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ORIGINAL RESEARCH ARTICLE

Evaluation of correlation between Diopsys® NOVA™ fixed-luminance flicker ERG and Diagnosys® Espion 2™ flicker ERG parameters

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Abstract

Purpose Diopsys® NOVA™ is a novel full-field electroretinography (ffERG) device that can make rapid measurements of retinal electrophysiologic function. Diagnosys® Espion 2™ is a clinical gold-standard ERG device. This study aimed to investigate whether light-adapted Diopsys® NOVA™ fixed-luminance flicker ffERG *magnitude* and *implicit time* (converted from *phase*) measurements correlate with light-adapted Diagnosys® Espion 2™ flicker ffERG *amplitude* and *implicit time* measurements, respectively.

Methods Twelve patients (22 eyes) with various retinal and uveitic diseases underwent light-adapted Diagnosys® Espion 2™ and Diopsys® NOVA™ fixed-luminance flicker testing. Diopsys® *magnitude* and *implicit time* (converted from *phase*) measurements were compared to Diagnosys® *amplitude* and *implicit time* measurements, and a Pearson correlation was used to evaluate any existing correlation. Groups were also compared using generalized estimating equations. Bland–Altman plots were utilized to determine agreement between the comparison groups.

Results Age of patients ranged from 14 to 87 years. 58% ($n=7/12$) of patients were female. A significant, *positive* correlation ($r=0.880$, $P<0.001$) was observed between *magnitude* (Diopsys®)

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and *amplitude* (Diagnosys®) measurements. *Amplitude* increases by 6.69 µV for each 1 µV increase in *Magnitude* (p-value < 0.001). A statistically significant, strong positive correlation was observed between Diopsys® *implicit time* measurements (converted from *phase*) and Diagnosys® *implicit time* measurements ($r = 0.814$, p-value < 0.001). For each 1 ms increase in Diopsys® *implicit time*, Diagnosys® *implicit time* increases by 1.13 ms (p-value < 0.001).
Conclusions There is a statistically significant positive correlation between light-adapted Diopsys® NOVA™ fixed-luminance flicker *amplitude* and Diagnosys® flicker *magnitude* values. Additionally, there is a statistically significant positive correlation between Diopsys® NOVA™ fixed-luminance flicker *implicit time* (converted from *phase*) and Diagnosys® flicker *implicit time* values. These results imply that the Diopsys® NOVA™ module, which utilizes the nonstandard shortened International Society for Clinical Electrophysiology of Vision (ISCEV) ERG protocol, can produce reliable light-adapted flicker ffERG measurements.

Keywords Fixed luminance · Flicker electroretinography · Full-field electroretinography · Diopsys® · Diagnosys®

Introduction

The electroretinogram (ERG) is an electrophysiological test measuring the electrical response of various retinal cells to light stimulus. ERG is used to objectively assess retinal function in hereditary retinal diseases such as Leber congenital amaurosis [1], retinitis pigmentosa [2, 3], cone-rod dystrophy [4], achromatopsia [5], as well as acquired retinal and uveitic diseases such as paraneoplastic and autoimmune retinopathies [6], central retinal vein occlusion [7], diabetic retinopathy [8, 9], and drug toxicities [10, 11].

Full-field electroretinography (ffERG) is used to obtain objective quantitative measurements of overall retinal electrophysiologic function [12]. Analysis of ffERG waveforms can be used to distinguish the functions of different retinal cell types, including photoreceptors, bipolar cells, and to a lesser extent, retinal ganglion cells, and amacrine cells [13]. Flicker ffERG, a part of standard ffERG testing, uses 28–33 Hz light frequency to stimulate light-adapted retina, producing waveforms that give insights into cone and bipolar cell function. Rods cannot respond to this frequency; therefore, flicker ffERG response is only generated from the cones and cone-related bipolar cells [14, 15]. Cone On- and Off-bipolar cells are principally responsible for generating flicker ERG waveforms [15]. This signal is additionally dependent on L- and M-cone cells, which are sensitive to long- and medium-wavelength light, respectively [15]. Short-wavelength-sensitive S-cone cells contribute minimally to the light-adapted flicker ERG [15].

Modifications to the International Society for Clinical Electrophysiology of Vision (ISCEV) Standards and Guidelines introduced a nonstandard abbreviated ERG protocol that reduces time of dark adaptation to 10 min, includes fewer dark-adapted ERG recordings, and does not require the use of mydriasis [15]. The Diopsys® NOVA™ device (Diopsys®, Inc., Pine Brook, NJ, USA) adheres to this nonstandard shortened ERG protocol as it does not require pupil dilation and does not include all ERG tests included in the ISCEV standard protocol.

To our knowledge, no previous study has provided the correlation of the Diopsys® NOVA™ device data with that of a standard ERG system. The index study aimed to investigate whether Diopsys® NOVA™ fixed-luminance flicker ffERG *magnitude* and *phase* measurements correlate with *amplitude* and *implicit time* measurements made by the Diagnosys® 30 Hz flicker ffERG test.

Methods

Setting of study and subjects

The index study was a retrospective analysis of patients with various retinal and uveitic pathologies who underwent both Diopsys® NOVA™ fixed-luminance flicker ffERG and Diagnosys® Espion 2™ 30 Hz flicker ffERG over a period of seven months at a tertiary care center (Byers Eye Institute at Stanford University). Diopsys® NOVA™ and Diagnosys® Espion 2™ ERG testing were performed in accordance with ISCEV standards [15]. Patients with an interim period of three or more months between the two tests were excluded. In addition, patients with

any surgical intervention or change in disease activity between testing sessions were excluded, including those with changes in visual acuity or disease activity markers such as cell count or macular edema.

Data collection and outcomes

We collected baseline patient characteristics, including age, sex, and underlying diseases. For the Diagnosys® flicker ERG, we collected *amplitude* and *implicit time* values. As for Diopsys® fixed-luminance flicker ERG, we collected *magnitude* and *phase* values.

Before undergoing Diagnosys® testing, patients were kept in a light-adapting room for at least 10 min. All Diagnosys® testing utilized a single Diagnosys® Espion 2™ device (Diagnosys® LLC, Lowell, MA, USA) and took place in the same clinical room under ambient light conditions. Patients were maximally dilated prior to Diagnosys® ERG testing. A patient's forehead and outer and inner canthi of both eyes were cleansed. The patient's eyes were then anesthetized with 0.5% proparacaine hydrochloride ophthalmic solution. Then, Dawson–Trick–Litzkow (DTL) electrodes were placed so that they touched the conjunctiva inferior to the limbus of each eye. A ground electrode was then applied to the center of the forehead. The patient's head was then placed in the Diagnosys® ColorDome™. Flicker testing utilized standard protocol testing with light fixed at a luminance of 3.0 cd s/m^2 flashed at 30 Hz (± 1%).

Prior to Diopsys® testing, patients were maximally dilated and kept in a light-adapting room for at least 10 min. To undergo Diopsys® NOVA™ fixed-luminance flicker ffERG, a patients forehead was cleansed with a lid scrub pad, followed by cleansing of the bottom eyelids close to the lash line. ERG lid electrodes were placed under each eyelid, close to the lash line, with lead wire attachments pointed outwards. A ground electrode was then applied to the forehead. Then, corresponding lead wires were attached to the different electrodes. The patient was then instructed to place the mini-ganzfeld stimulator gently covering their right eye first before light stimulation and measurement recordings by the machine. Light fixed at a luminance of 3 cd.s/m2 (600 cd/m^2 as 5 ms flashes) at 32 Hz on a white background with aluminance of 30 cd/m^2. *Magnitude* and *phase* values were then measured. This flash pattern was repeated for 20 s in each eye. If a recording had artifacts > 40%, the flicker test was repeated in accordance with Diopsys® guidelines.

Statistical analysis

Pearson correlation coefficient was used for correlation analysis of values measured by the Diopsys® NOVA™ fixed-luminance flicker ffERG and the Diagnosys® Espion 2™ 30 Hz flicker ffERG. To account for within-subject correlation between eyes, generalized estimating equations with robust standard errors were used. Bland–Altman plots were constructed to determine agreement between measurements made by the two electrophysiologic tests. Statistical analysis was performed using R (programming language). A *p*-value of less than 0.05 was considered statistically significant.

Results

Demographics

Twelve patients (22 eyes), all with various diseases affecting retinal function, were included in this study. These patients underwent Diopsys® fixed-luminance flicker ffERG and Diagnosys® Espion 2™ 30 Hz flicker ffERG testing. All patients had both tests performed within *three months* of each other. The mean difference between testing dates was 31.9 days (range, 0–81 days). The age of patients ranged from 14 to 87 years, and 58% were female (Table 1). Best-corrected visual acuity ranged from 20/20 to hand motion. The mean visual acuity was 0.71 logMAR.

Ocular diseases of patients

Ocular pathologies included retinal vasculitis ($n = 7/22$ eyes, 32%), melanoma associated retinopathy (MAR) ($n = 2/22$ eyes, 9%), idiopathic panuveitis ($n = 2/22$ eyes, 9%), autoimmune retinopathy ($n = 2/22$ eyes, 9%), autoimmune optic neuropathy ($n = 2/22$ eyes, 9%), non-proliferative diabetic retinopathy

Table 1 Overview of Patients and Ocular/Systemic Diseases

Case	Age	Sex	Diagnosis	Affected Eye(s)	Systemic Diseases
1	75	M	Melanoma associated retinopathy	OU	Metastatic cutaneous melanoma
2	25	F	Idiopathic retinal vasculitis	OU	Multiple sclerosis, Autoimmune hepatitis
3	46	M	Idiopathic panuveitis	OU	
4	41	F	Idiopathic retinal vasculitis	OS	
5	87	M	Autoimmune retinopathy	OU	
6	37	M	Idiopathic retinal vasculitis in patient with HLA-A 29 positivity	OU	
7	14	M	Autoimmune optic neuropathy	OU	
8	49	F	Non-proliferative diabetic retinopathy	OU	
9	85	F	Age-related macular degeneration	OU	
10	31	F	Tuberculous choroiditis	OU	Tuberculosis
11	69	F	Idiopathic retinal vasculitis	OU	Breast Cancer
12	81	F	Retinal vein occlusion	OD	

($n = 2/22$ eyes, 9%), age-related macular degeneration (n = 2/22 eyes, 9%), tuberculous choroiditis ($n = 2/22$ eyes, 9%), and retinal vein occlusion ($n = 1/22$ eyes, 5%) (Table 1). Four patients (33%) had known systemic associations including metastatic cutaneous melanoma, tuberculosis, breast cancer, multiple sclerosis, and autoimmune hepatitis (Table 1).

Correlation between flicker ERG measurements

Mean (SD) Diopsys® *magnitude* and Diagnosys® *amplitude* measurements were 11.76 ± 3.54 µV and 66.18 ± 25.66 µV, respectively. A statistically significant, strong *positive* correlation was observed between both measurements ($r = 0.880$, p-value < 0.001) (Fig. 1). For each 1 µV increase in magnitude, amplitude increases by 6.69 µV

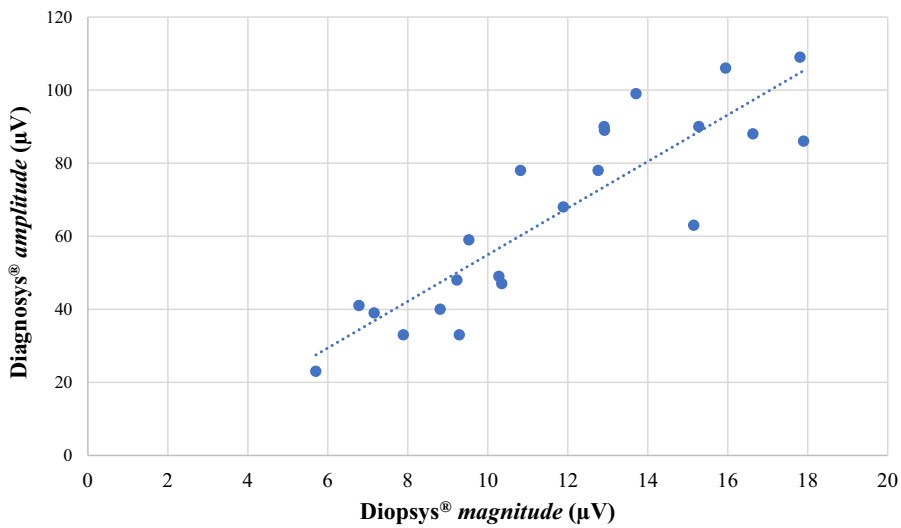


Fig. 1 Relationship between Diopsys® fixed-luminance flicker *magnitude* (µV) and Diagnosys® flicker *amplitude* (µV)

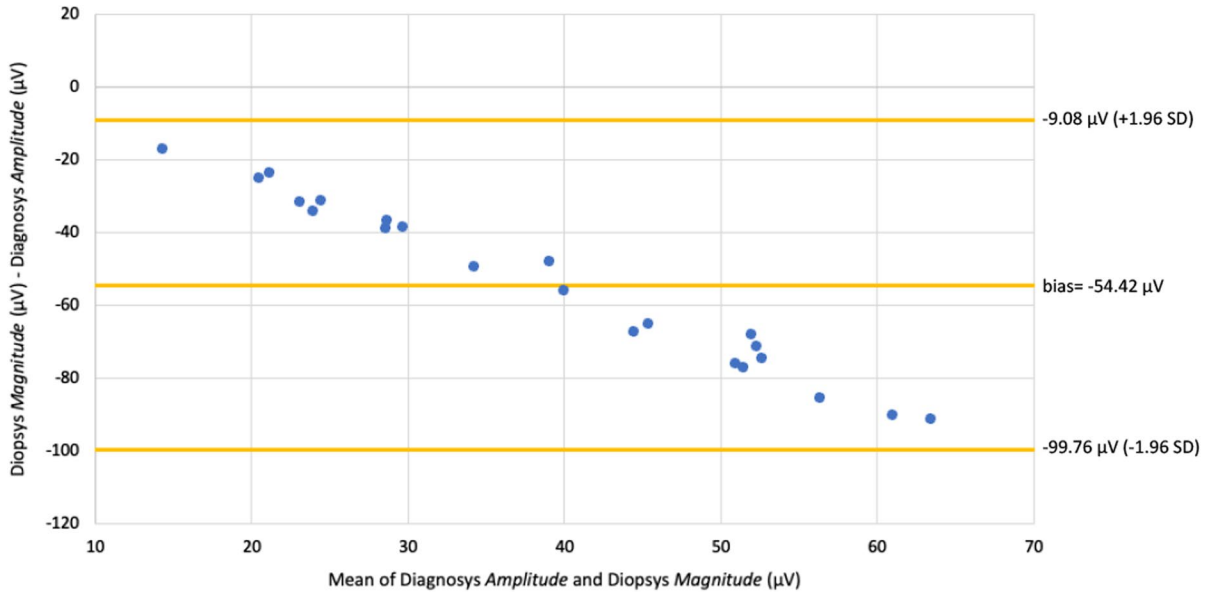


Fig. 2 Bland–Altman plot analyzing agreement between Diopsys® *magnitude* and Diagnosys® *amplitude* measurements

(p-value < 0.001). All data points lay within ± 1.96 SD of the mean difference, and there is a negative bias of − 54.42 µV for the Diopsys® NOVA™ fixed-luminance ffERG flicker magnitude measurements (Fig. 2). As *magnitude* and *amplitude* increased, the difference between these values also increased. Mean (SD) Diopsys® *phase* and Diagnosys® *implicit time* measurements were 301.76 ± 38.02° and 30.63 ± 4.28 ms, respectively. Mean (SD) Diopsys® *implicit time* measurements (converted from *phase*) was 5.06 ± 3.30 ms. A statistically significant, strong positive correlation was observed between Diopsys® *implicit time* measurements and Diagnosys® *implicit time* measurements ($r = 0.814$, p-value < 0.001) (Fig. 3). For each 1 ms increase in Diopsys® implicit time, Diagnosys® implicit time increases by 1.13 ms (p-value < 0.001). All but

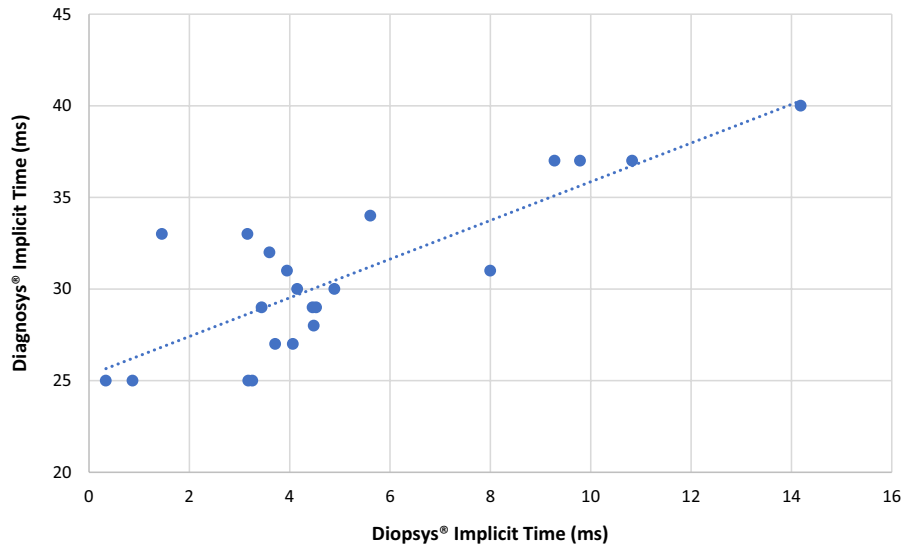


Fig. 3 Relationship between Diopsys® fixed-luminance *flicker implicit time* (milliseconds) and Diagnosys® *flicker implicit time* (milliseconds)

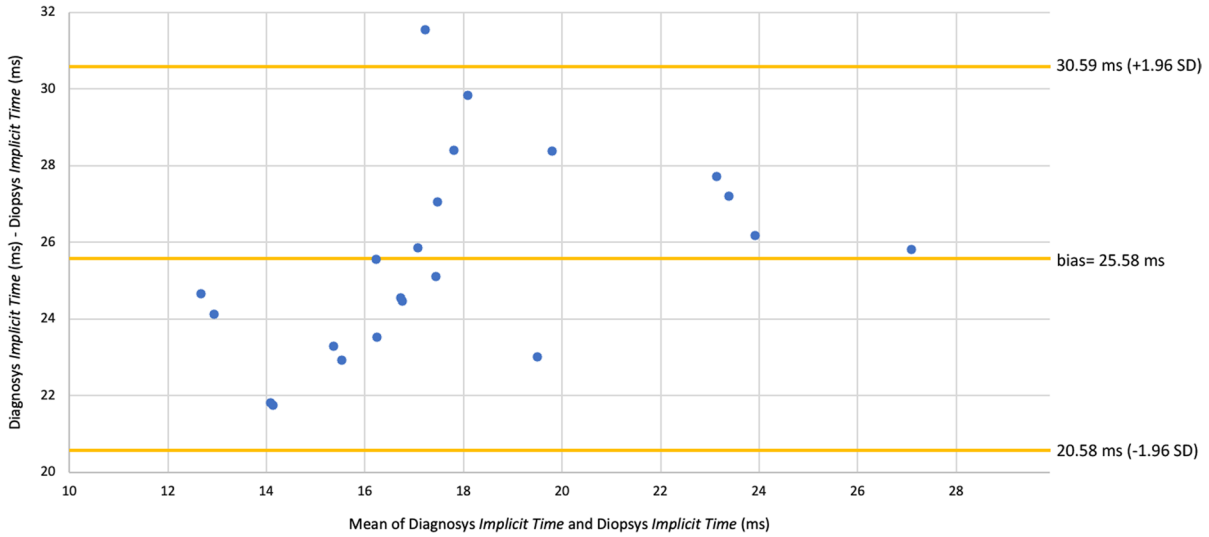


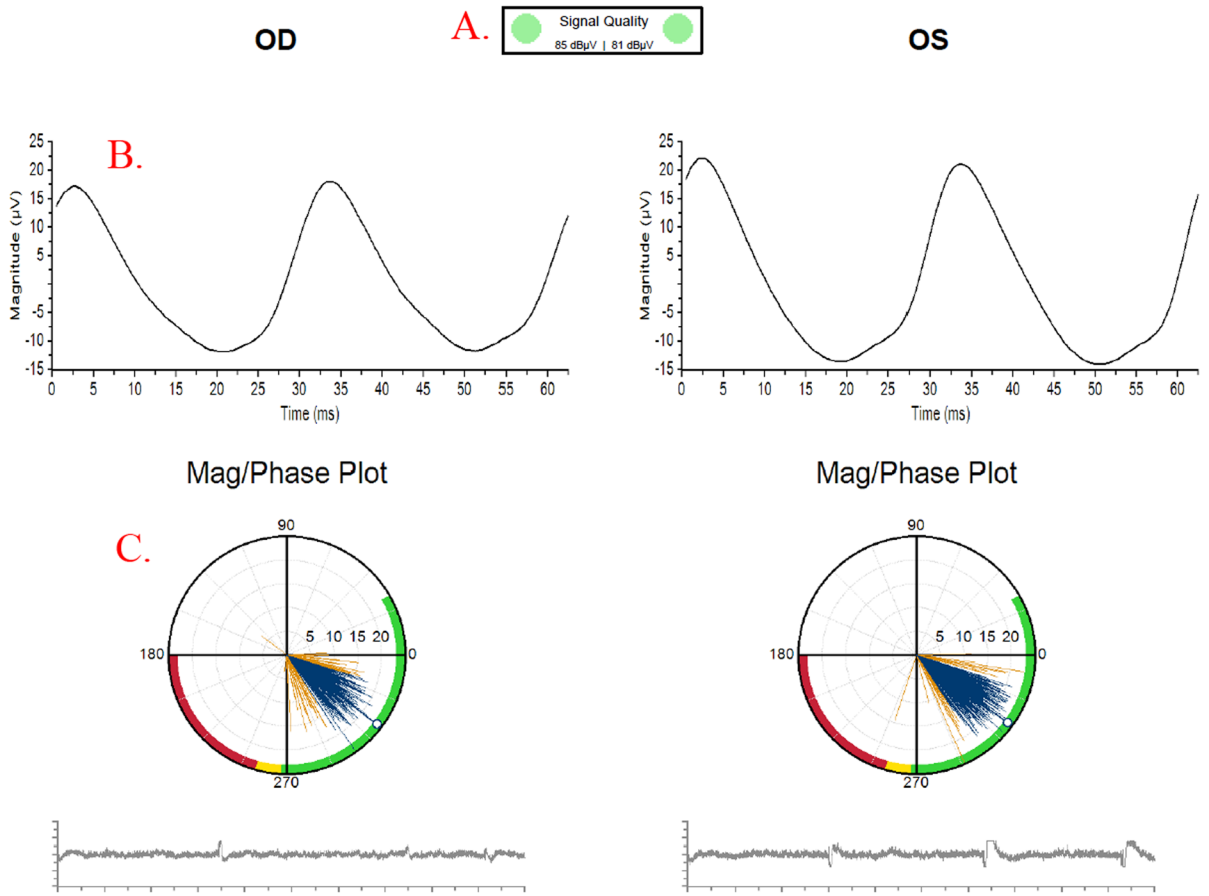
Fig. 4 Bland–Altman plot analyzing agreement between Diopsys® *implicit time* (converted from *phase*) and Diagnosys® *implicit time* measurements

one Bland–Altman data point lay within ± 1.96 SD of the mean difference, and there is a positive bias of 25.58 ms for the Diagnosys® fixed-luminance flicker implicit time measurements (Fig. 4). Figures 5 and 6 show representative reported results for Diopsys® NOVA™ fixed-luminance flicker and Diagnosys® Espion 2™ flicker, respectively.

Discussion

Our findings indicate that Diopsys® NOVA™ fixed-luminance flicker ERG *magnitude* measurements strongly correlate with Diagnosys® Espion 2™ flicker ERG *amplitude* measurements. Additionally, these two systems of measurement agree with one another. Our results also show that Diopsys® NOVA™ fixed-luminance flicker ERG *phase* values converted to *implicit time* values are strongly correlated with Diagnosys® Espion 2™ flicker ERG *implicit time* values, implying that Diopsys® NOVA™ fixed-luminance flicker ERG *phase* is consistent with Diagnosys® Espion 2™ flicker ERG *implicit time*. However, these two systems of measurement show biased (different) but correlated values. These results suggest that the Diopsys® NOVA fixed-luminance flicker ERG test provides reliable information about the timing and strength of light-adapted flicker responses.

According to the ISCEV Standard for Full-Field Clinical Electroretinography, reporting ERG results as frequency domain equivalents is accepted [15]. Diopsys® NOVA™ *magnitude* corresponds to the *amplitude* or responsivity of a subject's response to light stimulus. *Phase* relates to *implicit time*, where a *phase* closer to 360° corresponds to an *implicit time* closer to 0 s. Diopsys® NOVA™ utilizes frequency domain analysis to transform time domain units to *phase*. Throughout Diopsys® NOVA™ light-adapted flicker examination, frames of two responses (to two flashes) are translated from the time domain to the frequency domain, and *magnitude* and *phase* of the fundamental frequency are recorded. Once outliers have been eliminated, the means of the *mag*nitude and *phase* values are reported. To translate the 30-Hz flicker ERG signal into the frequency domain, a rapid Fourier transform is utilized, yielding a complex number. The *magnitude* of x is calculated as $\sqrt{(x_r^2 + x_i^2)}$ while the *phase* is calculated as $\arctan\left(\frac{x_i}{x_r}\right)$, where x_r and x_i are the real and imaginary components, respectively [17]. Magnitude and Phase for the Diopsys device are calculated from the first harmonic of the flicker frequency.



D.

Parameter	OD	OS	Asymmetry (%)
Magnitude (µV)	13.71	16.63	19
Phase (°)	322.48	323.39	0
Magnitude Variance Ratio	0.96	1.05	
Phase Variance Ratio	1.10	1.10	
Artifacts (%)	1.3	9.4	

Fig. 5 Representative Diopsys® NOVA™ fixed-luminance flicker ERG results. A. Indicator of signal quality showing the strength of the connection between the electrodes and a patient's head. B. Graph displaying flicker magnitude (µV) versus time (ms). C. Polar plot depiction of flicker measurements, where magnitude values are shown by radial line length and phase values are represented by the angle at which the radial line falls. Green signifies a 'within reference range' response, yellow signifies a 'borderline reference range' response, and red signifies an 'outside reference range' response. These reference ranges were established using a normative database compiled by the manufacture. D. Tabulated flicker data displaying mean magnitude (µV), mean phase (°), asymmetry in those measurements between eyes, magnitude variance ratio, phase variance ratio, and % of recorded artifacts

Observing Diopsys® NOVA™ fixed-luminance flicker ERG results, a reader can determine the normality of a *magnitude* or *phase* response by looking at the color of the result; green signifies a 'within reference range' response, yellow signifies a 'borderline reference range' response, and red signifies an

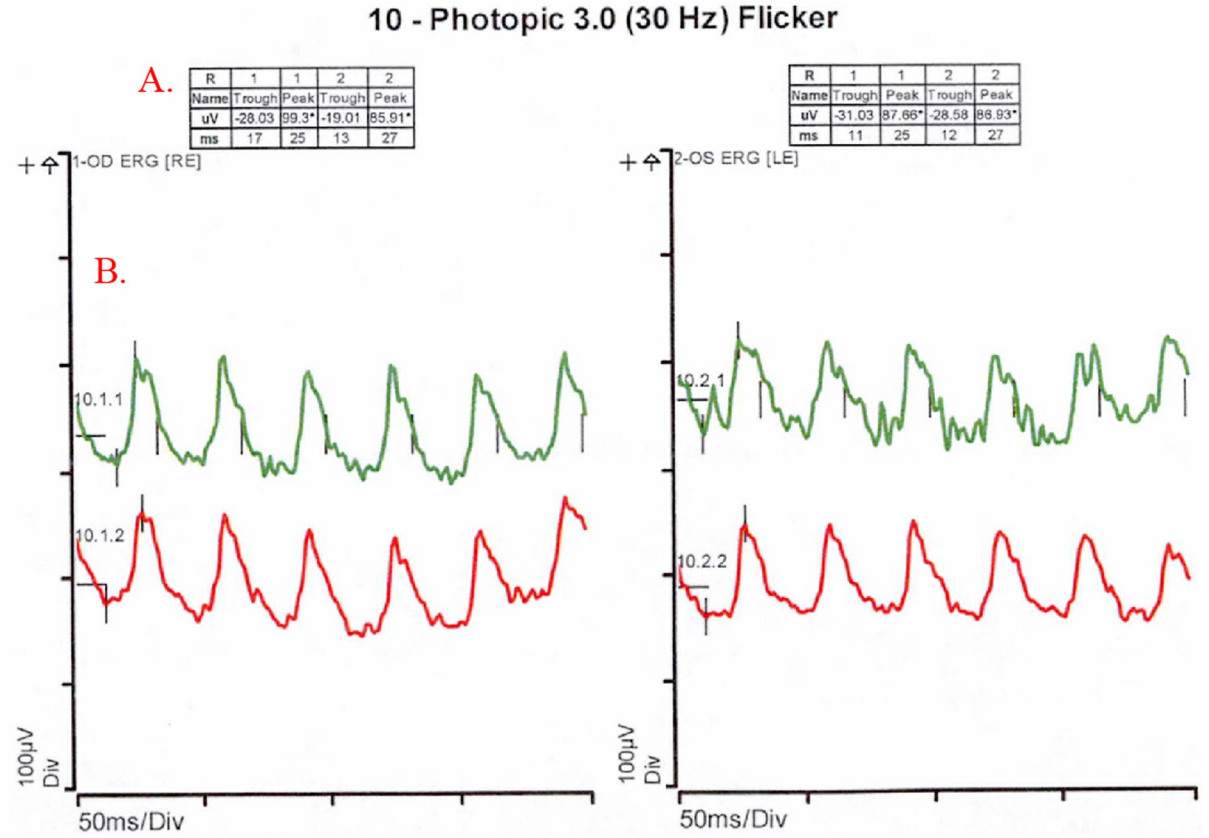


Fig. 6 Representative Diagnosys® Espion 2™ light-adapted 30 Hz flicker ERG results showing and tabulating amplitude and phase data and flicker waveforms. A. Tabulated flicker data displaying amplitude of troughs and peaks, as well as implicit time, of two recorded flicker waves. B. Graph displaying flicker amplitude (µV) versus time (ms) of two recorded flicker waves. The two waves are translated along the y-axis so that they do not overlap and can be interpreted independently

'outside reference range' response (Fig. 5). 'Within reference range' is considered a *magnitude* greater than 5.06 µV and a *phase* greater than 267.47°, 'borderline reference range' is considered a *magnitude* between 3.34 µV and 5.06 µV and a *phase* between 254.74° and 267.47°, and 'outside reference range' is considered a *magnitude* less than 3.34 µV and a *phase* less than 254.74°. These ranges are derived from a normative database compiled by the maker of the device, Diopsys®, and composed of 50 healthy patients (mean age: 53 years, 56% female) [14]. Additionally, the results are displayed in a polar plot (Fig. 5). From this image, one can determine the strength of a *magnitude* response by looking at the length of each radial line. One can also determine the *phase* response by identifying where along the circle the responses fall. As with the tabulated report of the data, green signifies a 'within reference range' response, yellow signifies a 'borderline reference range' response, and red signifies an 'outside reference range' response.

As the Diopsys® NOVA™ module utilizes skin electrodes placed on a patient's lower eyelids, no topical ophthalmic anesthetic is required. It is important to note, however, that utilization of skin electrodes, as opposed to corneal or conjunctival electrodes, results in reduced *amplitude* responses. In a study by Tang et al. [18], the authors compared RETeval sensor strip electrodes to Dawson–Trick–Litzkow (DTL) corneal electrodes for photopic negative response recordings using the LKC RETeval device. Similar to our experiment, they reported that the skin electrodes produced an attenuated signal compared to those recorded by DTL electrodes [18]. Additionally, as the Diopsys® NOVA™ fixed-luminance flicker test follows the non-standard abbreviated ISCEV protocol, the flicker test

can be performed in a timelier manner. Utilization of a more patient-friendly electrode and shorter ERG exam might be beneficial for pediatric and disabled patients [19–21].

Different from many other ERG systems, the Diopsys® NOVA™ module does not require maximal pupil dilation before ERG testing [15]. The Diopsys® NOVA™ fixed-luminance flicker flash stimulus is constant and does not adjust for changes in pupil size. While patients would benefit from avoiding risks associated with artificial dilation, such as impaired driving and acute closed-angle glaucoma, the intra-patient flicker ERG parameters will change with varying pupil size [22, 23]. This is in contrast to RETeval, another mydriasis-free ERG system, which adjusts retinal illumination according to pupil size [24]. As of 2022, the ISCEV Standards and Guidelines no longer require pupil dilation if stimulus and background light are intense enough to elicit ERG waveforms comparable to those obtained with dilated pupils [15]. Mobasserian et al.[25] showed a statistically significant difference in *magnitude* of Diopsys® NOVA™ flicker response before and after dilation. Given this finding, we elected to maximally dilate all patients before Diopsys® NOVA™ flicker testing to prevent interpatient ERG variability as a result of differences in pupil size.

One limitation of our study is the lack of cross-sectional Diagnosys® Espion 2™ or Diopsys® NOVA™ flicker testing. We attempted to mitigate this by excluding patients whose disease activity changed between testing dates. Patients included in this study had no changes in visual acuity or parameters of disease activity, such as cell count or macular edema, between testing sessions. Another limitation of our study was that our sample size was relatively small. Considering a type I error rate of 0.05, our sample size of 22 eyes is adequate to exceed a statistical power of 0.80. Future studies should utilize like-to-like comparisons between flicker parameters, have both experiments occur within a shorter time frame (preferably the same day), and involve a larger number of eyes.

Our study demonstrates that while the Diopsys® NOVA™ module is relatively new and includes several aspects atypical of traditional ERG systems, the fixed-luminance flicker parameters are significantly correlated with those of a standard ERG device commonly used in eye clinics across the globe. Previous studies have demonstrated the precision of the Diopsys® NOVA™ flicker test [14, 26]. There are other additional tests of the Diopsys® NOVA™ that were not evaluated for validity in this study. Other tests that the Diopsys® NOVA™ module can perform include visual evoked potential, multi-focal ERG, and pattern ERG. Future studies should look to evaluate agreement between these Diopsys® NOVA™ tests and those performed by gold-standard clinical ERG devices.

Conclusion

Diopsys® NOVA™ fixed-luminance flicker ERG results are concordant with Diagnosys® Espion 2™ flicker ERG measurements.

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Declarations

Conflict of interest Stanford University has received research support through the availability of the Diopsys® NOVA™ device for research purposes. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Stanford University School of Medicine and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Human rights and statement This article does not include any studies involving animals conducted by the authors.

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