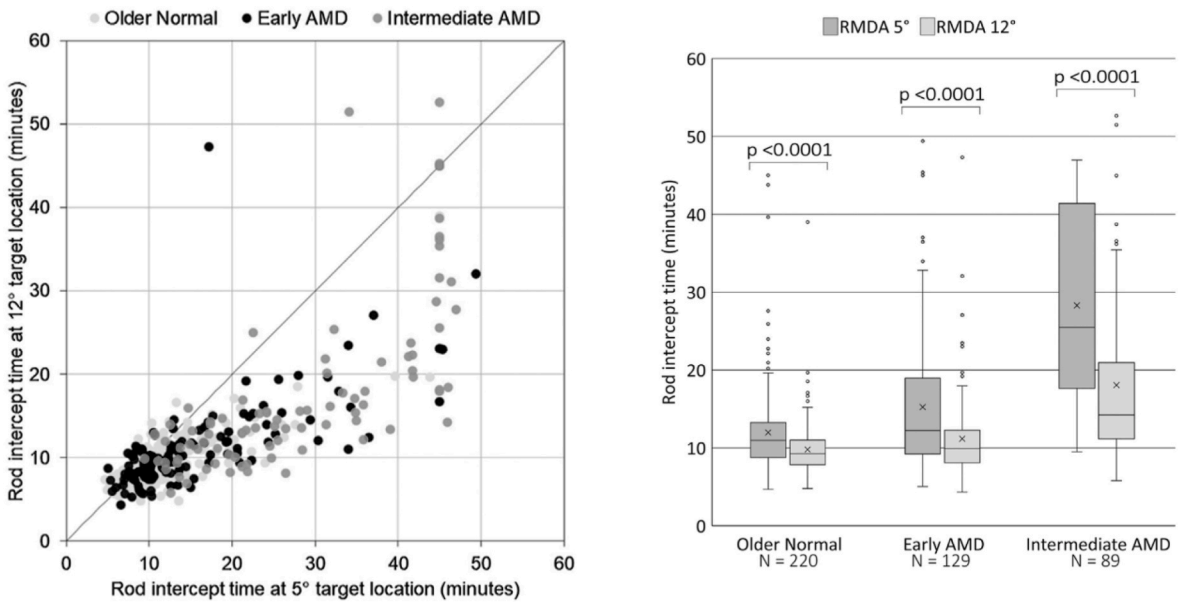


Fig. 6. Left: Scatter plot showing the relationship between rod intercept time (RIT) and the severity of AMD. Faster rod adaptation (lower RIT) corresponds to mild AMD, while slower rod adaptation (higher RIT) indicates intermediate AMD. Right: Testing closer to the fovea (5°) is associated with slower rod mediated dark adaptation (higher RIT) those subjects with and without AMD. (Owsley et al., 2023).

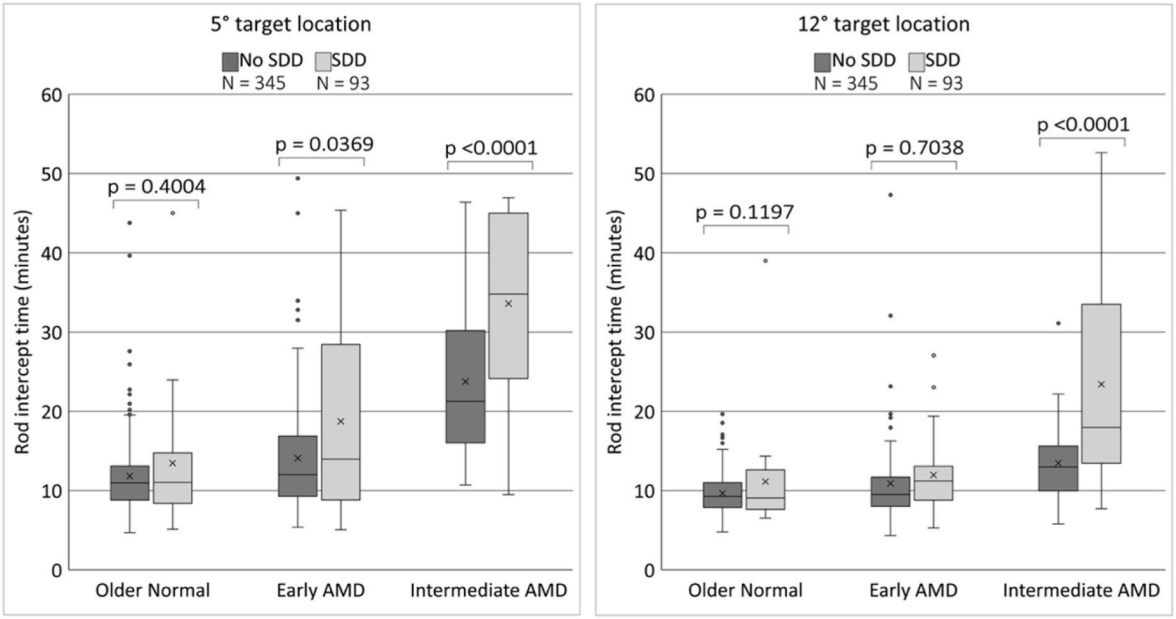


Fig. 7. Box plots comparing the distributions of RIT for eyes with no SDD (Subretinal Drusenoid Deposits) or Reticular Pseudodrusen and eyes with SDD stratified by AREDS 9-step AMD severity measured at 5° (left) and 12° (right). Note that in normal eyes at both 5° and 12°, SDD do not accentuate RIT. At 5°, SDD accentuates RIT for both early and intermediate AMD. At 12°, SDD accentuates RIT in intermediate AMD, but not in early AMD. Of the 438 eyes, overall, 99 (22.6 %) had SDD. For eyes in normal macular health, early AMD, and intermediate AMD, 20 (9.1 %), 32 (24.8 %), and 41 (46.1 %), respectively, had SDD. (Owsley et al., 2023).

if the baseline low-luminance deficit in visual acuity was worse than 0.40 logMAR units (i.e. 4 lines of worsening on the ETDRS carts with he filter interposed) (Sunness et al., 2008). Low luminance deficit was a stable and reproducible measure. Both baseline visual acuity score and the low luminance deficit in visual acuity were important predictors of future vision loss.

In the MACUSTAR study of subjects with iAMD, several visual function (VF) tests were conducted to assess the impact of intermediate disease on visual function: BCVA and LLVA in varied lighting conditions, Moorfields Acuity Test (MAT) for a sensitive assessment of VA with vanishing optotypes, Pelli Robson contrast sensitivity charts, and the (IReST) reading speed cards (Dunbar et al., 2022) (Fig. 8). The results demonstrated that the visual function of individuals with iAMD declined notably across multiple visual function parameters when compared with healthy controls, suggesting a significant broad impact of iAMD on visual function, with marked functional heterogeneity. Furthermore, some individuals with iAMD demonstrate visual functions as compromised as those of patients with late-stage AMD. Over time, all vision tests show an increasing proportion of patients exceeding the test-retest threshold (TRT). LLVA showed the largest growth in the proportion of patients surpassing TRT limits, which is especially significant given that LLVA has the broadest TRT margins. Additionally, visual function deficits

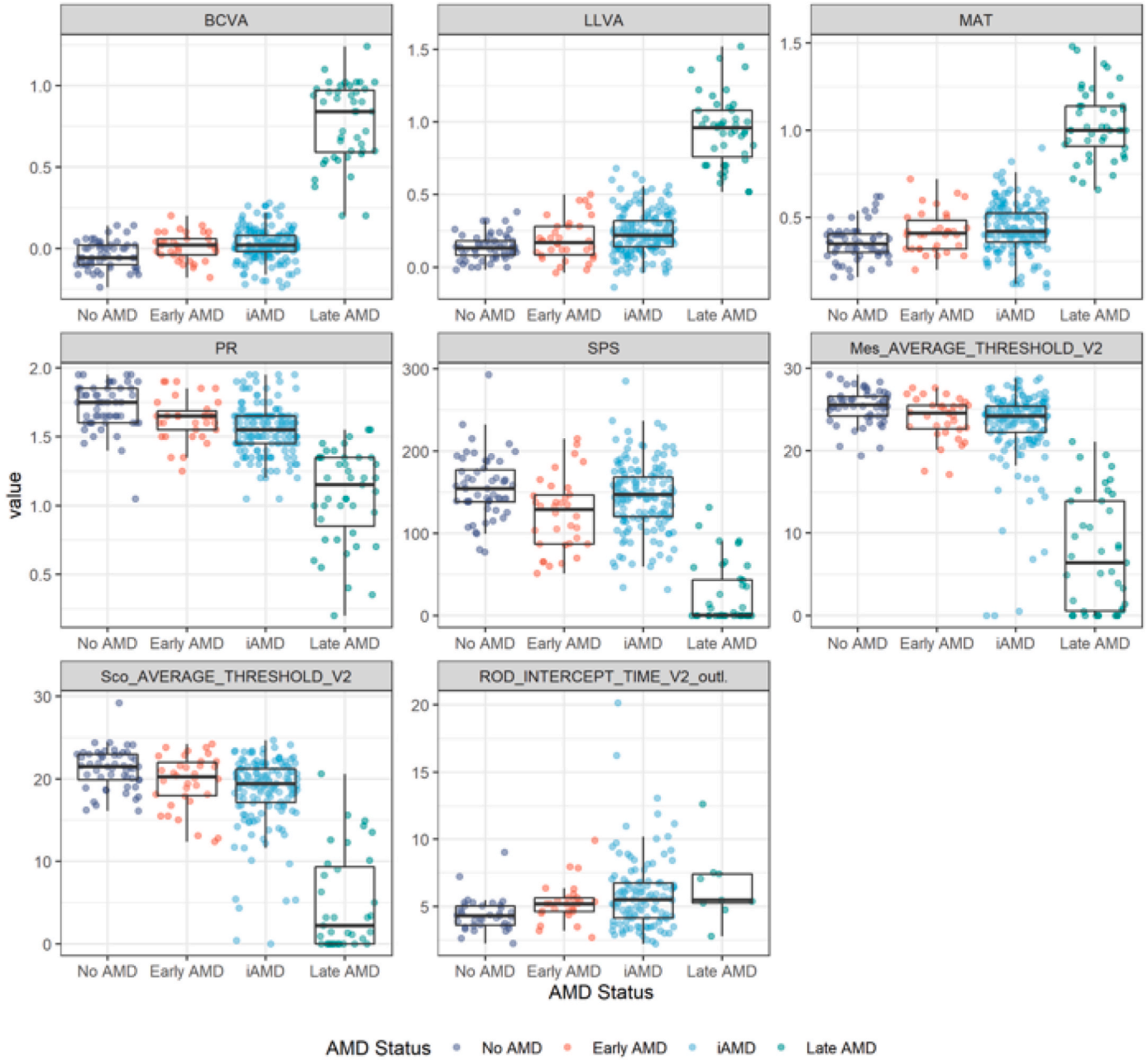


Fig. 8. Trajectory of deterioration within AMD severity groups over time using various visual tests. BCVA – Best Corrected Visual Acuity; LLVA – Low Luminous Visual Acuity, MAT- Moorfields Acuity Test: Y-axis Value defined logMAR; PR Pelli-Robson contrast sensitivity test; Y axis: logMAR in letters; SPS Small Print Standardized, Y-axis: Mesopic (Mes_AVERAGE_THRESHOLD_V2: average threshold and scotopic average threshold; Y-axis: decibels. Rod intercept time; Y-axis minutes.) These various functional tests demonstrated decline with increasing AMD severity. (Adapted from Higgins, 2023).

were shown to play a critical role in the prognosis of iAMD, with baseline RPD contributing to the progression.

The comprehensive assessment of visual functions in the MACUSTAR [NCT03349801; www.clinicaltrials.gov] study offers valuable insights for clinicians, for monitoring disease progression in iAMD and potentially for targeting treatment plans and contributes to setting informed expectations for patients with iAMD. These findings will also help refine the design of clinical trials. For example, possible endpoints in iAMD clinical trial design could include conversion to late-stage phenotypes of AMD, and these multiple visual function tests will be useful in studying interventions expected to slow functional decline or improve retinal function before conversion to late AMD.

4.4. *Fluorescence lifetime imaging ophthalmoscopy (FLIO)*

While high-resolution imaging techniques, such as OCT, are effective for detecting structural changes, metabolic markers could be especially important in earlier stages of AMD. Identifying a novel metabolic biomarker associated with the worsening stages of AMD could provide both a better understanding of the pathogenesis of AMD, and potentially, a new outcome variable.

Fluorescence lifetime imaging ophthalmoscopy (FLIO) is a relatively new imaging modality that captures both metabolic and structural alterations by measuring the metabolic state of molecules in vivo (Dysli et al., 2017). Using blue laser light (473 nm), natural fluorophores in the retina are excited. These excited fluorophores release energy in the form of light of longer wavelengths, which can be recorded in an

autofluorescence intensity image. The time between excitation and the detection of the autofluorescence signal, known as the fluorescence decay time, is referred to as the fluorescence lifetime (FLT) (Dysli et al., 2014).

Specific molecules have unique FLTs that are influenced by both the excitation wavelength and the metabolic environment. Therefore, the FLT signal detected from the retina provides a comprehensive view of both the structural and metabolic conditions with topographical precision. Current FLIO has spatial resolution comparable to other standard retinal images such as CFP, FAF, and *en face* OCT. The retina's main fluorophores are in the outer layers, primarily in the RPE. In addition to lipofuscin, other fluorophores such as melanin, collagen, elastin, and coenzymes involved in energy metabolism also contribute to the FLT signal (Dysli et al., 2017). Macular pigment, concentrated at the foveal center, is characterized by very short FLTs, and recent studies suggest that dietary intake of lutein and zeaxanthin influences macular pigment measurements (Jaggi et al., 2023).

Numerous studies utilizing FLIO in AMD have provided valuable insights across disease stages. (Dysli et al., 2016; Dysli et al., 2017; Lincke et al., 2021; Sauer et al., 2018a; Sauer et al., 2018b; Sauer et al., 2019) (Hammer et al., 2020), In early AMD, eyes with SDDs typically exhibit prolonged FLT, particularly in the perimacular region (Fig. 9). Interestingly, this slight FLT prolongation has also been observed in older individuals without evident signs of AMD, suggesting a possible relationship between FLT changes, aging, and early AMD. Although no direct correlation between these prolonged FLT and structural changes is established, the ring-like FLT pattern appears to align with the distribution of cone and rod photoreceptors.

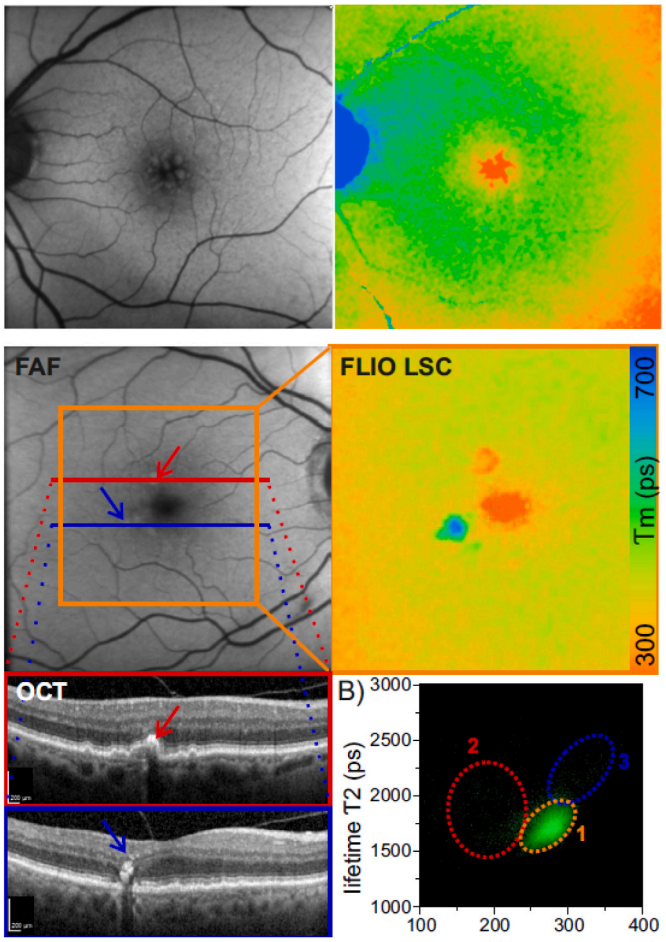


Fig. 9. FLIO of drusen in persons with iAMD demonstrating differences in drusen fluorescence (from: Dysli et al., 2017a,b.) PUT
Figures on top: Fundus autofluorescence intensity (FAF) and lifetime image (FLIO) of the left eye of a patient with intermediate age-related macular degeneration (AMD). Retinal fluorescence lifetimes in patients with early and intermediate AMD generally feature prolonged fluorescence lifetimes of the central retina. This is visible as a ring of prolonged FLT (color coded in green) around the central fovea (red/short fluorescence lifetimes). Figures below: FLIO in intermediate AMD demonstrating differences in drusen fluorescence. A soft druse exhibiting short fluorescence lifetimes (red) is highlighted with a red arrow. The area of prolonged FLT below corresponds to the hyperreflectivity the OCT images. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

In early AMD, drusen can exhibit either short or long FLTs, which may reflect differences in drusen location and composition (Fig. 10). (Dysli et al., 2017) In eyes with GA, FLT signals from the RPE are absent in the atrophic areas. Although GA regions appear dark on FAF intensity imaging, they still demonstrate significantly prolonged FLTs, likely originating from underlying choroid or connective tissue. (Dysli et al., 2016) Importantly, foveal sparing in macular GA is well-detected by FLIO, as the macular pigment in the foveal center produces short FLTs, even when this area is not easily distinguishable on autofluorescence intensity images (Fig. 10). Of particular prognostic interest is the border zone of GA, where a ring of intermediate FLTs may indicate the 'transition zone' of tissue in the process of becoming atrophic.

FLIO, therefore, offers the potential to reveal early metabolic changes and markers of AMD progression before they are detectable by other imaging techniques. However, the current FLIO system has limited image resolution (approximately 15 μm laterally and 300 μm axially) and cannot differentiate photons originating from distinct retinal layers. This spatial overlap, combined with the overlapping lifetimes and spectra of autofluorescent species in the retina and RPE, pose significant challenges for distinguishing underlying components, particularly in interpreting measurements from the short spectral channel. Recently, AO-enhanced FLIO has demonstrated the capability to improve image resolution to the cellular level, offering promise for enhancing the diagnostic power of FLIO (Tang et al., 2022; Kunala et al., 2024). Further research involving larger cohorts and longer follow-up periods is necessary to fully establish the prognostic value of FLIO in early AMD, to assess disease progression from intermediate to late stages, and to monitor of GA progression.

4.5. Optoretinography (ORG)

Optoretinography (ORG) is broadly defined as the "*optical measurements of changes in the retina in response to light stimulation*" (Kim et al., 2022; Williams et al., 2023; Jonnal, 2021). ORG is an optical analog to the electroretinogram (ERG), which measures diffuse electrical changes of the retina in response to light stimulation. Broadly, ORG signals can include any optical change, with the primary changes being intensity, phase, and polarization.

ORGs of different types, measured using a range of different technologies and under various different names, have been recorded for decades, but attention peaked when the phase-resolution capabilities of OCT combined with AO were leveraged to measure ORG functional responses from individual cones (Zhang et al., 2019). This OCT-based method can resolve physical changes in cone photoreceptors on a nanometer scale and over millisecond timescales in response to light stimulation. Over the last 5 years, multiple groups have measured ORGs using several different OCT-based approaches, with and without the use of AO. The primary focus has been on ORG measures from cones but, over the past few years, there have been reports of ORG signals from rods (Azimipour et al., 2020), ganglion cells and inner retinal layers (Pfäffle et al., 2019) as well as from the subretinal space and RPE (Tan et al., 2024).

There have been few reports on the clinical usefulness of ORG (Lassoued et al., 2021), (Wendel et al., 2024; Wongchaisuwat et al., 2024), with none to date in AMD. Nevertheless, ORG is a rapidly emerging field and the fact that OCT-based ORG systems can make

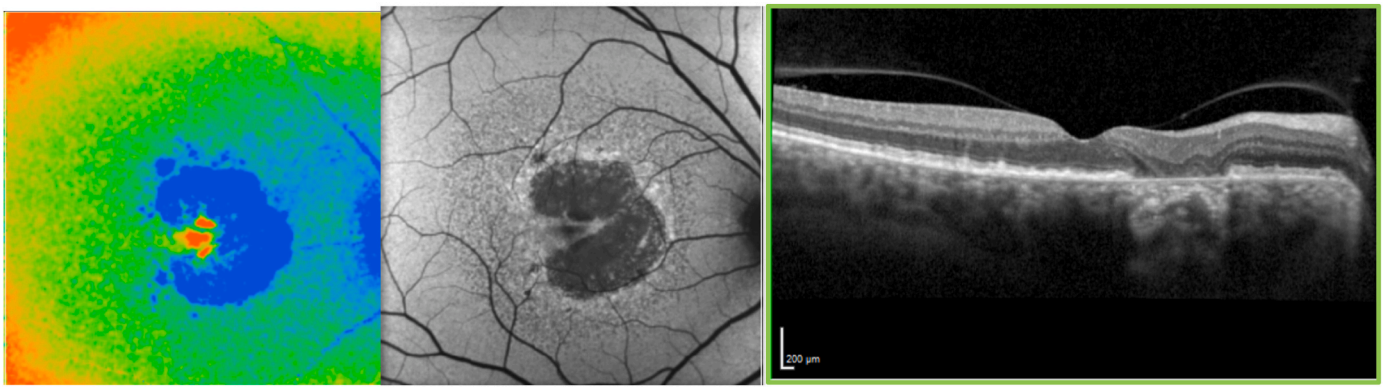


Fig. 10. FLIO of GA with foveal sparing in AMD
Lifetime image (left, FLIO) and fundus autofluorescence intensity (middle, FAF) of the left eye of a patient with advanced age-related macular degeneration (AMD) with geographic atrophy (GA). A ring shaped pattern of generally prolonged FLT of the central fovea is visible in green. Additionally, the blue area of long FLT indicates retinal atrophy around a central fovea with preserved macular/foveal pigment. A horizontal OCT scan of the fundus is shown to the right. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

objective measures of retinal function that are depth-resolved and have a spatial resolution that is many orders of magnitude better than ERG (the only other existing approach to measure functional retinal activity) make it a very promising technology. Moreover, ORG does not necessarily require the most high-end OCT systems, nor does it require the use of AO (Takeno et al., 2024; Zawadzki et al., 2024). Inevitably the value of ORG will emerge only after it is assessed in all stages of AMD.

5. Summary of functional tests

In summary, the discussions held at the RIMR meeting provided the opportunity to assess functional studies such as dark adaptation, mesopic and scotopic microperimetry, and FLIO for potential to provide structure-function correlations desired by industry investigators and regulatory agencies. Dark adaptation, although correlated with AMD stage, is a problematic endpoint in clinical trials because DA changes slowly and the technique is time consuming. Microperimetry has been a useful outcome variable in many clinical trials and new methodology may improve the structure-function correlations provided by this test. Finally, the promise of ORG is high but it has yet to be applied in AMD clinical trials. Development of more advanced methods to study structure such as high-resolution OCT and en face OCT offer an opportunity to better correlate structure and function in clinical trials and to better define useful biomarkers of visual outcome endpoints. Further research, using these evolving tools, is essential for improving early AMD detection, prediction of disease progression from intermediate to late stages, and the ability to monitor the advancement of GA over time. Documentation of the association of structure with function requires careful, well-powered prospective and/or retrospective cohort studies. For some outcomes, change in structure alone is sufficient (loss of functioning tissue, such as increase in GA area, is sufficient for regulatory purposes). To develop surrogate structural outcomes, it is necessary to document that the change in the surrogate leads to loss of visual function in a well-designed cohort study. Surrogates will speed clinical testing of new therapies. Once a surrogate is approved it is not necessary to have a functional outcome in addition to the surrogate primary outcome. However, functional outcome measurements are critical for clinical trials, even if they do not meet statistical significance, as important contributors to the totality of evidence for assessing safety and efficacy.

Discussions between clinical researchers, study sponsors, and regulatory agencies are crucial in mapping further research in this area of structure-function correlation measurements in clinical trials. A framework for regulatory control must be evidence-based. We need to work with regulatory agencies to determine acceptable endpoints for clinical trials of AMD interventions. Longitudinal data will be essential for establishing valid surrogate outcome variables for clinical trials in patients with non-neovascular AMD.

CRediT authorship contribution statement

Emily Y. Chew: Writing – review & editing, Writing – original draft, Conceptualization. **Catherine Cukras:** Writing – review & editing, Conceptualization. **Jacque L. Duncan:** Writing – review & editing, Conceptualization. **Chantal Dysli:** Writing – review & editing, Writing – original draft, Conceptualization. **Ye He:** Writing – review & editing, Conceptualization. **Erin Henry:** Writing – review & editing, Conceptualization. **Frank Holz:** Writing – review & editing, Conceptualization. **Eric Moult:** Writing – review & editing, Conceptualization. **Cynthia Owsley:** Writing – review & editing, Writing – original draft. **Austin Roorda:** Writing – review & editing, Writing – original draft, Conceptualization. **David Sarraf:** Writing – review & editing, Writing – original draft, Conceptualization. **Roy Schwartz:** Writing – review & editing, Writing – original draft, Conceptualization. **Richard Spaide:** Writing – review & editing, Conceptualization. **Lori Taylor:** Writing – review & editing, Conceptualization. **Michel Teussink:** Writing – review & editing, Writing – original draft, Conceptualization. **Yuhua Zhang:** Writing – review & editing, Conceptualization. **Giovanni Staurenghi:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Emily Y. chew reports a relationship with National Eye Institute that includes: employment. None If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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