Supplemental Material

Handheld Chromatic Pupillometry can Accurately and Rapidly Reveal Functional Loss in Glaucoma

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This online-only supplement includes:

Supplementary Methods:
Supplementary Methods 1. Details on ophthalmic examinations
Supplementary Methods 2. Design, operation and calibration of the handheld pupillometer
Supplementary Methods 3. Sample size calculation

Supplementary Figures:
Supplementary Figure 1. Mechanical design of the handheld chromatic pupillometer.
Supplementary Figure 2. Relative contribution of pupillometric, demographic and clinical features for the detection of glaucoma assessed using a gradient boosting machine (GBM) technique.
Supplementary Figure 3. Changes in the pupillary light response as a function of the severity of glaucoma.

Supplementary Tables:
Supplementary Table 1. Definitions of extracted pupillometric features.
Supplementary Table 2. Differences in main pupillometric features between controls and patients with glaucoma.
Supplementary Table 3. Classification performance of HCP.

Supplementary References

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

Supplementary Methods

Supplementary Methods 1. Details on ophthalmic examinations

All control participants underwent a comprehensive ophthalmological evaluation that included slit-lamp and gonioscopic examinations, best corrected visual acuity (BCVA) (LogMAR chart, Lighthouse Int., NY, USA), automated refraction (Canon RK 5 Auto Ref-Keratometer, Canon, Tochigiken, Japan), color vision testing (Ishihara plates, Kanehara & Co., Tokyo, Japan), intra-ocular pressure (IOP) measurement using Goldmann applanation tonometry, and peripapillary imaging using high definition OCT (Cirrus version 6.0, Carl Zeiss Meditec, Dublin, CA, USA). OCT results were validated only if the recording's signal strength was 6 or better. Participants also underwent SAP (Humphrey Visual Field (HVF) analyzer II model 750, Carl Zeiss Meditec, Dublin, CA, USA) with near refractive correction using the 24-2 Swedish Interactive Thresholding Algorithm (SITA) with stimulus size III. SAP testing was repeated if false positive or false negative rates exceeded 33%, or if the fixation loss rate was greater than 20%. Controls were defined as having an IOP less than 21 mmHg with open angles (on gonioscopy) in all quadrants, healthy optic nerves and normal visual fields. Patients with clinically confirmed glaucoma underwent the same battery of ophthalmological assessments as part of their clinical examination and follow-up, and were diagnosed by a fellowship trained specialist by the presence of glaucomatous optic neuropathy (loss of neuroretinal rim with a vertical cup-disc ratio of >0.7 and/or notching with nerve fibre layer defect attributable to glaucoma) with compatible visual field defect. Patients with an unconfirmed glaucoma diagnosis were excluded from this study.

Supplementary Methods 2. Design, operation and calibration of the handheld pupillometer

The handheld pupillometer is a lightweight system (~ 300g) designed to be ergonomic for self-administration and use in clinics (Supplementary Fig.1). Its rounded posterior handgrip allowed for comfortable handling, and its anterior silicone rubber eye cup allowed for light-isolation and comfortable positioning on the participant's orbital rim, covering the eye (Supplementary Fig.1). The eye cup is removable for sterilization and rotatable to be adapted to a participant's left or right eye. Light stimulations covering 50 degrees of the visual field (without considering any reflections off the interior of the device) were delivered using red-green-blue (RGB) light emitting diodes (LEDs) and were diffused by 2 diffusers before reaching the eye of the participant. The study eye of each participant was recorded under infra-red (IR) illumination, using an embedded camera that was pre-focused to capture the pupil and iris clearly in all participants, and fitted with an IR filter. The camera was oriented at a ~60 degree angle below the lower eyelid to reduce the potential impact of ptosis on measurement. The LEDs and camera were controlled using a Raspberry PI Zero-W single board computer (Raspberry Pi Foundation, UK) and powered by a rechargeable 3.7V lithium battery.

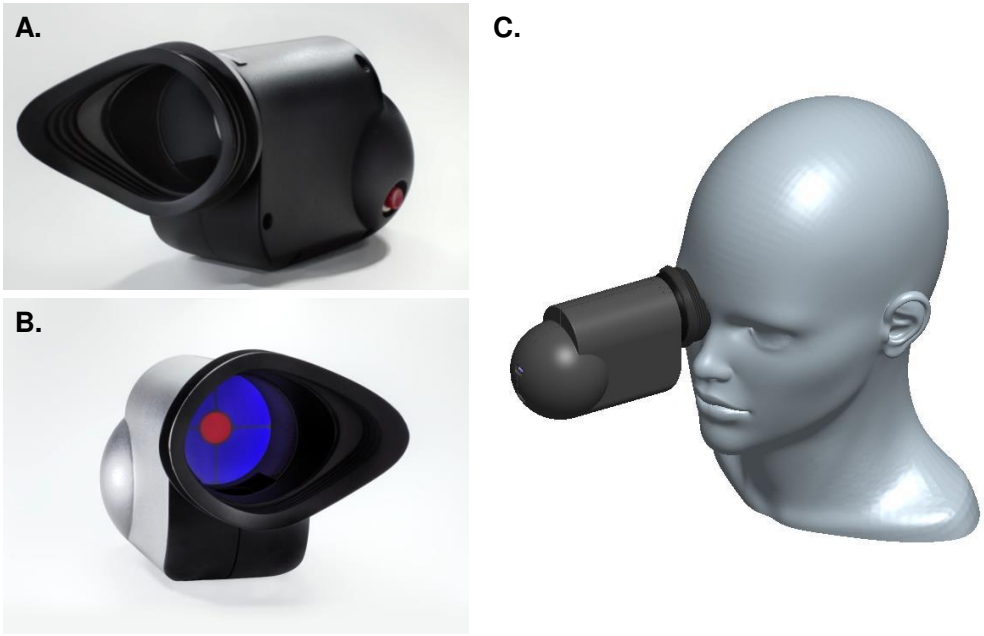
The pupillometer was operated remotely through a tablet (iPad Mini, Apple, CA, USA). A custom-built application allowed for a real-time monitoring of fixation, blinks and pupil detection. The same application was used for post-hoc analysis of the horizontal pupillary radius as a function of time and light stimulus. Whenever the participant could not maintain fixation or blinked excessively, the testing procedure was repeated.

The device's light characteristics (intensity and spectra) were calibrated with the sensor placed at the patient's eye level using a calibrated radiometer (ILT5000, International Light Technologies, Peabody, MA, USA) and spectroradiometer (ILT950, International Light Technologies, Peabody, MA, USA), respectively.

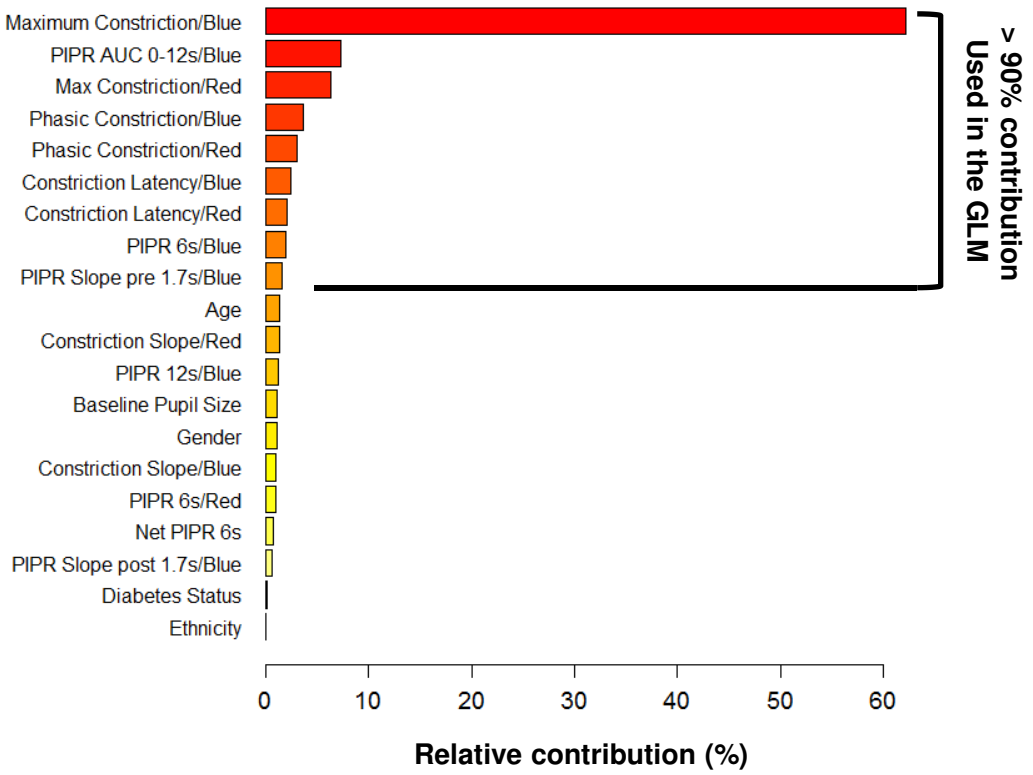
Supplementary Methods 3. Sample size calculation

The intended sample size for this study was calculated as follows: For $\alpha = 0.05$, a balanced disease prevalence of 50% and a predicted sensitivity and specificity of 90%, the adequate sample size of participants (controls and glaucoma) to achieve adequate HCP sensitivity and specificity assessment with a maximum marginal error of 5%, was 277 participants.[1] Taking into account a predicted 20% exclusion rate of healthy controls and a higher exclusion rate (60%) in consecutively recruited patients from the glaucoma clinics, we targeted a sample of 387 participants to be screened including 166 potential controls and 221 potential glaucoma patients.

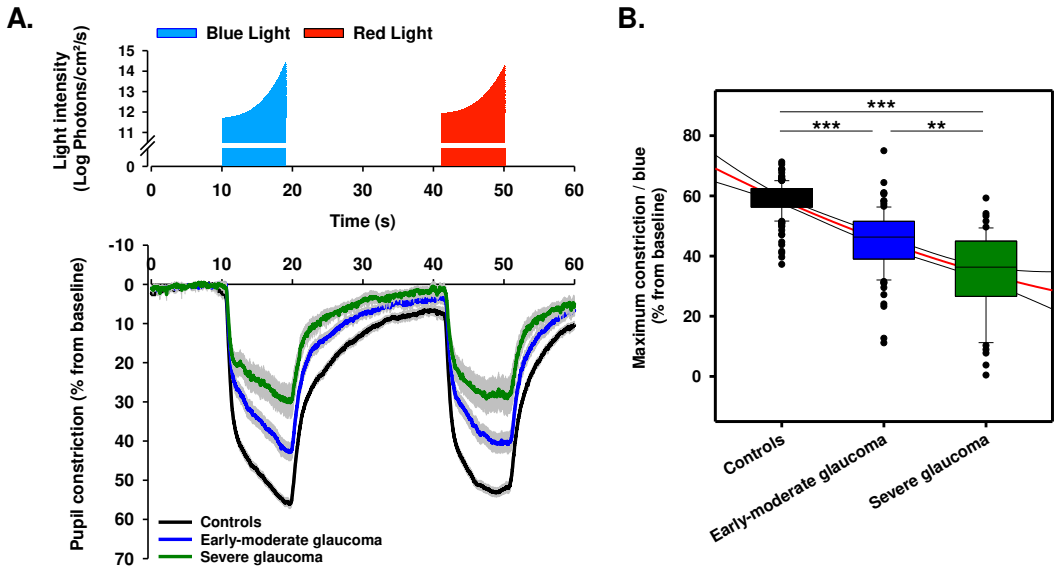
Supplementary Figures



Supplementary Figure 1. Mechanical design of the handheld chromatic pupillometer. A. Photograph showing the overall shape of the device and eye cup. **B.** Photograph showing one of diverse light stimulation paradigms (central, peripheral, quadrants and full field) allowed by the device. **C.** Illustration simulating a monocular self-administration of the pupillometric examination procedure by a patient. The fellow eye is typically covered by the patient's hand which is not shown here for illustration clarity purposes. All rights for the photographs and illustration shown in this figure belong to the Singapore Eye Research Institute.



Supplementary Figure 2. Relative contribution of pupillometric, demographic and clinical features for the detection of glaucoma assessed using a gradient boosting machine (GBM) technique. The variables with the highest contribution to the classification model were maximum constriction to blue light (62.2%), PIPR AUC 0-12s (7.3%) and maximum constriction to red light (6.3%). Age and gender contributed 1.4% and 1.1% to the model, respectively, while the relative contributions of diabetes-status and ethnicity were negligible (0 to 0.1%). Top pupillometric contributing features were utilized to develop a generalized linear model for glaucoma classification.



Supplementary Figure 3. Changes in the pupillary light response as a function of the severity of glaucoma. A. Average baseline-adjusted pupillary light responses in patients with early-moderate and severe glaucoma compared to controls. Multiple features of the pupillometric traces were affected by the severity of the disease. **B.** Gradual decrease in the maximum constriction to blue light as a function of the severity of the disease ($H = 182.4$, $P < 0.001$). The red and black lines represent the linear regression (adjusted $R^2 = 0.52$, $P < 0.001$) and 95%CI, respectively. Statistical comparisons between groups were performed using a Kruskal-Wallis One Way ANOVA on Rank. Post-hoc analysis was done pairwise using Dunn's method. **: $P < 0.01$; ***: $P < 0.001$.

Supplementary Tables

Supplementary Table 1. Definitions of extracted pupillometric features.

Pupillometric features (unit)	Definition
Baseline pupil size (pixels)	Median horizontal pupil radius assessed in darkness during 5 seconds prior to blue light onset
Phasic constriction (%)	Median of the baseline-adjusted pupil size 0.5 to 2.5 seconds after light onset
Constriction latency (s)	Time required from light onset for the baseline-adjusted pupillary constriction to reach 10% in amplitude
Constriction slope (%/s)	Slope of gradual pupillary constriction during the last 6 seconds of light exposure
Maximum constriction (%)	Maximum amplitude of constriction at light offset
Slope pre *or* post 1.7s after light offset (%/s)	Slope of pupil redilation before or after 1.7 seconds following blue light offset
PIPR 6s (%)	Median of the baseline-adjusted pupil size 5 to 7 seconds after light offset
PIPR 12s (%)	Median of the baseline-adjusted pupil size 11 to 13 seconds after blue light offset
PIPR AUC 0 – 12 s (%.s)	Area under the pupillary response curve 0 to 12 seconds after blue light offset
Net PIPR 6s (%)	PIPR 6s (blue) – PIPR 6s (red)

Abbreviations: AUC: area under the receiver operating characteristic curve; PIPR: post illumination pupillary response.

Supplementary Table 2. Differences in main pupillometric features between controls and patients with glaucoma.

	Pupillometric features	Control	Glaucoma
Blue light	Constriction latency, s	0.46 (0.17)	0.71 (0.52)***
	Phasic constriction, %	33.7 (10.7)	20.9 (13.5)***
	Maximum constriction, %	59.6 (6.1)	42.5 (14.2)***
	PIPR slope pre 1.7s, %/s	-11.0 (6.0)	-8.3 (7.6)***
	PIPR slope post 1.7s, %/s	-1.3 (0.5)	-0.8 (0.5)***
	PIPR 6s, %	20.5 (7.7)	11.1 (8.7)***
	PIPR AUC 0-12s, %.s	282.3 (71.1)	168.7 (104.7)***
Red light	Constriction latency, s	0.57 (0.25)	0.80 (0.74)***
	Phasic constriction, %	34.2 (10.4)	22.8 (14.8)***
	Maximum constriction, %	56.0 (7.9)	41.0 (16.7)***
	PIPR 6s, %	16.7 (7.7)	10.0 (9.9)***
Overall	Baseline pupil size, pixels	55.5 (13.3)	47.0 (15.0)***
	Net PIPR 6s, %	3.85 (8.2)	1.56 (5.5)***

Data are represented as median (IQR). Statistical comparisons between were performed using a Mann Whitney U test. ***: $P < 0.001$. **Abbreviations: AUC:** area under the receiver operating characteristic curve; **PIPR:** post illumination pupillary response.

Supplementary Table 3. Classification performance of HCP

A. Cross tabulation results

		Reference Standard	
		Glaucoma	Controls
HCP	Glaucoma	131	20
	Controls	18	153

B. Classification performance metrics

	HCP
AUC	0.94 (0.91 - 0.96)
Sensitivity, %	87.9 (81.6 - 92.7)
Specificity, %	88.4 (82.7 - 92.8)
PPV, %[a]	21.8 (15.5 - 29.7)
NPV, %[a]	99.5 (99.2 - 99.7)
Accuracy, %[a]	88.4 (84.4 - 91.7)

Data are represented as average (95%CI). [a] values calculated at a disease prevalence of 3.54%. **Abbreviations: AUC:** area under the receiver operating characteristic curve; **NPV:** negative predictive value; **PPV:** positive predictive value.

Supplementary References

1 Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. *J Biomed Inform* 2014;**48**:193–204. doi:10.1016/j.jbi.2014.02.013