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Functional imaging of the human papilla and peripapillary region based on flicker-induced reflectance changes

Mireille Crittin*, Charles E. Riva

Institut de Recherche en Ophtalmologie, Laboratoire d'Optique et de Biophysique, Av. Grand-Champsec 64, 1950 Sion, Switzerland

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Abstract

A non-invasive functional imaging technique of the nervous tissue of the human eye fundus based on two wavelength light reflectance changes (ΔR %) is presented. The effect of 30° diffuse luminance flicker field made of two spectral components (569 and 600 nm) on ΔR was studied in six normal volunteers. Group averaged ΔR was significant and similar in all quadrants of the optic disc at 569 nm with a mean equal to − 10.7% for the tissue of the optic disc and − 3.6% for the peripapillary regions. At 600 nm, the ΔR were also significant but smaller, i.e. − 3.5% for the disc tissue and − 1.7% for the peripapillary region. The changes at 569 and 600 nm represent most probably blood volume changes. The 600 nm reflectance did not show evident features suggesting the presence of flicker-induced oxygenation changes.

Keywords: Functional imaging; Fundus reflectance; Diffuse luminance flicker; Optic disc; Blood volume

Activity-dependent changes in intrinsic optical properties of cortex have been shown to be useful for monitoring changes in cerebral activity. These property modifications induce changes in the light intensity reflected from the active tissue. Of particular interest are the signal generated by the activity-induced transition from oxyhaemoglobin to haemoglobin of the blood in the capillaries and the signal originating from changes in light absorption as a result of the changes in blood volume. While the reflectance of light at 570 nm, an isobestic wavelength, is the most suitable to reveal the blood volume changes, an illumination at 600 nm appears to be more favourable for detecting changes in blood oxygen delivery [4,6,7].

In spite of its potential to shed light on the mechanisms underlying the neuro-vascular coupling in the retina and optic nerve and on the effect of pathological alterations on this coupling, the imaging of activity-dependent intrinsic signals from these neural tissues of the eye has not been achieved yet. This paper, therefore, represents an attempt to derive the time course as well as obtain two-dimensional maps of the activity-dependent changes in reflectance of the optic disc and peripapilla of the human eye by adapting the reflectance-based technique mentioned above.

Fundus reflectance measurements were performed with a previously described reflectometer [2]. Briefly, a 30° field centred at the optic disc was illuminated with light composed of two spectral components, namely 569 and 600 nm. These wavelengths were selected by filtering the fundus illumination light delivered by the internal halogen lamp of a fundus camera (FF 450 IR, Zeiss, Jena, Germany) with a dual-band pass filter (each bandwidth at half − height = 5 nm). The retinal irradiance of ~50 $\mu W/cm^2$ of both 569 and 600 nm lights was well under the maximum permissible level [1]. For simultaneous detection of images at the two wavelengths with a computer controlled intensified CCD video camera (4 Quik E/01-25, Stanford Computer Optics, Munich, Germany), an image splitter (High-Efficiency MultiSpec Patho-Imager, Optical Insights, Santa Fe, NM) was mounted in front of the video camera. Both images were acquired at a rate of approximately five images per second.

The reflectance measurements were performed from the optic disc tissue and from a region less than 1/4 diameter outside the disc (peripapillary region) in one eye of each of six normal volunteers (mean age 28 years, range 22–38 years), all with excellent target fixation. The research followed the tenets of the Declaration of Helsinki and

* Corresponding author. Tel.: +41-27-205-79-00; fax: +41-27-205-79-01.

E-mail address: mireille.crittin@iro.vsnet.ch (M. Crittin).

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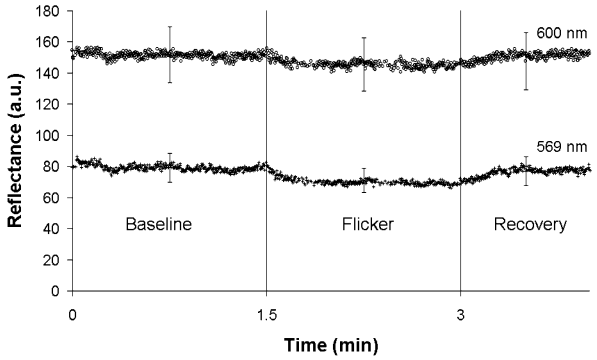

Fig. 1. Typical temporal time course of the optic disc tissue reflectance (R in arbitrary units). R decreases during flicker stimulation at 569 nm (+) and 600 nm (○). For both wavelengths, no significant changes were found for the groups between the R averaged during the last 30 s of baseline and of recovery. Typical error bars are shown (± SD).

informed consent was obtained from each subject. Thirty minutes before starting the measurements, the pupil of the tested eye was dilated with two drops of Tropicamide 0.5% (Novartis Ophthalmics AG, Hettlingen, Switzerland).

The measurement protocol consisted of a cycle made up of three consecutive phases, each of 60–90 s duration. During phase 1 (baseline) and phase 3 (recovery), the illumination light was maintained steady. During phase 2, square-wave modulation of the two-wavelengths fundus illumination at a frequency of 8 Hz using a chopper wheel generated the diffuse flicker stimulus and associated rise in retinal activity. This cycle was repeated three to four times consecutively with 10 min of rest between cycles.

The acquired fundus reflectance images were processed off-line using macros written with IDL (Research Systems, Inc., Boulder, CO) and Image-Pro Plus (Media Cybernetics, Inc., Silver Spring, MD). The reflection of a uniformly diffuse white reflectance standard (Spectralon, reflectance 99%, Labsphere, Inc., North Sutton, NH) was used to calibrate the reflectance of acquired fundus images. To compensate for unavoidable eye motions, a fully automatic registration was performed on the images. This registration allowed images to be aligned with accuracy better than one pixel, corresponding to 12 μm of the eye fundus [11]. The registration transformations were restricted to translations and rotations.

Table 1
Group averaged ΔR (%) at the optic disc tissue in response to flicker

Regions	ΔR (%)	
	569 nm	600 nm
Temporal superior	−10.9 ± 3.5	−3.1 ± 1.4
Temporal inferior	−11.1 ± 3.7	−4.0 ± 2.4
Nasal inferior	−10.2 ± 5.1	−3.8 ± 2.4
Nasal superior	−10.5 ± 4.6	−3.1 ± 2.3

For each subject, an average ΔR was calculated for each optic disc quadrant from four boxes of approximately 80×80 μm^2 area.

Fig. 1 shows a typical time course of the reflectance (R) averaged over a ~350 μm diameter area of the temporal rim tissue of the optic disc at the two wavelengths. For both wavelengths, R decreased in response to flicker and then returned to pre-stimulation levels following cessation of the stimulus. Most of the decrease during stimulation as well as most of the increase during recovery took place during the first 20 s after onset and cessation of the stimulus. To improve the signal-to-noise ratio of the reflectance, the images obtained during the last 30 s of each phase and at each wavelength were averaged. As we could experimentally confirm, this process did not result in a loss of spatial resolution, a consequence of the image registration being performed prior to averaging.

The changes in R (ΔR) were expressed in % as $100 \times (R_f - R_b)/R_b$, where R_b is the average of R over the last 30 s of baseline and R_f is the average of R during the last 30 s of flicker. To statistically assess the flicker-induced ΔR in each quadrant of the optic disc and in the peripapillary region, we averaged ΔR over four sites with an area of ~80×80 μm^2 for each optic disc quadrant and of ~180×180 μm^2 for the peripapillary region, avoiding visible retinal vessels (as exemplified in Fig. 2A). Using the first cycle of measurements for each subject, significant group averaged ΔR values were found for both wavelengths,

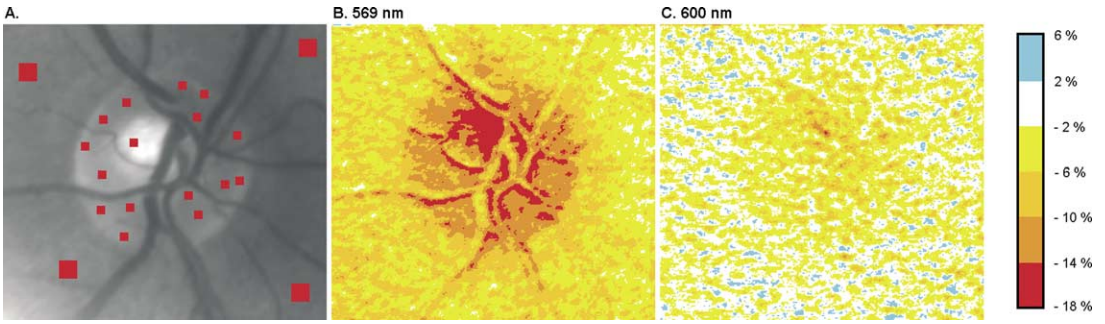

Fig. 2. (A) Black/white fundus image obtained by averaging 200 single images at 569 nm. Typical sites for quantitative assessment of local ΔR are displayed in red. Functional images at 569 nm (B) and at 600 nm (C) measured in one subject. ΔR (%) $= 100 \times (R_f - R_b)/R_b$, where R_b and R_f are the reflectance averaged images during the last 30 s of baseline and flicker, respectively. The colour intervals for images (B) and (C) are shown in the pseudo-colour scale on the right. Regions corresponding to maximum ΔR are shown in red, whereas regions of minimum ΔR are in white.

Table 2
Group average and range of ΔR (%) between the last 30 s of both baseline and flicker phases

	Optic disc tissue		Peripapillary region	
	569 nm	600 nm	569 nm	600 nm
ΔR (%)	−10.7 ± 4.0	−3.5 ± 1.9	−3.6 ± 2.6	−1.7 ± 1.4
Range (%)	−15.5 to −6.1	−6.2 to −1.6	−6.9 to 0.02	−4.1 to 0.06
P value	0.005	0.020	0.026	0.044

For each subject, a mean change in ΔR was calculated for both the optic disc (16 sites) and the peripapillary region (four sites).

for all regions of the tissue of the optic disc and peripapillary region (paired t-test between the last 30 s of baseline and flicker, $P < 0.05$). The group averaged ΔR values were not significantly different between the four locations on the optic disc for both wavelengths (Table 1), as assessed by one-factorial analysis of variance (ANOVA). Furthermore, for all regions, no significant changes were found between the baseline and the recovery period (paired t-test, $P > 0.1$). Averaged over all sites of the optic disc tissue and peripapillary region, respectively, the group mean ΔR decreased significantly by 10.7 ± 4.0% at 569 nm and 3.5 ± 1.9% at 600 nm (mean ± SD) for the optic disc tissue and by 3.6 ± 2.6% at 569 nm and 1.7 ± 1.4% at 600 nm for the peripapillary region (Table 2).

Fig. 2B,C exhibit typical colour coded maps of ΔR obtained from one subject with the fundus shown in Fig. 2A. There is a clear overall decrease in reflectance (see colour code on the right of Fig. 2C), more marked at 569 nm than at 600 nm. At the shorter wavelength, the decrease reaches values between 10 and 15% in the disc tissue. The large ΔR along some of the vessels represents most probably a vasodilatation, an effect previously described by others [3,9].

For each subject, the time courses of the reflectance of the disc tissue (averaged over the 16 sites) at 569 and 600 nm for the first 40 s of the flicker phase were fitted with a three-parameter exponential decay (SigmaPlot). The time constants (τ) of the reflectance decreases were calculated from the curves with significant fit coefficients. The group averaged τ values (± SD, $n = 6$) at 569 and 600 nm were found to be 10.9 ± 4.5 and 9.3 ± 3.9 s, respectively, i.e. not statistically significantly different from each other ($P = 0.4$). Furthermore, careful comparison of both reflectance time courses during the first 5 s of stimulation did not reveal any delay between the 600 and 569 nm curves nor a peak in the early part of the 600 nm curve, both characteristics of the intrinsic signals from the cortex [4]. These two features in the 600 nm reflectance were attributed to oxygenation changes in the capillaries and venules [4].

The decrease in reflectance during flicker at both wavelengths can be interpreted based on the model of Hickam, Frayser and Ross [8] as representing an increase in blood volume. This model assumes that most of the light returning from the optic disc has passed through the blood column and has been reflected back through the blood again. The smaller decrease in reflectance at 600 nm is due to the fact that the extinction coefficient of blood at 600 nm is smaller than that at 569 nm. This blood volume increase conformed to recent laser Doppler flowmetry (LDF) measurements from the optic disc in humans [5,10], showing that blood volume increases during flicker with a τ of 12.7 s (9.7–18 s) [10], a value similar to the τ obtained from the reflectance change.

Using the data obtained from the three to four cycles in each eye, we determined the mean ΔR (± SD) over these cycles in response to flicker for each subject (Table 3) and derived a coefficient of variation (CV = 100 × SD/mean) as a measure of the reproducibility of the ΔR. For the tissue on the optic disc CV = 13% and 30% at 569 and 600 nm, respectively. It is much larger for the peripapillary region due to the smallness of the changes.

This study demonstrates for the first time the feasibility of imaging changes in intrinsic optical properties of the human optic disc and peripapillary region in response to increased retinal activity. In its present stage of development, the technique appears suitable for investigating how the functional maps at 569 nm, which reflects blood volume changes, vary when the characteristics of the stimulus and/or the physiological conditions of the subjects are modified. Improvement of the signal-to-noise ratio of the 600 nm signal is needed in order to ascertain whether this signal contains information on the change in tissue oxygenation in response to neural stimulation.

Table 3
Mean over the cycles of ΔR (± SD) in (%) in response to flicker for each subject

Subject	Cycle numbers	Tissue on the optic disc		Peripapillary region	
		569 nm	600 nm	569 nm	600 nm
1	3	−7.3 ± 0.4	−2.6 ± 1.7	0.1 ± 0.9	−0 ± 0.3
2	4	−7.7 ± 2.1	−3.8 ± 1.1	−2.4 ± 1.4	−2.4 ± 2.2
3	3	−16.4 ± 1.2	−7.3 ± 1.7	−7.8 ± 1.7	−4.8 ± 2.3
4	3	−9.4 ± 1.6	−3.6 ± 0.6	−2.9 ± 1.2	−1.7 ± 0.5
5	3	−14.5 ± 2.4	−7.5 ± 1.7	−5.5 ± 1.5	−4.7 ± 1.1
6	4	−8.8 ± 0.5	−2.4 ± 0.5	−2.3 ± 0.7	−1.7 ± 0.7

Each ΔR represents an average over all sites measured on the disc or in the peripapillary region.

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