Report on Statistics

Dynamic pupillometry in video-based oculography

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The ability for the pupils to dilate and constrict (open and close) are controlled by the autonomic nervous process, which again is controlling specific muscles around the pupil to relax or contract. This muscular reflex is light dependent. In darkness the pupil dilates and in brightness it constricts. The ocular autonomic nervous system influences several other functions of the eye too, which includes pupil diameter, ocular accommodation (maintaining clear vision), ocular blood flow and intraocular pressure. The 'swinging flashlight test' is a manual and subjective test used to assess and detect a pupil defect, which is called the relative afferent pupil defect. The purpose is to detect differences and responses between the two eyes, and how they respond to light. In this trial we tested whether video-based oculography (VOG) is suitable to be used in a clinical setting to examine the pupillary responses of glaucoma patients. The variables measured were pupil diameters, latencies and velocities during light exposure. The medical product BulbiCAM from Bulbitech AS was used for the trial. The study was designed as a latin square design to test and retest the subjects up to eight times for intra-rater reliability using two BulbiCAMs. Twothird of the subjects were also tested for stability of the devices. Statistical analysis applying the agreement index (AI) for reliability and stability where performed. Our preliminary results indicate that high-frame rate VOG is a promising tool to measure and quantify individual dynamic pupillometry in a clinical setting.

BACKGROUND
The 'swinging flashlight test' is regularly used in an ophthalmic clinic for examination of the Relative Afferent Pupil Defect (RAPD). If RAPD is present in the patient it can be an important indicator in the clinical evaluation. RAPD can be found in both glaucoma and age-related macular degeneration (AMD) patients, or in patients with neurological diseases. The presence of RAPD indicates a reduced afferent input from the retina or from the optic nerve.[1-4] Several studies have researched the use of VOG pupillometry and the accuracy within glaucoma patients. RAPD testing in VOG pupillometry demonstrated a 56% RAPD in the glaucoma population, compared to 29% detection using the swinging flashlight method.[5,6]
To clinically assess the pupils, the pupillary light reflex and its neural pathway is being used, where light is exposed in the eye of the patient. The reflex constricts the pupil in response to the light, to protect the retina, and the opposite happens in darkness, where the pupil opens to let more light enter the retina (figure 1). Pupillary light reflex is used to assess if there is a suspicion on optic nerve lesions, oculomotor nerve injury, autonomic nerve damage, brain stem lesions, as in tumors and some neurological diseases, including certain medications (e.g. barbiturates). The test is also often used as a first examination of the patients, as it often is affected in eye or neurological pathologies. Pupillary abnormalities are then graded in four grades:

Grade I -Initial weak constriction with greater re-dilatation
Grade II -Initial stall (stop) and greater re-dilatation
Grade III -Immediate pupillary dilatation
Grade IV -Immediate pupillary dilatation following 6 seconds of illumination

The golden standard of quantifying pupil RAPD is by using the Neutral Density Filters (NDF). The different filters stop light from reaching the eye and the retina. The swinging flashlight test is then assessed by increasing the density of the filters in front of the healthy eye, to observe the other eye until the abnormal pupil signs disappear. NDF contains 10 filters used to increase density. A log unit ranging from 0.3 to 3.0 in steps of 0.3 is used to quantify the RAPD.

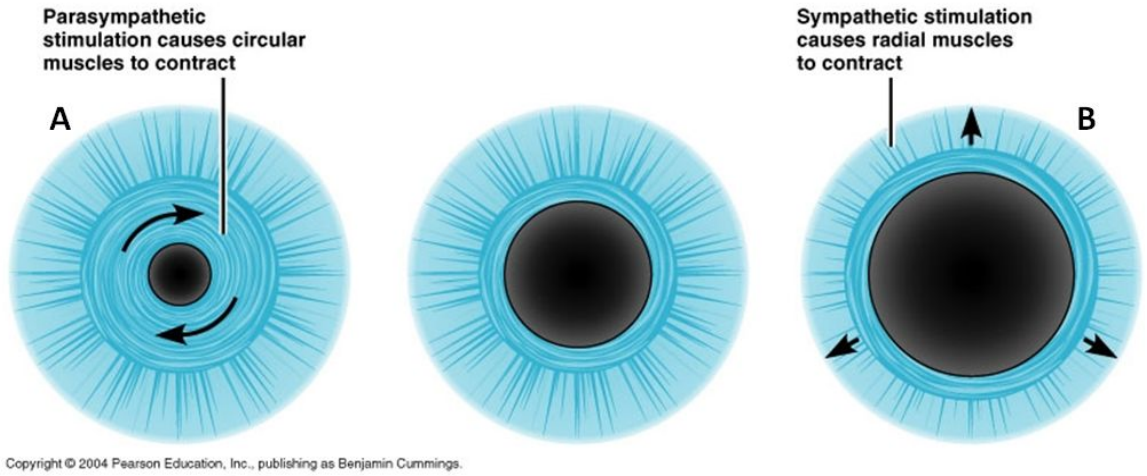


Figure 1 *The light reflex.* ***A:*** *Light exposure to the eye resulting in constriction of the pupil.* ***B:*** *Darkness resulting in dilatation of the pupil. (Pearson Education Inc., publishing as Benjamin Cummings)*

METHOD

In this trial we tested the reproducibility, reliability and the stability of two different BulbiCAMs, without any grading or correlation with a quantifying NDF. Variables measured were pupil diameter during constriction and dilatation phase, pupillary latencies and constriction velocities and how light exposed to one eye responded in a constriction in the other eye.

An orthogonal nested Latin square design was used to assess six glaucoma patients, classified as mild to severe glaucoma, in 3 different age groups. Three normal subjects were used as controls. Due to the stratification in the design a total of at least three subjects was included in the substrata, equally divided on the predefined age and disease stage stratum.

The study was performed at the Medical Research Foundation, Sankara Nethralaya Eye Hospital, Chennai, India. The study follows the Helsinki declaration and was ethically approved by the regional ethical board.

Subjects underwent a test-retest procedure of four times for inter-rater reliability in two different BulbiCAM devices, and two third of the population underwent eight times the test for stability investigation of the devices.

A suspended head mounted VOG device from Bulbitech AS (BulbiCAM) was used. The BulbiCAM is based on a two screen solution, which enables both monocular or binocular stimuli to be presented. In this trial binocular stimulation of the eyes was performed. Eye pupil values were measured with a high-speed digital infrared camera, which measures eye movements at a 400-Hz frame rate. Data was presented in both diameter graphs and in velocity graphs for the two eyes. Diameters, latencies and peak velocities were given in numbers (Figure 2).

On the screens, a picture of a butterfly was presented for the test subject, which would try to avoid blinking during the picture presentation. There was an option to run the test in two sequences, which in total took 50 seconds. Data from the two sequences was exported for statistical analysis. A total of four trials were done, with a total of 8 sequences exported for analysis.

Any patient blinks or smaller computer noise were filtered out from the data, but denoted in the diagram with small red squares in the x-axis of the pupil diameter graph. This also corresponded to the graph of velocity.

A total of eight variables were measured, consisting of six pupil diameters and two latencies. Variables can be seen in table 1.

Table 1: Pupillary variables

	Pupil variables during dynamic pupillometry
D1	The diameter of the right and the left pupil during the dilation phase, measured a few milliseconds before the eyes were exposed to light.
L1	Latency of the right and left pupil at the moment of starting to constrict. This was in the algorithm automatically detected in the velocity graph. Latency is denoted in milliseconds.
D2	The diameter of the right and the left pupil in the constriction phase, measured a few milliseconds before the eyes were exposed to darkness.
L2	Second latency of the right and left pupil at the moment of starting to constrict. Only the left eye was exposed to light.
D3	The diameter of the right and left pupil in the constriction phase. Only the left eye was exposed to light.
D4	The diameter of the right and left pupil in the constriction phase. Only the right eye was exposed to light.
D5	The diameter of the right and left pupil in the constriction phase. Only the left eye was exposed to light.
D6	The diameter of the right and left pupil in the constriction phase. Only the right eye was exposed to light.

Table 1 *Pupil variables explained*

Figure 2 *presenting the pupil diagram for the left and the right eye. Upper graph providing information on pupil dynamics shown in millimetres during light stimulus. Lower graph providing information on velocities on pupil constriction and dilatation. In figures presenting the measured pupil diameter (millimetre), latency (milliseconds) and peak velocity (millimetre/sec). The stimulus that was presented to the eyes (light or dark screens) can be seen on the x-axis.*

RESULTS

Statistical evaluation of the pupil variables was made for investigating the reproducibility, reliability and stability in two BulbiCAM devices.
Statistical evaluation of the variables was statistically evaluated for reliability and stability.

Statistical method
All results on assumed continuously distributed variables are expressed by Mean values, Standard Deviation (SD) and 95% Confidence interval[8]. Comparison of devices was performed by using analysis of variance with repeated measurement[9]. Contingency Table Analysis was used for categorical data [10]. Differences between devices were considered significant if the p-value was less or equal to the level of 5 %. Pair of observations performed on the same patient on two different devices was used for analysis and estimation of device agreement. The mean of the paired observation ($Mean_{pairs}$) was plotted against the mean difference within pairs ($Mean_{diff}$). The results are graphically given by the Bland &Altman agreement plot as $Mean_{diff} \pm 2*SD_{diff}$ against $Mean_{pairs}$ [11,12]. Additionally, the number of outliers and the agreement coefficient $AI = 1 – [2*SD_{diff} / Mean_{pairs}]$ is given[13]. In order to investigate the stability of the devices, the pair of observations in the same patient on two different devices was repeated at least four times. The stability of agreement was analysed using the same procedure as described for the agreement analyse above.

Categorization of the Agreement Index
<0.40 (Poor), [0.40 - 0.60> (moderate), [0.60 - 0.70> (Good). [0.70 - 0.8> (Very good) og >0.80 (Excellent)

The agreement index of the pupil variables between devices for pupil sizes (D1, D2, D3, D4, D5, and D6) for the left eye was 0,80. For the right eye it was 0,87. The stability agreement index of the pupil sizes for the left eye was 0,86. For the right eye it was 0,85.
The agreement index of the pupil latencies for the left eye was 0,91. For the right eye it was 0,86. The stability agreement index of the pupil latencies for the left eye was 0,92. For the right eye it was 0,89.
The agreement index of the pupil peak velocities for the left eye was 0,81. For the right eye it was 0,84. The stability agreement index of the pupil peak velocities for the left eye was 0,85. For the right eye it was 0,83.
In table 2,3,4,5,6 and 7 the agreement index between devices, and the agreement index for stability can be observed. Included are also Altman plots and line of equality.

CONCLUSION
In conclusion, our preliminary results indicate that high-frame-rate VOG, is a promising tool to measure and quantify individual pupil values in a dynamic pupillometry.

The authors declare no conflicts of interest.

Agreement and stability of agreement between devices

2.1: Agreement between devices on pupil size

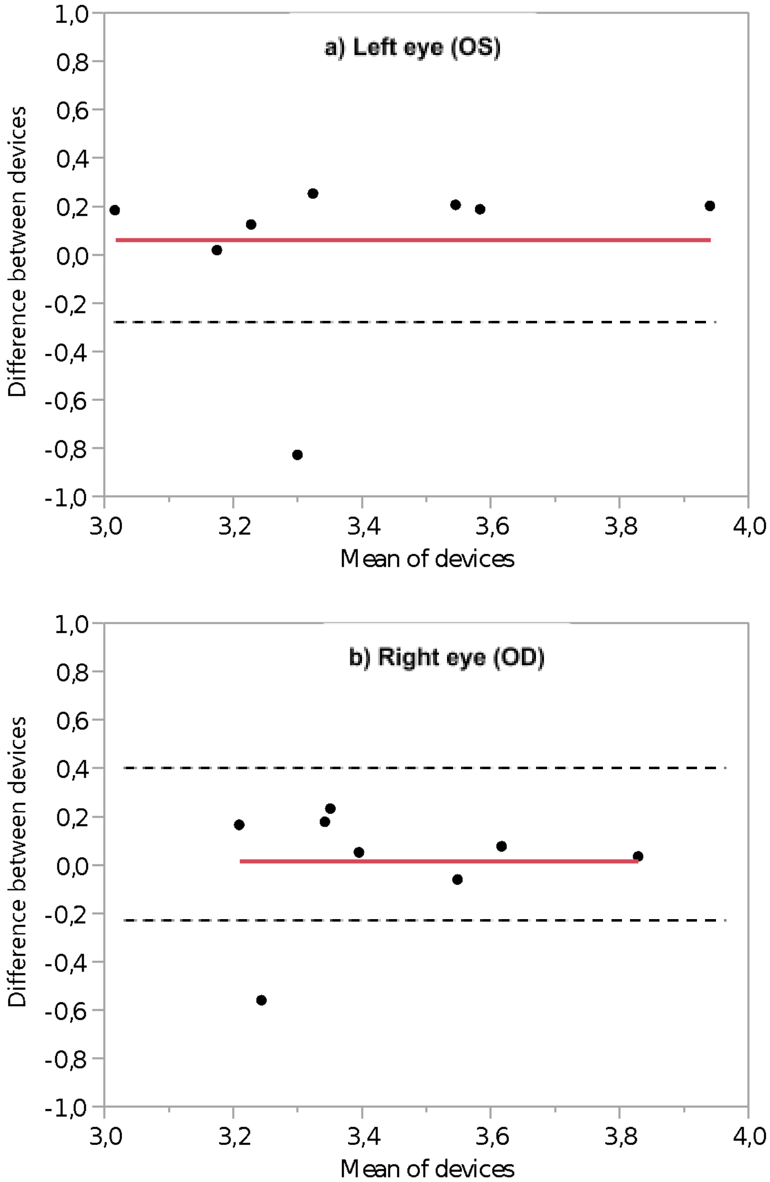
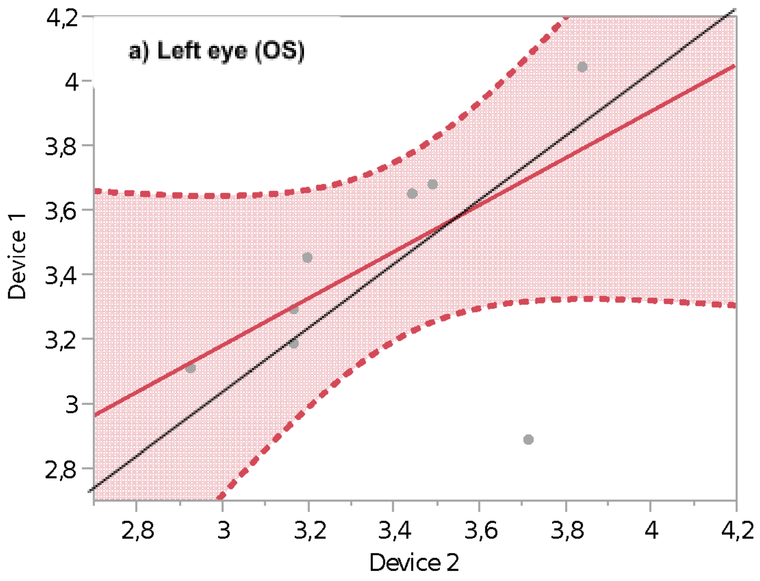


Figure 3: Agreement plot between devices on pupil size measured on a) left eye and b) right eye. The line represents the mean measurement on the two devices, the dotted line the agreement limits and the dots the group of observations



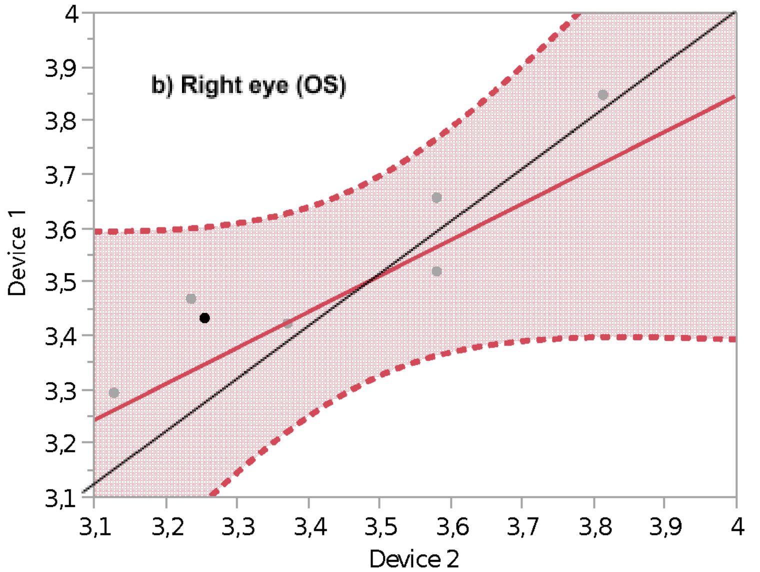
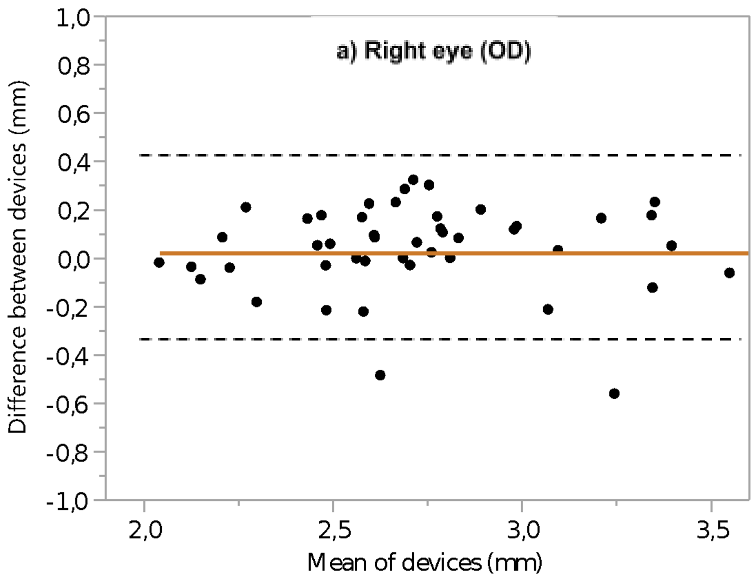


Table 3: Agreement between devices on Pupil size

Variable	Parameters	Estimation
	Mean of measurements (SD)	3.45 (0.32)
	Difference between measurements (SD)	0.06 (0.34)
Pupil size	Agreement Index (AI)	0.80
Left eye	% outliers	1/18=5.6%
	Correlation between measurements	0.59
	Correlation between mean and absolute difference value	-0.06
	Mean of measurements (SD)	3.49 (0.23)
	Difference between measurements (SD)	0.01 (0.23)
Pupil size	Agreement Index (AI)	0.87
Right eye	% outliers	1/18= 5.6%
	Correlation between measurements	0.60
	Correlation between mean and absolute difference value	-0.65

2.2: Stability of agreement between devices on pupil size



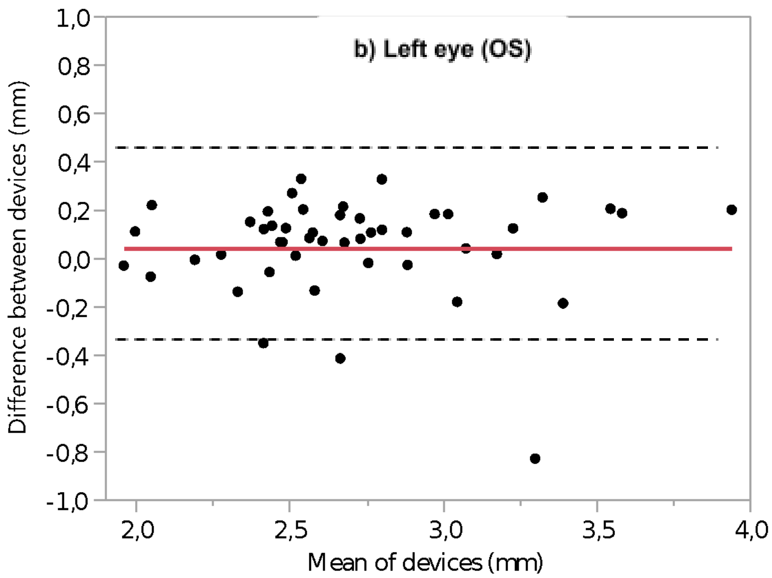


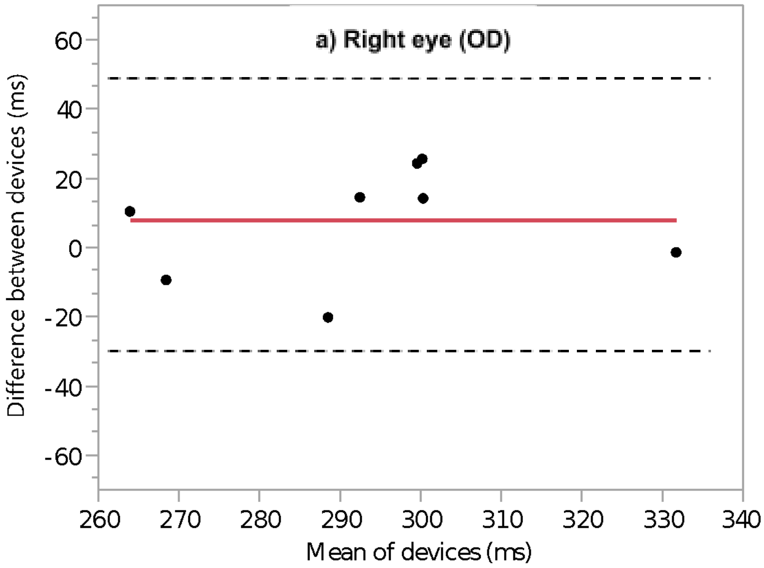
Figure 4: Stability agreement plot between devices on pupil size measured on a) left eye and b) right eye. The line represents the mean measurement of all measurements on the two devices, the dotted line the agreement limits and the dots the group of observations.

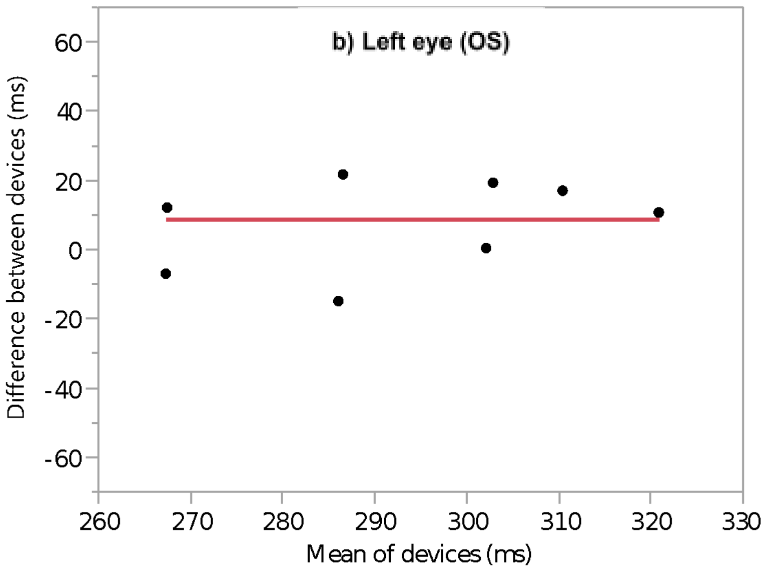
Table 4: Stability of agreement between devices on pupil size

Variable	Parameters	Estimation
	Mean of measurements (SD)	2.77 (0.42)
	Difference between measurements (SD)	0.02 (0.19)
Pupil size	Agreement Index (AI)	0.86
Right eye	% outliers	2/54 = 3.7%
	Correlation between measurements	0.58
	Correlation between mean and absolute difference value	-0.005
	Mean of measurements (SD)	2.72(0.44)
	Difference between measurements (SD)	0.04 (0.20)
Pupil size	Agreement Index (AI)	0.85
Left eye	% outliers	2/54=3.7%
	Correlation between measurements	0.75
	Correlation between mean and absolute difference value	0.25

III: Pupil latency; Agreement and stability of agreement between devices

3.1: Agreement between devices on latency





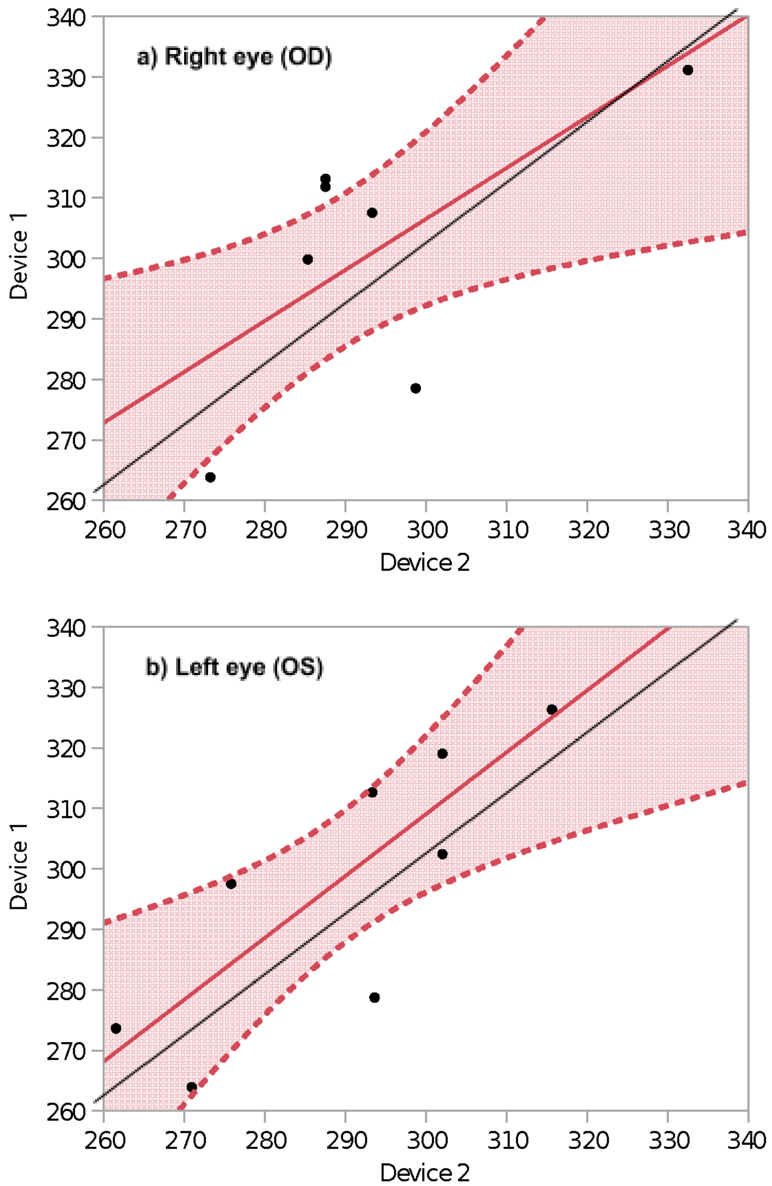
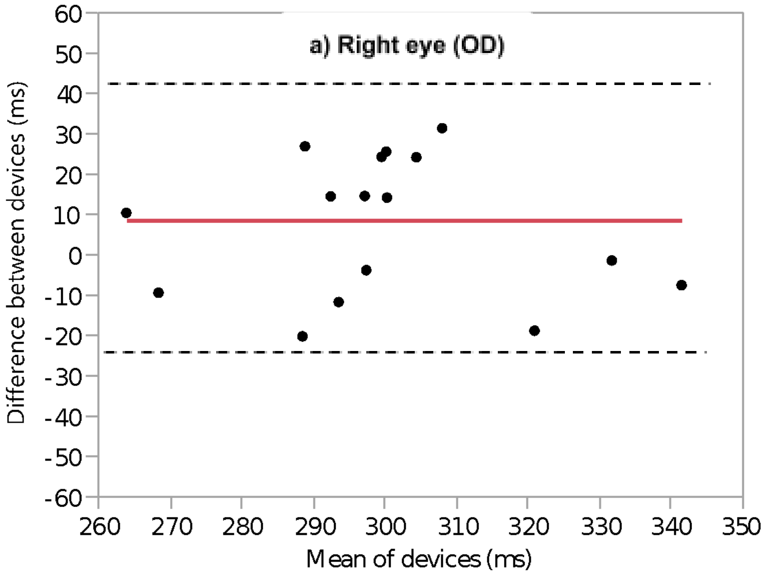


Table 5: Agreement between devices on Pupil latency

Variable	Parameters	Estimation
Pupil latency Left eye	Mean of measurements (SD)	294 (18.5)
	Difference between measurements (SD)	8.5 (13.0)
	Agreement Index (AI)	0.91
	% outliers	0%
	Correlation between measurements	0.80
	Correlation between mean and absolute difference value	0.10
Pupil latency Right eye	Mean of measurements (SD)	294 (19.9)
	Difference between measurements (SD)	7.8 (15.4)
	Agreement Index (AI)	0.86
	% outliers	0%
	Correlation between measurements	0.75
	Correlation between mean and absolute difference value	-0.12

3.2: Stability of agreement between devices on pupil latency



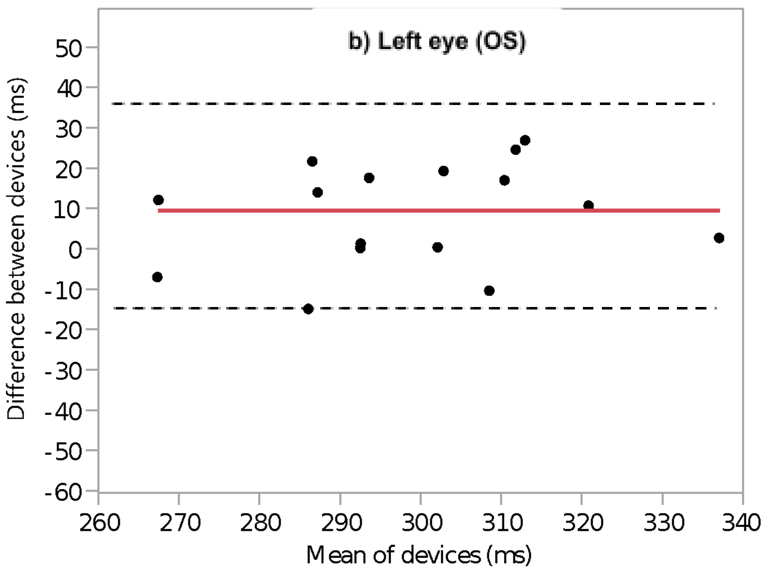
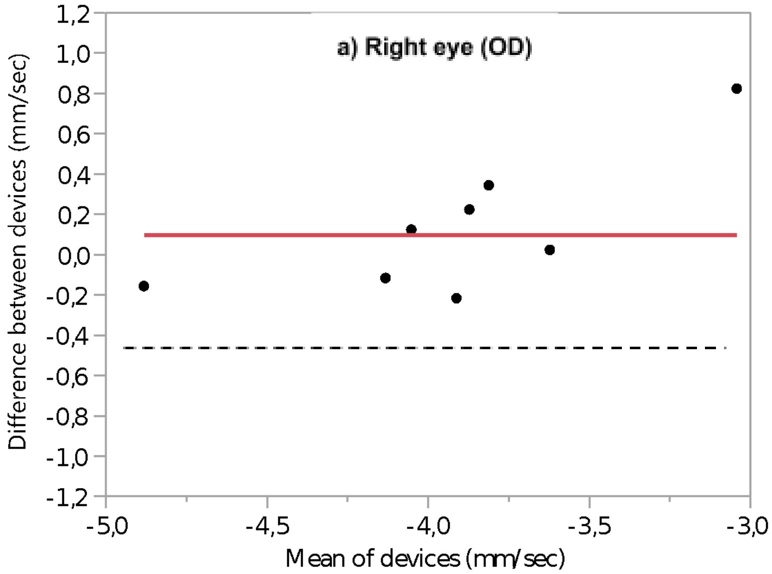


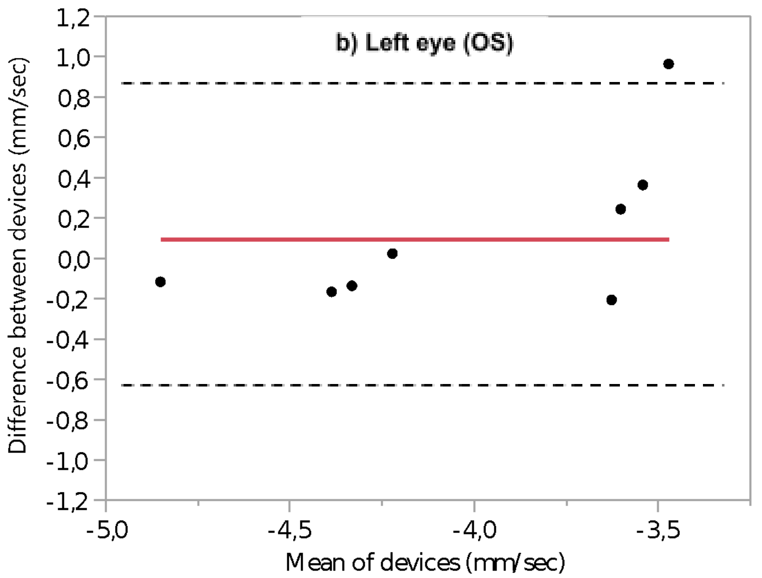
Table 6: Stability of agreement between devices on pupil latency

Variable	Parameters	Estimation
Pupil latency Right eye	Mean of measurements (SD)	299.3 (18.8)
	Difference between measurements (SD)	8.4 (17.0)
	Agreement Index (AI)	0.89
	% outliers	0%
	Correlation between measurements	0.66
	Correlation between mean and absolute difference value	-0.14
Pupil latency Left eye	Mean of measurements (SD)	298.8 (17.4)
	Difference between measurements (SD)	9.4 (12.4)
	Agreement Index (AI)	0.92
	% outliers	0%
	Correlation between measurements	0.78
	Correlation between mean and absolute difference value	0.20

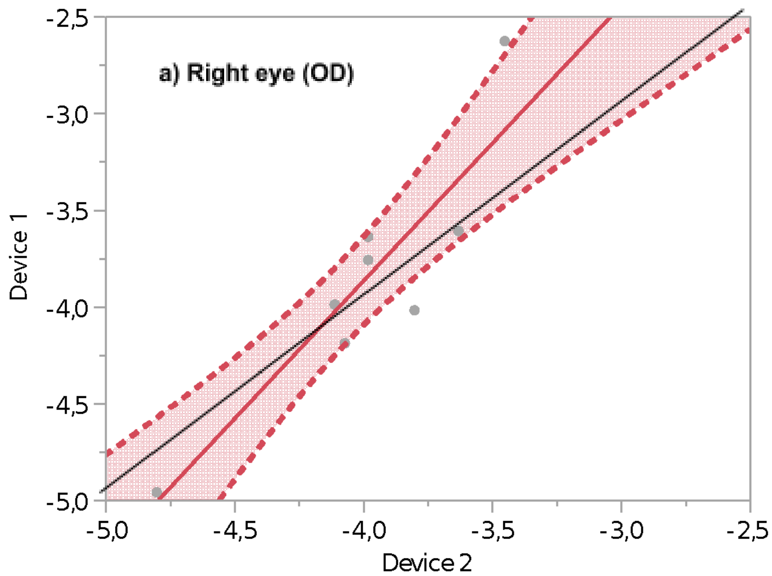
IV: Peak velocity; Agreement and stability of agreement between devices

4.1: Agreement between devices on Peak velocity





b) Left eye (OS)
Difference between devices (mm/sec)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
-5,0
-4,5
-4,0
-3,5
Mean of devices (mm/sec)



a) Right eye (OD)
Device 1
-2,5
-3,0
-3,5
-4,0
-4,5
-5,0
-5,0
-4,5
-4,0
-3,5
-3,0
-2,5
Device 2

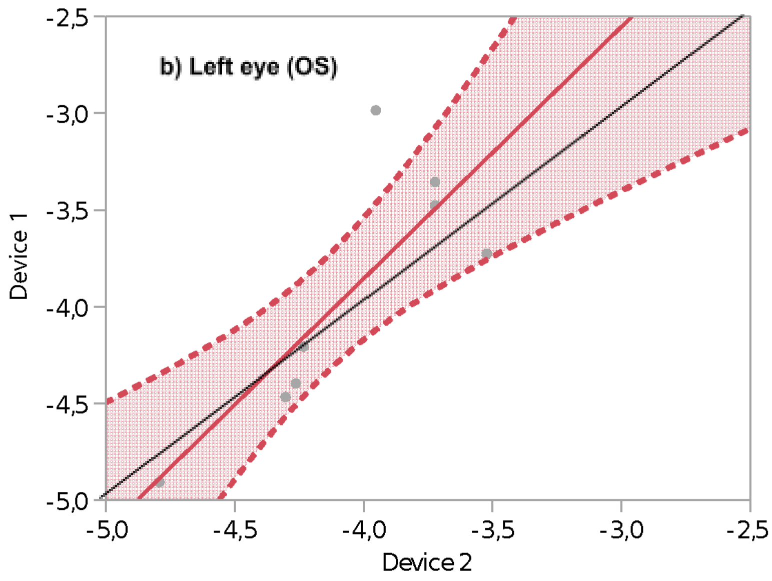
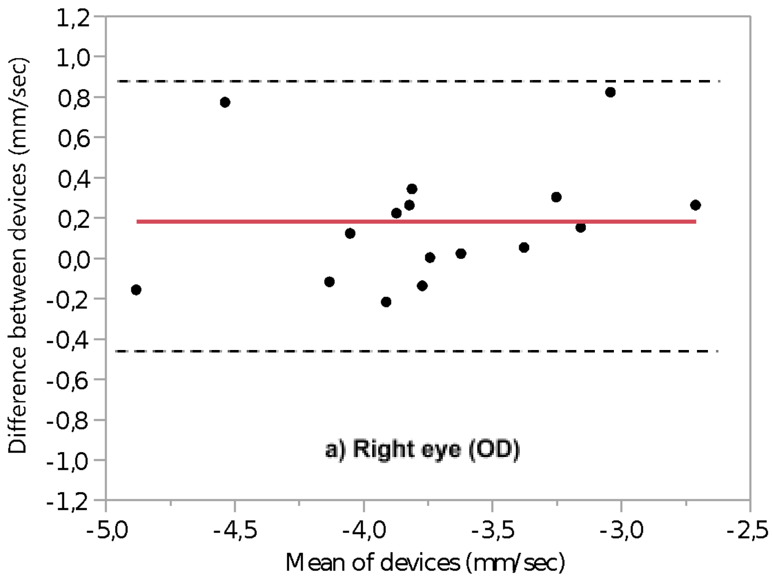


Table 7: Agreement between devices on Peak velocity

Variable	Parameters	Estimation
Peak velocity Right eye	Mean of measurements (SD)	-4.02 (0.58)
	Difference between measurements (SD)	0.096 (0.33)
	Agreement Index (AI)	0.84
	% outliers	1/9=11.1%
	Correlation between measurements	0.76
	Correlation between mean and absolute difference value	-0.60
Peak velocity Left eye	Mean of measurements (SD)	-4.10 (0.55)
	Difference between measurements (SD)	-0.091 0.38)
	Agreement Index (AI)	0.81
	% outliers	1/9=11.1%
	Correlation between measurements	0.64
	Correlation between mean and absolute difference value	-0.61

4.2: Stability of agreement between devices on Peak velocity



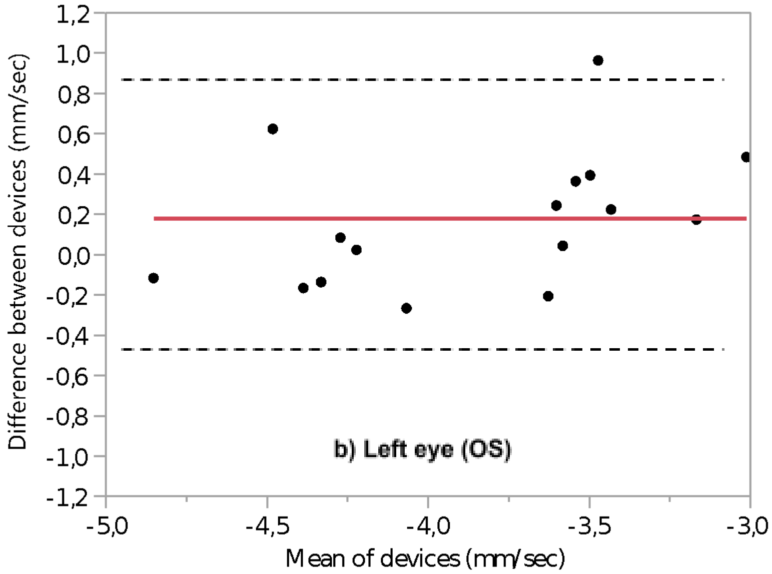


Table 8: Stability of agreement between devices on Peak velocity

Variable	Parameters	Estimation
Peak velocity Right eye	Mean of measurements (SD)	-3.84 (0.61)
	Difference between measurements (SD)	0.18 (0.33)
	Agreement Index (AI)	0.83
	% outliers	0
	Correlation between measurements	0.87
	Correlation between mean and absolute difference value	0.08
Peak velocity Left eye	Mean of measurements (SD)	-3.94 (0.57)
	Difference between measurements (SD)	0.18 (0.34)
	Agreement Index (AI)	0.83
	% outliers	1/18=5.6%
	Correlation between measurements	0.85
	Correlation between mean and absolute difference value	-0.22

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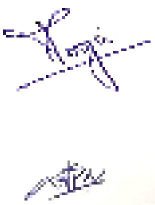
Signature:		*Signature:*				*Signature:*	
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