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ORIGINAL RESEARCH

# Dynamic Pupillary Responses in Age-Related Macular Degeneration: A Controlled Clinical Study Using High-Frequency Video-Oculography

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Video abstract

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**Purpose:** To investigate whether dynamic pupillary responses differ between patients with age-related macular degeneration (AMD) and healthy controls (HC), and to evaluate their potential as functional biomarkers using high-frequency, VR-based video-oculography.

**Methods:** A controlled clinical study included 17 AMD patients and 17 age-matched HCs; four AMD participants were excluded for low recording quality. Dynamic pupillary responses were recorded with the BulbiCam video-oculography system (400 Hz), which presented independent monocular light stimuli through multiple permutations of bright (300 cd/m²) and dark (5 cd/m²) conditions. Measured variables included pupil diameter, latency, peak velocity, and pupil diameter–time integral (PDTI). Each eye was tested separately, and repeated sessions were analysed for reliability (intraclass correlation coefficient, ICC), repeatability (agreement index, AI), and stability (stability index, SI). Group differences were assessed using analysis of variance (ANOVA) and receiver operating characteristic (ROC) analysis.

**Results:** AMD eyes showed larger steady-state pupil diameter and higher PDTI than controls ($p < 0.05$). First peak velocity was reduced in the worst eye only, while latencies were unchanged. PDTI and diameter demonstrated high reliability and stability across repetitions, and ROC analysis confirmed effective group discrimination.

**Conclusion:** High-frequency VR pupillometry detected reproducible functional alterations in AMD, consistent with impaired macular photoreceptor input but preserved reflex transmission. PDTI and diameter serve as diagnostic (population-level) and monitoring (patient-level) biomarkers, offering a non-invasive and objective method for AMD detection and follow-up in clinical and research settings.

**Keywords:** age-related macular degeneration, AMD, biomarkers, Bulbicam, pupillometry, retinal function, video-oculography

## Introduction

The pupillary light reflex (PLR) is an autonomic circuit that adjusts pupil diameter to ambient luminance. Cones signal through ON cone bipolar cells, while rods connect via rod bipolars and the AII amacrine relay[1] into the ON pathway; both streams converge on pupil-projecting retinal ganglion cells (RGCs). Most of these are intrinsically photosensitive (ipRGCs). M1 ipRGCs combine rod–cone input with intrinsic melanopsin currents: extrinsic drive produces a rapid phasic constriction, whereas melanopsin mediates a sustained, blue-weighted post-illumination pupil response (PIPR) that maintains constriction under steady or bright light. Only ~3000 fibres (≈0.3% of the optic nerve) project to pupillary centres, the majority being M1 ipRGCs, with smaller contributions from M2 ipRGCs and conventional ON RGCs.[2,3]

Although primarily governed by the PLR, pupil size is modulated by accommodation, convergence, circadian rhythms,[4] emotional state,[5] pharmacological agents, and systemic or neurodegenerative disease. Parkinson's disease,[6] diabetes mellitus,[7] ageing,[8] and hypoxia[9] all alter pupil behaviour, underscoring that pupil dynamics reflect both retinal and systemic inputs and can serve as a sensitive window into ocular and neurological health.[10,11]

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Age-related macular degeneration (AMD) is a progressive retinal disorder that damages the macula, where high-acuity vision depends on cone photoreceptors, the retinal pigment epithelium (RPE), and the choriocapillaris. Early AMD is characterised by drusen and RPE dysfunction; advanced dry AMD leads to photoreceptor atrophy and geographic tissue loss. These structural changes disrupt both rod- and cone-mediated pathways, weakening the circuits that normally feed into pupil-projecting RGCs. While AMD has traditionally been assessed with visual acuity and structural imaging, its impact on pupil dynamics is poorly characterised. Reports include enlarged baseline pupil diameter[12] and delayed constriction latency,[13] and several studies suggest the PLR may provide a sensitive biomarker of functional impairment in AMD.[14] "The PLR can be used as a biomarker of retinal health and aging".[2]

Reliable functional biomarkers are essential to detect subtle but clinically significant changes in visual function before overt structural damage occurs — a key challenge in early AMD. Manual clinical assessment of pupil responses is limited by semi-quantitative grading and high inter-observer variability, making it unsuited to capture the rapid dynamics critical for accurate evaluation. Automated pupillometry using video-based eye tracking overcomes these limitations, offering high-resolution, objective, and reproducible measurements while minimising operator bias. It enables detection of minute changes in pupil behaviour, and repeated measures enhance stability and interpretability compared with single snapshots.[15–18]

The Bulbicam (BCAM) eye-tracking system (Bulbitech, Trondheim, Norway) is a CE-marked, video-based platform operating at 400 Hz. The primary aim of this study is to validate BCAM-derived pupil metrics for differentiating dry AMD patients from healthy controls, with comprehensive evaluation of reliability, repeatability, and stability to establish robust biomarkers for both population-level studies and individual monitoring.

# Material and Methods

## Study Population

Patients with a prior diagnosis of AMD were recruited from the ophthalmology outpatient clinic at Oslo University Hospital–Ullevål (OUH). Eligible participants were aged 18 years or older and free of other ocular disease or systemic conditions known to affect macular or pupillary function (eg, Parkinsonism, diabetes mellitus). Exclusion criteria included: best-corrected visual acuity < 0.1 in either eye; use of ocular or systemic medication influencing pupil function; inability to perform eye movements; grossly abnormal ocular appearance; pupillary abnormalities due to nerve damage or mechanical injury; and cataracts beyond the incipient stage, among others.

Seventeen patients with AMD were enrolled, of whom four were excluded from pupillography due to blepharochalasis, leaving 13 participants (8 females, 5 males). Their mean age was 66.1 years (range 49.6–80.2) and mean disease duration 5.2 years (0.2–13.1). Each patient was matched with a healthy control (HC) of similar age and sex who met the same eligibility criteria, except for the absence of ophthalmic or neurological disease. The HC group comprised 13 individuals (8 females, 5 males) with a mean age of 63.4 years (48.1–84.5).

All participants provided written informed consent. The study was approved by the OUH data protection officer. The Regional Ethics Committee deemed the project outside its mandate, as it involved no intervention or change in patient care. The study complied with the Declaration of Helsinki and was registered on ClinicalTrials.gov (NCT05441072) and EudraCT (2021–006258-30).

## Study Design

Design: This was a controlled, non-randomised parallel-group study, reflecting the inherent differences between the AMD and HC cohorts. Each AMD participant underwent six repeated measurements, while HC participants completed two.

## Device Setup

All recordings were performed with the BCAM system. BCAM applies dark pupil/bright pupil and corneal reflex methods for video-oculography, acquiring gaze data at 400 frames per second. The configuration consists of a single infrared eye-tracking camera and two display screens, enabling independent stimulus presentation to either eye while tracking both. Device documentation (STED record, version 1.1) has been provided to reviewers for context but is not

part of the published supplementary material. Detailed technical specifications are provided in the BulbiHub software versions 221031 and 221216 were used.

## Eye Classification

Classification of the best and worst eye in the AMD group was performed retrospectively after data collection. The primary criterion was multimodal clinical imaging according to the Modified International Criteria (MIC) for AMD staging. If both eyes received the same MIC grade, visual acuity served as the secondary criterion.

## Clinical Procedure

Baseline Ophthalmic Examination: All participants underwent a standard ophthalmic assessment without cycloplegia, including:

- Best-corrected visual acuity (BCVA) measured with a standard ETDRS chart.
- Contrast sensitivity with a Pelli-Robson chart.
- Intraocular pressure using an iCare tonometer.
- Optical coherence tomography (OCT; RS-3000, NIDEK Co).
- Fundus photography (Optos California).
- Slit-lamp examination of the anterior segment.

## BCAM Setup

BCAM examinations were conducted in a moderately lit room with no external light sources. No cycloplegics were used. Once seated, participants were exposed only to monitor illumination. They were positioned comfortably in chairs with backrests and armrests, with the forehead supported against the stationary BCAM. BulbiHub software recorded inter-pupillary distance and refraction. Trial lenses were placed in the BCAM to ensure clear screen viewing without accommodation or induced miosis. Full setup and calibration procedures were followed according to the BCAM Instructions for Use (Revision 02). A copy was provided to reviewers for methodological verification.

## Examination Protocol

Each AMD participant completed three BCAM sessions per day over two consecutive days; in some cases, all three sessions were performed within a single day. Each session lasted approximately 15 minutes and was followed by a rest interval. HC participants underwent two BCAM sessions completed within one day. Calibration was performed automatically by the BCAM software, and all sessions were conducted by the same operator. Before each task, participants received standardised instructions in Norwegian.

## BCAM Stimulus Protocol

The BCAM pupil test evaluated dynamic pupillary responses under controlled lighting conditions. A structured sequence of 10 light permutations was presented (Table 1), alternating illumination of the right eye (OD) and left eye (OS) between 300 cd/m² and 5 cd/m². Measurements were taken at the transition points between intervals to capture immediate pupil responses.

## Variables Measured

- *Pupil Diameter (mm)*: The average pupil diameter at each transition point was recorded in millimetres, representing the steady-state response under each lighting condition. Note on indexing. In the current software version, pupil diameter variables were labelled with a one-step offset (eg, "Diameter 05" corresponds to the 3.0-s timepoint, the junction between segments 1 and 2). This was a labelling artefact only: the underlying time-locked data were correct and used in all analyses.
- *Latency (ms)*: We defined PLR latency as the time to the first jerk peak (third derivative of diameter), an onset marker used in oculomotor signal detection.[19] Derivative-based onset metrics reduce sensitivity to baseline offsets compared with amplitude thresholds in related domains.[20] We therefore adopted a jerk-based criterion and validated its behaviour in our data using simulations and test–retest analyses.

**Table 1** Sequence of Stimulus Segments and Extracted Pupil Metrics

| Segment | Second | OD Display Brightness ($cd/m^2$) | OS Display Brightness ($cd\ m^{-2}$) | Diam. Variable Suffix | Peak Velocity in Segment Prefix | Latency in Segment Suffix | PDTI in Segment Suffix |
|---|---|---|---|---|---|---|---|
| *1* | 0–3 | 5 | 5 | 03 | | | 00_03 |
| *2* | 3–5 | 300 | 300 | 05 | First | 03 | 03_05 |
| *3* | 5–8 | 5 | 5 | 08 | | | 05_08 |
| *4* | 8–10 | 5 | 300 | 10 | Second | 08 | 08_10 |
| *5* | 10–13 | 5 | 5 | 13 | | | 10_13 |
| *6* | 13–15 | 300 | 5 | 15 | Third | 13 | 13_15 |
| *7* | 15–17 | 300 | 300 | 17 | | | |
| *8* | 17–20 | 5 | 300 | 20 | | | |
| *9* | 20–22 | 300 | 300 | 22 | | | |
| *10* | 22–25 | 300 | 5 | 25 | | | |

**Notes**: Diameter variable labels reflect a one-step indexing offset in the software. This was a labelling artefact only; all data were correctly time-locked. PDTI XX–YY denotes the pupil diameter–time integral from XX to YY s (mm s). The 25-s test comprised a continuous sequence of ten consecutive stimulus segments (rows shown in order). In each segment, pupil variables were extracted: diameter, maximum constriction velocity (suffix = stimulus change time), latency (suffix = stimulus change time), and pupil diameter–time integral (PDTI). Representative raw traces of pupil diameter and velocity across one full stimulus sequence are shown in Supplementary Figure 1.

- *Peak Velocity (mm/s)*: The maximum rate of pupillary constriction or dilation immediately after a change in stimulus was calculated in millimetres per second. By convention, constriction velocities are negative.
- *Pupil Diameter-Time Integral (mm·s) (PDTI)*: For each stimulus interval, the pupil diameter trace was integrated with respect to time. This gives the *cumulative* "exposure" *of the pupil diameter over that period* (in millimetres × seconds), ie the area under the diameter–time curve. PDTI is therefore not a geometric area of the pupil but a temporal integral of its size, reflecting how large the pupil remained, and for how long, during the interval. PDTI divided by the time period yields the average diameter over that period.

## Statistical Analysis

The primary aim of this study was to validate the BCAM pupil procedure and to identify diagnostic and monitoring biomarkers for AMD. In such studies, it is essential to minimise false-positive biomarkers without overlooking clinically meaningful ones.

Sample size was determined based on the clinically relevant difference (CRD) between AMD and HC groups, expressed in standard deviations (SD). With a CRD of 2 SD, a significance level of 5% ($\alpha = 0.05$), and power of 90% ($\beta = 0.10$), at least 12 AMD patients and 12 healthy controls are required. To also evaluate reliability and stability, a somewhat larger sample was considered appropriate.

If the CRD is reduced to 1.5 SD at the same significance level and power, the required number of participants increases to 16 per group. Finally, with $\alpha = 0.05$, power = 0.80, and CRD = 1 SD, a minimum of 16 AMD patients and 16 age-matched healthy controls are needed.

## Validation Framework

Continuously distributed variables are presented as mean values with 95% confidence intervals (CIs). Dispersion is reported using either SD or standard error (SE). Normality is assessed with the Shapiro–Wilk test; non-normal variables are log-transformed. All tests are two-tailed with a 5% significance threshold. Group differences are evaluated using analysis of variance (ANOVA) and receiver operating characteristic (ROC) analysis. Changes within groups are assessed with repeated-measures ANOVA.

## Biomarker Concept

In classical usage, a biomarker is broadly defined as any measurable indicator of a biological process, whether or not it is linked to disease. In this study, however, we apply the term in a stricter, operational sense: the pupillography-derived

variables are considered *functional biomarkers of foveal performance in AMD* only to the extent that they, in this study, have utility in distinguishing healthy from sick on a group level, or in monitoring on a patient level.

## Reliability and Repeatability

The intraclass correlation coefficient, ICC, model 3.1 was used to assess *between-subject reliability*, ie, the degree to which measurements are consistent across different individuals. Bland–Altman plots were used for intra-patient repeatability, showing the mean difference between repeated measures together with the limits of agreement, which are calculated as the mean plus or minus two times the within-subject standard deviation, $SD_w$. Negative ICC values can occur when within-subject variability exceeds between-subject variability. In this context they were interpreted as indicating no reliability.

The *Agreement Index*, AI, was additionally calculated to provide a normalised within-patient precision score.[21,22]

$$AI = 1 - \frac{2 \cdot SD_w}{Mean}$$

The *Stability Index*, SI quantified consistency of repeated measurements over time:

$$SI = 1 - \frac{SD_w}{SD_b}$$

where $SD_b$ is the between-patient standard deviation. AI and SI can occasionally fall outside the range [0,1] if within-subject variability is large relative to between-subject variability. Classification therefore relied on point-estimate thresholds as prespecified, not on bounds.[21,22]

The Statistical Methodology section of the Supplementary Materials contains further explanation, including derivations, conceptual background, and boundary conditions for these metrics. The Biomarker Decision Rules of the Supplementary Materials describe the framework for determining which variables are biomarkers.

# Results

Validity: Most PDTI variables discriminated AMD from controls, beginning with the first PDTI measure (Figure 1a) and the second PDTI measure (Figure 1b). The first peak velocity measure in the worst eye also met validity criteria (Figure 1c), while the diameter variables showed broad discrimination (Figure 1d and e). Together, these ROC analyses indicate that PDTI and Diameter variables, as well as the first peak velocity, are valid markers, whereas latency variables did not achieve discrimination. Validity analyses were performed on 12 AMD–HC pairs, as one pair was excluded due to velocity traces outside the predefined physiological bounds. Reliability and stability analyses retained 13 pairs wherever available, with N=12 for velocity variables where the same exclusions applied.

Reliability results are shown in Figure 2. The first and second PDTI measures were repeatable (Figure 2a and b), the first peak velocity measure demonstrated acceptable repeatability (Figure 2c), and both diameter variables were consistent across patients (Figure 2d and e).

Stability outcomes are summarised in Figure 3. PDTI variables remained stable across repeated sessions (Figure 3a and b), the best eye first peak velocity measure showed limited stability (Figure 3c), and both diameter measures were classified as excellent (Figure 3d and e).

Tables 2–4 provide the supporting statistical detail in the same order (validity, reliability, stability), and Table 5 integrates these findings into the final biomarker classification.

PDTI variables: In the worst eye, all six significantly discriminated AMD from HC (Table 2: all $p \leq 0.05$; AUC lower 95% CI > 0.50). In the best eye, five of six reached significance, with PDTI 05_08 failing validity (AMD–HC CI crossed zero; AUC lower CI ≤ 0.50). Repeatability analysis (Table 3) showed strong between-patient reliability (ICC > 0.5) and within-patient agreement (AI > 0.5) for all valid variables. Stability indices were consistently above threshold (Table 4). Accordingly, 11 of 12 PDTI variables were classified as biomarkers at both population and patient levels (Table 5).

Velocity variables: Of three peak pupil velocity (PPV) measures, only the first PPV in the worst eye reached validity (Table 2: $p \leq 0.05$; AUC lower CI > 0.50). It also met repeatability thresholds (ICC > 0.5; AI > 0.5, Table 3), but its stability

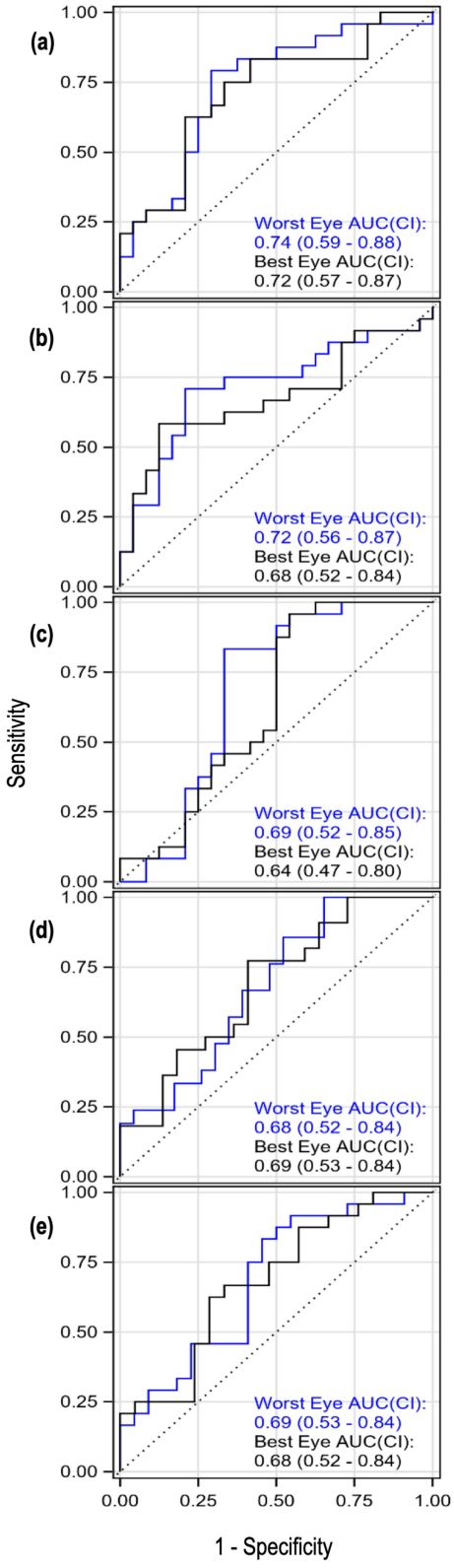


**Figure 1** ROC curves for representative pupil variables (Pupil Diameter-Time Integral (PDTI), Peak velocity, Diameter). Subpanels (**a**–**e**) correspond to five representative variables selected for illustration. Full analyses for all 44 variables are provided in Tables 2–5. PDTI XX-YY is the pupil diameter-time integral from XX to YY s (mm*s). A. PDTI 00–03 — PDTI between 0–3 s. B. PDTI 03–05 — PDTI between 3–5 s. C. First Peak Velocity — peak constriction velocity between 0–3 s. D. Diameter 03 — pupil diameter at second 3. E. Diameter 05 — pupil diameter at second 5. Each variable is plotted for the worse (blue) and best (black) eye. Area under the curve (AUC) and 95% confidence intervals are displayed. AUC values above 0.7 suggest moderate discriminatory power.
**Note**: Diameter labels reflect a one-step indexing offset (see Methods and Table 1).

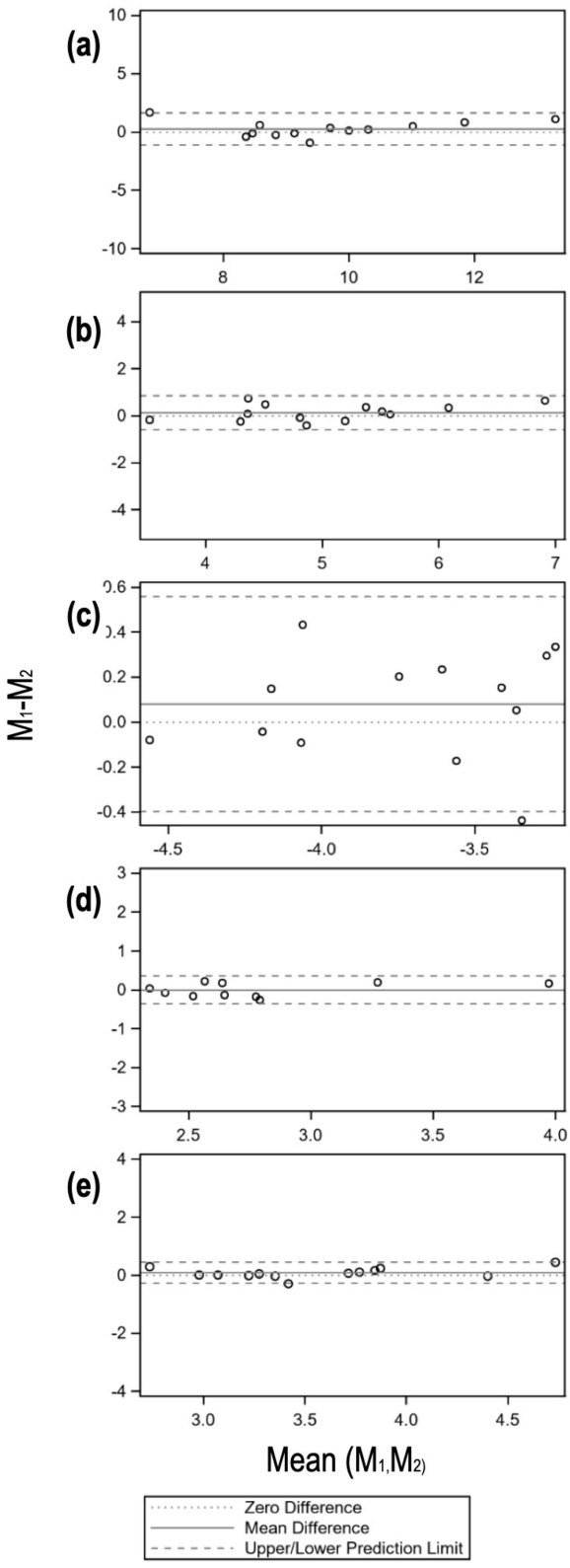


**Figure 2** Bland–Altman plots assessing test–retest agreement. Repeatability and agreement indices (ICC, AI) are shown for the same representative variables as in Figure 1. Full analyses for all 44 variables are provided in Tables 2–5. Each panel displays the agreement between two measurement sessions ($M_1$ and $M_2$) for a candidate variable. PDTI XX-YY is the pupil diameter-time integral from XX to YY s (mm·s). (**a**) PDTI 00–03 — PDTI between 0–3 s. (**b**) PDTI 03–05 — PDTI between 3–5 s. (**c**) First Peak Velocity. (**d**) Diameter 03. (**e**) Diameter 05. The vertical axis shows the difference ($M_1 - M_2$), and the horizontal axis shows the average of the two measurements. The solid line represents the mean difference; dashed lines indicate the 95% limits of agreement.

**Note**: Diameter labels reflect a one-step indexing offset (see Methods and Table 1).


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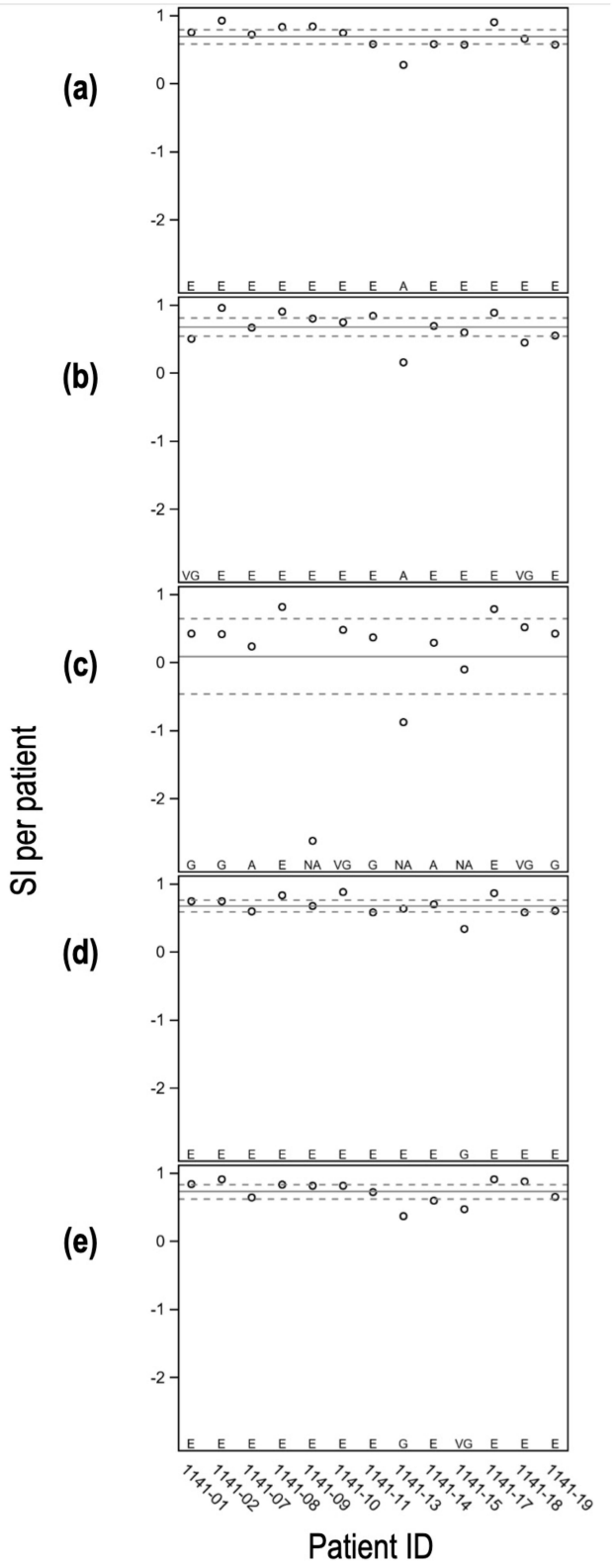


**Figure 3** Stability classifications (SI) for representative pupil variables. Subpanels (**a–e**) correspond to the same variables as in Figure 1. Full analyses for all 44 variables are provided in Tables 2–5. PDTI XX-YY is the pupil diameter-time integral from XX to YY s (mm·s). A. PDTI 00–03 — PDTI between 0–3 s. B. PDTI 03–05 — PDTI between 3–5 s. C. First Peak Velocity. D. Diameter 03. E. Diameter 05. An SI value closer to 1 indicates higher stability. The dashed horizontal line shows the group mean; dotted lines represent ±1 standard deviation. Letters beneath each datapoint denote expert qualitative ratings of stimulus quality: E = Excellent, VG = Very Good, G = Good, A = Acceptable, NA = Not Acceptable.

**Note**: Diameter labels reflect a one-step indexing offset (see Methods and Table 1).

**Table 2** Validation of Bulbicam Tests

| Pupil test | Eye | Variable | AMD Patients | | Healthy Controls | | AMD-HC | | | ROC Analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Mean | SE | Mean | Lower 95% CI | Higher 95% CI | AUC | Lower 95% CI | Higher 95% CI |
| *Pupil diameter-time integral (PDTI) (mm s)* | Worst Ey (N = 12) | PDTI 00_03 | 9.73 | 0.31 | 8.46 | 0.31 | 1.27 | 0.37 | 2.17 | 0.74 | 0.59 | 0.88 |
| | | PDTI 03_05 | 5.06 | 0.15 | 4.51 | 0.15 | 0.55 | 0.12 | 0.99 | 0.72 | 0.56 | 0.87 |
| | | PDTI 05_08 | 8.80 | 0.26 | 8.03 | 0.26 | 0.77 | 0.03 | 1.51 | 0.68 | 0.53 | 0.83 |
| | | PDTI 08_10 | 5.31 | 0.16 | 4.67 | 0.16 | 0.64 | 0.19 | 1.08 | 0.72 | 0.57 | 0.87 |
| | | PDTI 10_13 | 9.33 | 0.28 | 8.32 | 0.28 | 1.01 | 0.22 | 1.79 | 0.71 | 0.56 | 0.86 |
| | | PDTI 13_15 | 5.41 | 0.18 | 4.81 | 0.18 | 0.60 | 0.09 | 1.11 | 0.69 | 0.54 | 0.85 |
| | Best Eye (N = 12) | PDTI 00_03 | 9.74 | 0.33 | 8.40 | 0.33 | 1.34 | 0.39 | 2.29 | 0.72 | 0.57 | 0.87 |
| | | PDTI 03_05 | 4.96 | 0.19 | 4.41 | 0.19 | 0.56 | 0.01 | 1.11 | 0.68 | 0.52 | 0.84 |
| | | PDTI 05_08 | 8.66 | 0.30 | 7.88 | 0.30 | 0.78 | −0.07 | 1.63 | 0.63 | 0.46 | 0.80 |
| | | PDTI 08_10 | 5.32 | 0.17 | 4.59 | 0.17 | 0.73 | 0.24 | 1.22 | 0.72 | 0.57 | 0.87 |
| | | PDTI 10_13 | 9.35 | 0.28 | 8.21 | 0.28 | 1.14 | 0.34 | 1.95 | 0.70 | 0.55 | 0.85 |
| | | PDTI 13_15 | 5.42 | 0.19 | 4.75 | 0.19 | 0.67 | 0.14 | 1.20 | 0.70 | 0.54 | 0.85 |
| *Latency (ms)* | Worst Eye (N = 12) | Latency 03 | 233 | 5.9 | 237 | 6.1 | −4.1 | −21.2 | 13.0 | 0.51 | 0.34 | 0.69 |
| | | Latency 08 | 257 | 4.9 | 263 | 5.1 | −5.6 | −20.0 | 8.7 | 0.63 | 0.46 | 0.80 |
| | | Latency 13 | 230 | 11.1 | 242 | 11.4 | −11.7 | −43.8 | 20.2 | 0.53 | 0.36 | 0.69 |
| | Best Eye (N = 12) | Latency 03 | 229 | 3.2 | 231 | 3.4 | −2.8 | −12.3 | 6.7 | 0.55 | 0.38 | 0.73 |
| | | Latency 08 | 262 | 11.2 | 272 | 11.4 | −9.5 | −41.7 | 22.6 | 0.57 | 0.40 | 0.74 |
| | | Latency 13 | 231 | 11.1 | 252 | 10.9 | −20.9 | −52.3 | 10.4 | 0.63 | 0.47 | 0.79 |
| *Peak velocity (mm/s)* | Worst Eye (N = 12) | First PV | −3.72 | 0.16 | −3.14 | 0.16 | −0.58 | −1.05 | −0.11 | 0.69 | 0.52 | 0.85 |
| | | Second PV | −3.31 | 0.14 | −2.97 | 0.14 | −0.34 | −0.75 | 0.07 | 0.63 | 0.46 | 0.79 |
| | | Third PV | −2.48 | 0.15 | −2.32 | 0.14 | −0.16 | −0.57 | 0.25 | 0.55 | 0.38 | 0.73 |
| | Best Eye (N = 12) | First PV | −3.64 | 0.18 | −3.16 | 0.18 | −0.48 | −0.99 | 0.03 | 0.64 | 0.47 | 0.80 |
| | | Second PV | −3.33 | 0.15 | −2.99 | 0.15 | −0.34 | −0.77 | 0.08 | 0.63 | 0.47 | 0.79 |
| | | Third PV | −2.54 | 0.14 | −2.24 | 0.14 | −0.30 | −0.71 | 0.11 | 0.61 | 0.45 | 0.78 |

(*Continued*)

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**Table 2** (Continued).

| Pupil test | Eye | Variable | AMD Patients | | Healthy Controls | | AMD-HC | | | ROC Analysis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Mean | SE | Mean | Lower 95% CI | Higher 95% CI | AUC | Lower 95% CI | Higher 95% CI |
| *Diameter (mm)* | Worst Eye (N = 12) | Diameter 03 | 2.83 | 0.10 | 2.53 | 0.09 | 0.29 | 0.03 | 0.56 | 0.68 | 0.52 | 0.84 |
| | | Diameter 05 | 3.59 | 0.11 | 3.22 | 0.11 | 0.37 | 0.05 | 0.69 | 0.69 | 0.53 | 0.84 |
| | | Diameter 08 | 2.29 | 0.06 | 2.08 | 0.07 | 0.21 | 0.03 | 0.40 | 0.71 | 0.56 | 0.87 |
| | | Diameter 10 | 3.46 | 0.10 | 3.06 | 0.10 | 0.40 | 0.10 | 0.69 | 0.72 | 0.56 | 0.87 |
| | | Diameter 13 | 2.52 | 0.08 | 2.31 | 0.08 | 0.21 | −0.01 | 0.43 | 0.68 | 0.52 | 0.83 |
| | | Diameter 15 | 3.50 | 0.11 | 3.12 | 0.11 | 0.38 | 0.06 | 0.70 | 0.71 | 0.56 | 0.86 |
| | | Diameter 17 | 2.64 | 0.09 | 2.42 | 0.09 | 0.22 | −0.03 | 0.47 | 0.63 | 0.47 | 0.79 |
| | | Diameter 20 | 2.31 | 0.07 | 2.09 | 0.07 | 0.22 | 0.03 | 0.41 | 0.69 | 0.54 | 0.85 |
| | | Diameter 22 | 2.72 | 0.08 | 2.50 | 0.08 | 0.22 | −0.02 | 0.46 | 0.69 | 0.54 | 0.85 |
| | | Diameter 25 | 2.36 | 0.06 | 2.21 | 0.07 | 0.16 | −0.03 | 0.34 | 0.67 | 0.51 | 0.83 |
| | Best Eye (N = 12) | Diameter 03 | 2.79 | 0.10 | 2.50 | 0.10 | 0.30 | 0.02 | 0.58 | 0.69 | 0.53 | 0.84 |
| | | Diameter 05 | 3.60 | 0.12 | 3.19 | 0.13 | 0.41 | 0.06 | 0.77 | 0.68 | 0.52 | 0.84 |
| | | Diameter 08 | 2.33 | 0.06 | 2.04 | 0.06 | 0.29 | 0.11 | 0.46 | 0.75 | 0.60 | 0.89 |
| | | Diameter 10 | 3.51 | 0.11 | 3.04 | 0.11 | 0.46 | 0.15 | 0.78 | 0.72 | 0.57 | 0.87 |
| | | Diameter 13 | 2.53 | 0.07 | 2.27 | 0.07 | 0.26 | 0.05 | 0.48 | 0.69 | 0.54 | 0.84 |
| | | Diameter 15 | 3.53 | 0.12 | 3.08 | 0.12 | 0.45 | 0.11 | 0.79 | 0.70 | 0.55 | 0.85 |
| | | Diameter 17 | 2.65 | 0.09 | 2.38 | 0.09 | 0.27 | 0.02 | 0.52 | 0.62 | 0.45 | 0.78 |
| | | Diameter 20 | 2.32 | 0.07 | 2.07 | 0.07 | 0.25 | 0.06 | 0.45 | 0.67 | 0.52 | 0.83 |
| | | Diameter 22 | 2.75 | 0.08 | 2.44 | 0.08 | 0.31 | 0.08 | 0.54 | 0.69 | 0.54 | 0.84 |
| | | Diameter 25 | 2.37 | 0.06 | 2.14 | 0.06 | 0.23 | 0.07 | 0.39 | 0.70 | 0.55 | 0.85 |

**Notes**: Sample size for validity analysis was N = 12 AMD–HC pairs, as measurements outside predefined physiological limits were automatically excluded by the algorithm. PDTI XX–YY denotes the pupil diameter–time integral from XX to YY s (mm·s); constriction velocities are expressed as negative values. Comparison of patients with age-related macular degeneration (AMD) and age-matched healthy controls (HC), including receiver operating characteristic (ROC) analysis. Results are given as mean values with standard error (SE) and 95% confidence intervals (CI). The number of AMD–HC pairs is shown in the Eye column. By convention, constriction velocities are negative.

**Table 3** Reliability of the Bulbicam Tests

| Pupil test | Eye | Variable | Measure 1 | | Measure 2 | | Measure 1 – Measure 2 | | | ICC | AI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Mean | SE | Mean | Lower 95% CI | Higher 95% CI | | |
| *Pupil diameter-time integral (PDTI) (mm s)* | Worst Eye (N = 12) | PDTI 00_03 | 9.81 | 0.47 | 9.53 | 0.47 | 0.275 | −1.11 | 1.66 | 0.92 | 0.86 |
| | | PDTI 03_05 | 5.10 | 0.25 | 4.96 | 0.25 | 0.135 | −0.60 | 0.87 | 0.92 | 0.86 |
| | | PDTI 05_08 | 8.74 | 0.41 | 8.74 | 0.41 | −0.002 | −1.21 | 1.20 | 0.96 | 0.91 |
| | | PDTI 08_10 | 5.34 | 0.26 | 5.17 | 0.26 | 0.170 | −0.59 | 0.93 | 0.95 | 0.89 |
| | | PDTI 10_13 | 9.44 | 0.42 | 9.10 | 0.42 | 0.345 | −0.87 | 1.56 | 0.96 | 0.91 |
| | | PDTI 13_15 | 5.52 | 0.28 | 5.20 | 0.28 | 0.321 | −0.51 | 1.15 | 0.95 | 0.88 |
| | Best Eye (N = 12) | PDTI 00_03 | 9.91 | 0.50 | 9.54 | 0.50 | 0.372 | −1.09 | 1.84 | 0.95 | 0.88 |
| | | PDTI 03_05 | 5.14 | 0.31 | 4.85 | 0.31 | 0.284 | −0.61 | 1.17 | 0.81 | 0.72 |
| | | PDTI 05_08 | 8.79 | 0.50 | 8.51 | 0.50 | 0.285 | −1.18 | 1.75 | 0.88 | 0.80 |
| | | PDTI 08_10 | 5.48 | 0.28 | 5.16 | 0.28 | 0.324 | −0.49 | 1.13 | 0.93 | 0.86 |
| | | PDTI 10_13 | 9.55 | 0.42 | 9.18 | 0.42 | 0.371 | −0.86 | 1.60 | 0.96 | 0.91 |
| | | PDTI 13_15 | 5.66 | 0.30 | 5.18 | 0.30 | 0.480 | −0.39 | 1.35 | 0.90 | 0.82 |
| *Latency (ms)* | Worst Eye (N = 12) | Latency 03 | 233 | 5.2 | 232 | 5.2 | 0.8 | −14.3 | 15.8 | 0.38 | 0.82 |
| | | Latency 08 | 255 | 6.2 | 257 | 6.2 | −1.8 | −19.9 | 16.3 | 0.89 | 0.92 |
| | | Latency 13 | 210 | 13.1 | 231 | 13.1 | −20.7 | −58.8 | 17.4 | −0.40 | 0.29 |
| | Best Eye (N = 12) | Latency 03 | 229 | 4.7 | 227 | 4.7 | 1.9 | −11.8 | 15.7 | −0.35 | 0.76 |
| | | Latency 08 | 255 | 9.7 | 267 | 9.7 | −11.8 | −40.2 | 16.5 | 0.22 | 0.67 |
| | | Latency 13 | 235 | 5.3 | 224 | 5.3 | 11.1 | −4.5 | 26.7 | 0.60 | 0.86 |
| *Peak velocity (mm/s)* | Worst Eye (N = 12) | First PV | −3.70 | 0.12 | −3.78 | 0.12 | 0.079 | −0.28 | 0.44 | 0.85 | 0.87 |
| | | Second PV | −3.43 | 0.13 | −3.35 | 0.13 | −0.078 | −0.46 | 0.30 | 0.63 | 0.76 |
| | | Third PV | −2.59 | 0.14 | −2.56 | 0.14 | −0.028 | −0.45 | 0.40 | 0.86 | 0.79 |
| | Best Eye (N = 12) | First PV | −3.47 | 0.20 | −3.73 | 0.20 | 0.263 | −0.33 | 0.86 | 0.48 | 0.58 |
| | | Second PV | −3.26 | 0.20 | −3.38 | 0.20 | 0.129 | −0.46 | 0.72 | 0.32 | 0.49 |
| | | Third PV | −2.51 | 0.20 | −2.68 | 0.20 | 0.163 | −0.42 | 0.75 | 0.31 | 0.35 |

(*Continued*)

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**Table 3** (Continued).

| Pupil test | Eye | Variable | Measure 1 | | Measure 2 | | Measure 1 – Measure 2 | | | ICC | AI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Mean | SE | Mean | Lower 95% CI | Higher 95% CI | | |
| *Diameter (mm)* | Worst | Diameter 03 | 2.79 | 0.16 | 2.79 | 0.16 | −0.002 | −0.47 | 0.46 | 0.93 | 0.87 |
| | Eye | Diameter 05 | 3.61 | 0.16 | 3.53 | 0.16 | 0.084 | −0.38 | 0.55 | 0.95 | 0.90 |
| | (N = 12) | Diameter 08 | 2.27 | 0.09 | 2.29 | 0.09 | −0.027 | −0.30 | 0.25 | 0.88 | 0.86 |
| | | Diameter 10 | 3.49 | 0.17 | 3.43 | 0.17 | 0.065 | −0.43 | 0.56 | 0.97 | 0.92 |
| | | Diameter 13 | 2.56 | 0.11 | 2.45 | 0.11 | 0.109 | −0.22 | 0.43 | 0.93 | 0.88 |
| | | Diameter 15 | 3.56 | 0.16 | 3.42 | 0.16 | 0.143 | −0.32 | 0.61 | 0.98 | 0.93 |
| | | Diameter 17 | 2.68 | 0.14 | 2.57 | 0.14 | 0.111 | −0.30 | 0.53 | 0.88 | 0.81 |
| | | Diameter 20 | 2.34 | 0.10 | 2.28 | 0.10 | 0.065 | −0.23 | 0.36 | 0.92 | 0.87 |
| | | Diameter 22 | 2.76 | 0.13 | 2.65 | 0.13 | 0.112 | −0.27 | 0.50 | 0.90 | 0.84 |
| | | Diameter 25 | 2.39 | 0.09 | 2.33 | 0.09 | 0.053 | −0.21 | 0.32 | 0.94 | 0.91 |
| | Best | Diameter 03 | 2.82 | 0.15 | 2.72 | 0.15 | 0.094 | −0.36 | 0.55 | 0.9 | 0.84 |
| | Eye | Diameter 05 | 3.63 | 0.17 | 3.54 | 0.17 | 0.092 | −0.41 | 0.60 | 0.96 | 0.91 |
| | (N = 12) | Diameter 08 | 2.39 | 0.10 | 2.33 | 0.10 | 0.065 | −0.24 | 0.37 | 0.82 | 0.82 |
| | | Diameter 10 | 3.57 | 0.18 | 3.47 | 0.18 | 0.098 | −0.42 | 0.62 | 0.98 | 0.93 |
| | | Diameter 13 | 2.62 | 0.11 | 2.47 | 0.11 | 0.159 | −0.17 | 0.49 | 0.90 | 0.85 |
| | | Diameter 15 | 3.59 | 0.17 | 3.44 | 0.17 | 0.149 | −0.35 | 0.65 | 0.98 | 0.92 |
| | | Diameter 17 | 2.74 | 0.15 | 2.61 | 0.15 | 0.132 | −0.30 | 0.56 | 0.89 | 0.81 |
| | | Diameter 20 | 2.41 | 0.11 | 2.29 | 0.11 | 0.117 | −0.21 | 0.45 | 0.88 | 0.83 |
| | | Diameter 22 | 2.76 | 0.13 | 2.69 | 0.13 | 0.076 | −0.30 | 0.45 | 0.95 | 0.90 |
| | | Diameter 25 | 2.44 | 0.09 | 2.33 | 0.09 | 0.106 | −0.16 | 0.38 | 0.92 | 0.85 |

**Note**. Reliability analyses included 12 eyes per condition, as measurements outside predefined physiological limits were automatically excluded by the algorithm. Diameter variables are subject to a one-step indexing offset in the software; this affects labelling only, as all data are correctly time-locked. PDTI XX–YY represents the pupil diameter–time integral from XX to YY s (mm·s). Repeated-measure agreement across sessions in eyes with age-related macular degeneration (AMD) and matched controls. Reliability is expressed as the intraclass correlation coefficient (ICC) and the within-patient agreement index (AI). Standard error (SE) represents variability across repeated samples. By convention, constriction velocities are negative.

**Table 4** Stability of Bulbicam Tests

| Pupil test | Eye | Variable | M1 | M2 | M3 | M4 | M5 | M6 | SI | | | Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Mean | Lower 95% CI | Higher 95% CI | |
| *Pupil diameter-time integral (PDTI) (mm s)* | Worst Eye (N = 12) | PDTI 00_03 | 9.8 | 9.5 | 9.1 | 9.3 | 9.1 | 9.5 | 0.69 | 0.59 | 0.80 | Excellent |
| | | PDTI 03_05 | 5.1 | 5.0 | 4.8 | 4.8 | 4.6 | 4.9 | 0.68 | 0.54 | 0.81 | Excellent |
| | | PDTI 05_08 | 8.7 | 8.7 | 8.5 | 8.6 | 8.4 | 8.8 | 0.77 | 0.70 | 0.85 | Excellent |
| | | PDTI 08_10 | 5.3 | 5.2 | 5.0 | 5.0 | 4.9 | 5.2 | 0.67 | 0.60 | 0.74 | Excellent |
| | | PDTI 10_13 | 9.4 | 9.1 | 8.9 | 9.0 | 8.6 | 9.2 | 0.72 | 0.66 | 0.78 | Excellent |
| | | PDTI 13_15 | 5.5 | 5.2 | 5.3 | 5.3 | 5.1 | 5.4 | 0.74 | 0.67 | 0.81 | Excellent |
| | Best Eye (N = 12) | PDTI 00_03 | 9.9 | 9.5 | 9.1 | 9.4 | 9.2 | 9.6 | 0.72 | 0.64 | 0.80 | Excellent |
| | | PDTI 03_05 | 5.1 | 4.9 | 4.8 | 4.8 | 4.7 | 5.0 | 0.65 | 0.51 | 0.79 | Excellent |
| | | PDTI 05_08 | 8.8 | 8.5 | 8.4 | 8.6 | 8.5 | 9.0 | 0.73 | 0.62 | 0.84 | Excellent |
| | | PDTI 08_10 | 5.6 | 5.2 | 4.9 | 5.0 | 5.0 | 5.3 | 0.62 | 0.52 | 0.73 | Excellent |
| | | PDTI 10_13 | 9.6 | 9.2 | 8.9 | 8.9 | 8.7 | 9.3 | 0.68 | 0.57 | 0.78 | Excellent |
| | | PDTI 13_15 | 5.7 | 5.2 | 5.2 | 5.2 | 5.2 | 5.5 | 0.68 | 0.57 | 0.79 | Excellent |
| *Latency (ms)* | Worst Eye (N = 12) | Latency 03 | 234 | 234 | 233 | 229 | 219 | 217 | 0.20 | −0.07 | 0.45 | Acceptable |
| | | Latency 08 | 256 | 258 | 255 | 257 | 244 | 275 | 0.30 | −0.14 | 0.75 | Good |
| | | Latency 13 | 210 | 231 | 242 | 230 | 217 | 229 | 0.60 | 0.18 | 1.00 | Excellent |
| | Best Eye (N = 12) | Latency 03 | 230 | 228 | 234 | 245 | 229 | 225 | −0.70 | −1.82 | 0.44 | Not acceptable |
| | | Latency 08 | 256 | 268 | 270 | 254 | 258 | 256 | −0.50 | −1.48 | 0.47 | Not acceptable |
| | | Latency 13 | 235 | 224 | 257 | 232 | 223 | 230 | 0.00 | −0.77 | 0.70 | Not acceptable |
| *Peak velocity (mm/s)* | Worst Eye (N = 12) | First PV | −3.7 | −3.8 | −3.2 | −3.6 | −3.7 | −3.6 | 0.10 | −0.46 | 0.65 | Not acceptable |
| | | Second PV | −3.4 | −3.4 | −3.3 | −3.3 | −3.3 | −3.2 | 0.32 | 0.15 | 0.50 | Acceptable |
| | | Third PV | −2.6 | −2.5 | −2.7 | −2.5 | −2.7 | −2.7 | 0.32 | 0.07 | 0.56 | Acceptable |
| | Best Eye (N = 12) | First PV | −3.5 | −3.7 | −3.5 | −3.7 | −3.8 | −3.8 | 0.61 | 0.44 | 0.78 | Excellent |
| | | Second PV | −3.3 | −3.4 | −3.2 | −3.4 | −3.4 | −3.3 | 0.56 | 0.37 | 0.74 | Excellent |
| | | Third PV | −2.5 | −2.7 | −2.6 | −2.5 | −2.6 | −2.8 | 0.28 | 0.00 | 0.58 | Acceptable |

(*Continued*)

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**Table 4** (Continued).

| Pupil test | Eye | Variable | M1 | M2 | M3 | M4 | M5 | M6 | SI | | | Classification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Mean | Lower 95% CI | Higher 95% CI | |
| *Diameter (mm)* | Worst | Diameter 03 | 2.8 | 2.8 | 2.8 | 2.8 | 2.7 | 2.9 | 0.68 | 0.59 | 0.77 | Excellent |
| | Eye | Diameter 05 | 3.6 | 3.5 | 3.4 | 3.5 | 3.3 | 3.5 | 0.73 | 0.63 | 0.83 | Excellent |
| | (N = 12) | Diameter 08 | 2.3 | 2.3 | 2.2 | 2.3 | 2.2 | 2.4 | 0.66 | 0.54 | 0.79 | Excellent |
| | | Diameter 10 | 3.5 | 3.4 | 3.4 | 3.3 | 3.2 | 3.5 | 0.77 | 0.71 | 0.83 | Excellent |
| | | Diameter 13 | 2.6 | 2.5 | 2.4 | 2.5 | 2.4 | 2.6 | 0.73 | 0.65 | 0.81 | Excellent |
| | | Diameter 15 | 3.6 | 3.4 | 3.4 | 3.4 | 3.3 | 3.5 | 0.76 | 0.69 | 0.83 | Excellent |
| | | Diameter 17 | 2.7 | 2.6 | 2.7 | 2.6 | 2.6 | 2.7 | 0.69 | 0.60 | 0.78 | Excellent |
| | | Diameter 20 | 2.3 | 2.3 | 2.3 | 2.3 | 2.2 | 2.4 | 0.69 | 0.60 | 0.78 | Excellent |
| | | Diameter 22 | 2.8 | 2.7 | 2.6 | 2.6 | 2.6 | 2.7 | 0.70 | 0.61 | 0.79 | Excellent |
| | | Diameter 25 | 2.4 | 2.3 | 2.3 | 2.3 | 2.3 | 2.4 | 0.67 | 0.56 | 0.77 | Excellent |
| | Best | Diameter 03 | 2.9 | 2.8 | 2.7 | 2.8 | 2.6 | 2.9 | 0.63 | 0.52 | 0.74 | Excellent |
| | Eye | Diameter 05 | 3.6 | 3.5 | 3.4 | 3.5 | 3.2 | 3.5 | 0.76 | 0.66 | 0.86 | Excellent |
| | (N = 12) | Diameter 08 | 2.4 | 2.3 | 2.2 | 2.3 | 2.2 | 2.4 | 0.66 | 0.50 | 0.81 | Excellent |
| | | Diameter 10 | 3.5 | 3.4 | 3.4 | 3.4 | 3.3 | 3.5 | 0.75 | 0.68 | 0.81 | Excellent |
| | | Diameter 13 | 2.6 | 2.5 | 2.4 | 2.5 | 2.5 | 2.7 | 0.69 | 0.58 | 0.80 | Excellent |
| | | Diameter 15 | 3.6 | 3.4 | 3.4 | 3.4 | 3.4 | 3.5 | 0.73 | 0.66 | 0.81 | Excellent |
| | | Diameter 17 | 2.7 | 2.6 | 2.7 | 2.6 | 2.6 | 2.8 | 0.71 | 0.62 | 0.79 | Excellent |
| | | Diameter 20 | 2.4 | 2.3 | 2.3 | 2.3 | 2.2 | 2.4 | 0.70 | 0.57 | 0.83 | Excellent |
| | | Diameter 22 | 2.8 | 2.7 | 2.6 | 2.6 | 2.6 | 2.7 | 0.74 | 0.66 | 0.81 | Excellent |
| | | Diameter 25 | 2.4 | 2.3 | 2.3 | 2.3 | 2.3 | 2.4 | 0.66 | 0.54 | 0.77 | Excellent |

**Notes**: For validity, complete AMD–HC pairs were required; one pair was excluded (N=12). Some SI values extend outside [0,1] due to sampling variability; see *Methods* for details. PDTI XX–YY represents the pupil diameter–time integral from XX to YY s (mm·s). Six repeated measurements (M1–M6) are shown as mean values. Stability is expressed by the Stability Index (SI) with 95% confidence intervals (CI), and classification is based on SI thresholds. By convention, constriction velocities are negative.


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**Table 5** Biomarker Classification

| Pupil test | Eye | Variable | Patient-HC 95% CI | ROC/ Anova | Repeatability | | Relia-bility | Stabi-lity | Biomarker Classification | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Excludes zero | Pat vs HC | Between | Within | | | Popu-lation | Patient |
| *Pupil diameter-time integral (PDTI) (mm s)* | Worst Eye (N = 13) | PDTI 00_03 | + | + | + | + | + | + | + | + |
| | | PDTI 03_05 | + | + | + | + | + | + | + | + |
| | | PDTI 05_08 | + | + | + | + | + | + | + | + |
| | | PDTI 08_10 | + | + | + | + | + | + | + | + |
| | | PDTI 10_13 | + | + | + | + | + | + | + | + |
| | | PDTI 13_15 | + | + | + | + | + | + | + | + |
| | Best Eye (N = 13) | PDTI 00_03 | + | + | + | + | + | + | + | + |
| | | PDTI 03_05 | + | + | + | + | + | + | + | + |
| | | PDTI 05_08 | - | + | + | + | + | + | - | - |
| | | PDTI 08_10 | + | + | + | + | + | + | + | + |
| | | PDTI 10_13 | + | + | + | + | + | + | + | + |
| | | PDTI 13_15 | + | + | + | + | + | + | + | + |
| *Latency (ms)* | Worst Eye (N = 13) | Latency 03 | - | - | - | + | - | + | - | - |
| | | Latency 08 | - | - | + | + | + | + | - | - |
| | | Latency 13 | - | - | - | + | - | + | - | - |
| | Best Eye (N = 13) | Latency 03 | - | - | - | + * | - | - | - | - |
| | | Latency 08 | - | - | - | + * | - | - | - | - |
| | | Latency 13 | - | - | + | + * | + | - | - | - |
| *Peak velocity (mm/s)* | Worst Eye (N = 12) | First PV | + | + | + | + * | + | - | + | + |
| | | Second PV | - | - | + | + | + | + | - | - |
| | | Third PV | - | - | + | + | + | + | - | - |
| | Best Eye (N = 12) | First PV | - | - | - | + | - | + | - | - |
| | | Second PV | - | - | - | + | - | + | - | - |
| | | Third PV | - | - | - | + | - | + | - | - |

(*Continued*)

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**Table 5** (Continued).

| Pupil test | Eye | Variable | Patient-HC 95% CI | ROC/ Anova | Repeatability | | Relia-bility | Stabi-lity | Biomarker Classification | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Excludes zero | Pat vs HC | Between | Within | | | Popu-lation | Patient |
| *Diameter (mm)* | Worst Eye (N = 13) | Diameter 03 | + | + | + | + | + | + | + | + |
| | | Diameter 05 | + | + | + | + | + | + | + | + |
| | | Diameter 08 | + | + | + | + | + | + | + | + |
| | | Diameter 10 | + | + | + | + | + | + | + | + |
| | | Diameter 13 | - | + | + | + | + | + | + | + |
| | | Diameter 15 | + | + | + | + | + | + | + | + |
| | | Diameter 17 | - | - | + | + | + | + | - | - |
| | | Diameter 20 | + | + | + | + | + | + | + | + |
| | | Diameter 22 | - | + | + | + | + | + | + | + |
| | | Diameter 25 | - | + | + | + | + | + | + | + |
| | Best Eye (N = 13) | Diameter 03 | + | + | + | + | + | + | + | + |
| | | Diameter 05 | + | + | + | + | + | + | + | + |
| | | Diameter 08 | + | + | + | + | + | + | + | + |
| | | Diameter 10 | + | + | + | + | + | + | + | + |
| | | Diameter 13 | + | + | + | + | + | + | + | + |
| | | Diameter 15 | + | + | + | + | + | + | + | + |
| | | Diameter 17 | + | + | + | + | + | + | + | + |
| | | Diameter 20 | + | + | + | + | + | + | + | + |
| | | Diameter 22 | + | + | + | + | + | + | + | + |
| | | Diameter 25 | + | + | + | + | + | + | + | + |

**Notes**: For validity, complete AMD–HC pairs were required; one pair was excluded (N = 12). PDTI XX–YY represents the pupil diameter–time integral from XX to YY s (mm·s). A "+" sign indicates that the variable satisfies predefined criteria for a viable biomarker (eg, between-group differentiation, intra-individual repeatability); "–" indicates the criteria were not met. An asterisk (*) indicates the variable is not stable For validity, criteria were satisfied if *either* the 95% CI for the AMD–HC difference excluded zero *or* ROC/ANOVA analysis was significant (AUC lower CI > 0.50 or $p < 0.05$). Concordant evidence from both strengthened confidence but was not required for classification.

was "Not acceptable" (SI ≤ 0.14, Table 4). The second and third PPV variables failed validity in both eyes (Table 2). Thus, only 1 of 6 velocity measures is a candidate biomarker, with limited robustness for longitudinal use (Table 5).

Diameter variables: In the worst eye, 9 of 10 diameter variables discriminated AMD from HC (Table 2: $p \leq 0.05$; AUC lower CI > 0.50). Diameter 17 failed (AMD–HC CI crossed zero; AUC lower CI ≤ 0.50). Diameters 22 and 25 had marginal CIs but were retained because ROC analyses confirmed discrimination. In the best eye, all 10 diameter variables achieved validity. Repeatability (Table 3) was strong for both between-patient (ICC > 0.5) and within-patient (AI > 0.5) measures. Stability was classified as "Excellent" across both eyes (Table 4). Thus, 19 of 20 diameter variables were accepted as reliable biomarkers at both levels, with only Diameter 17 excluded (Table 5).

Latency variables: None of the three latency measures in either eye discriminated AMD from HC (Table 2: all $p > 0.05$; AUC lower CI including 0.50). Although some showed repeatability (eg, Latency 08 in the worst eye, Latency 13 in the best eye; Table 3), validity was not met. Since validity is required for biomarker classification, all six latency variables were excluded (Table 5).

Overall summary: Across all 44 candidate variables (12 PDTI, 6 Velocity, 20 Diameter, 6 Latency), 30 qualified as diagnostic and monitoring biomarkers. These included 11 PDTI variables and 19 Diameter variables, which consistently demonstrated validity, repeatability, and stability. One Velocity variable (first PPV, worst eye) is considered a tentative biomarker but with limited stability. No latency variables qualified.

# Discussion

This study demonstrates that dynamic pupillometry can detect consistent functional differences between AMD patients and healthy controls. PDTI and diameter were significantly larger in AMD. Comparable alterations in baseline diameter have been observed previously in AMD using lower-frequency pupillometry.[12,14] The first peak velocity was reduced in AMD, but only in the worst eye, and showed limited stability across sessions. Other velocity measures were not valid, whereas latencies were largely preserved. Taken together, these findings indicate that macular disease alters the magnitude and dynamics of pupillary constriction without delaying its onset, identifying PDTI and diameter as the most robust functional biomarkers.

The combined pattern of larger steady-state diameter, higher PDTI, and reduced first peak velocity is consistent with diminished phasic constrictor drive and preserved reflex latency.[12,14] Under photopic conditions, this pattern accords with reduced cone-mediated input to pupil-projecting retinal ganglion cells, reflecting macular dysfunction in AMD. The preserved latency suggests that afferent transmission within the pupillary light reflex pathway remains intact, whereas the reduced constriction amplitude indicates attenuated retinal output rather than delayed conduction.

## Strengths and Limitations

A strength of this study is the use of high-frequency video-oculography with repeated measurements, which allowed precise capture of small pupillary changes and robust estimates of intra- and inter-patient variability. Masking of AMD grading until after testing minimized bias, and the structured stimulus sequence ensured reproducible test conditions.

Limitations include the relatively small sample size and the cross-sectional design, which precluded analysis of longitudinal change. Medication use that could affect autonomic tone was not controlled, although such effects are likely evenly distributed between groups. Only white-light stimulation was applied, limiting separation of cone- and melanopsin-driven responses, and choroidal contributions were not directly measured. Finally, the study relied primarily on absolute pupil size rather than constriction amplitudes or full waveform metrics, which may also contain clinically relevant information. An indexing artefact in the software labelled diameter variables one step offset from their true timepoints. This did not affect analyses, as all data were time-locked correctly, but we note it here for clarity.

## Future Directions

Larger, longitudinal cohorts are needed to establish whether pupillary alterations predict AMD progression or correlate with visual outcomes. Chromatic stimuli and macula-targeted stimulus fields could help disentangle cone, rod, and melanopsin contributions. Future work should also test whether these metrics can detect subclinical AMD changes or monitor treatment efficacy.

## Clinical Perspective

Dynamic pupillometry provides a rapid, non-invasive, and objective functional assessment that complements OCT and visual acuity. The BCAM platform delivers results in under three minutes without dilation or darkroom requirements and can be run by trained technicians, making it feasible for outpatient screening or monitoring.

# Conclusions

The combination of PDTI, diameter, and peak velocity offers a novel set of functional biomarkers for detecting and monitoring AMD. These variables demonstrate high repeatability, stability, and differentiation power, rendering them promising non-invasive metrics for assessing AMD-related changes in autonomic pupillary function. Future longitudinal studies will be crucial in determining their predictive value for disease progression and treatment response. Large language models (ChatGPT, OpenAI) were used to assist the lead author during manuscript preparation, including text drafting, structural editing, and literature search support. All analytical content, interpretations, and conclusions were developed, verified, and approved by the authors.

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# Disclosure

Bjørn A. Helland-Hansen is employed as Chief Medical Officer at Bulbitech AS, reports grants from Norwegian Research Council, and owns shares, as well as IP (patent: US20240335111A1 – "Eye Testing Device"). Goran Petrovski serves on the Advisory Board of Bulbitech AS on a *pro bono* basis. The authors report no other conflicts of interest in this work.

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