In Search of an Optoretinogram

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1. Introduction

Physical measures of retinal events, such as the electroretinogram and retinal densitometry, have proved to be quite useful in the study of visual function. Inspired by a number of in vitro studies which have demonstrated changes in the optical scattering properties of photoreceptors during transduction[1-6] , we attempted to observe similar changes in vivo in the human eye. The elusive signal which we sought was dubbed the *optoretinogram,* which, like the electroretinogram, would give a measure of some aspect of retinal activity, but would be based on optical rather than electrical signals. Unlike the electroretinogram, which only gives a gross signal for the whole eye, the optoretinogram could conceivably provide spatial information as well.

A small intense infrared spot was scanned in one dimension across a small area of retina, and the reflected light was collected by a sensitive detector. Modulations of the detector output at the scanning frequency (and harmonics) indicate nonuniformities in the reflected radiation, which in general are large due to retinal features such as blood vessels. In order to measure a change in retinal reflectance due to visible stimulation, a light/dark edge was presented in the middle of the scanned area of retina, and the phase of this edge was reversed periodically. The difference between the modulations in the two stimulus phases was accumulated digitally; static features generate signals that do not depend on the phase of the visible stimulus, and thus cancel in the difference.

2. Optical System

A schematic diagram of the optical system is shown in figure 1. Infrared light was emitted by LED1. This LED was packaged without a bonded lens; the source area was quite small, approximately 0.1 by 0.7 mm. Lens L1 formed an image of the source magnified 10x at position R' which was conjugate with the retina. En route from L1 to R', the beam was bounced off a half circular mirror at mirror/aperture MA1 (see detail in figure 1), and a scanning mirror SM2. Lens L2 was located one focal length from R' and two focal lengths from MA1 and two focal lengths from the subject, thus imaging the source on the subject's retina and MA1 in the subject's pupil. Mirror/aperture MA1 consisted of a small aluminum plate with a 3mm counter sunk hole forming the aperture. A small mirror clamped to the back of the plate covered half of the aperture. This half-circular mirror was imaged in the subject's pupil to form the entrance pupil for the infrared beam. The aperture portion of MA1 similarly formed the exit pupil. Light reflected from the retina followed the same path out, passing through the aperture portion of MA1. Lens L3 imaged the retina on the surface of a silicon avalanche photodiode D1. Mirror M3 bent the beam to make the system more compact. Filter F1 blocked short wavelength light from the detector.

The visible stimulus was a small checkerboard presented in Maxwellian view. Lens L2 served as the Maxwellian view lens for this stimulus. The visible beam was combined with the infrared beam using an optical flat as a beam splitter at BS1. Visible light was produced by tungsten lamp T1, and collimated by lens L4. The collimated beam passed through green filter F2, and a film image of a checkerboard grating at G1. Lens L5 imaged the lamp filament at scanning mirror SM4, which was located two focal lengths from lens L2 and was thus conjugate with the subject's pupil. Grating G1 was positioned so that the image formed by lens L5 was located at aperture stop A2, conjugate with the retina. Scanning mirror SM4 actually consisted of two small scanning mirrors mounted close together to allow X-Y scanning. Rectangular aperture stop A2 was used to mask off the edges of the checkerboard grating.

Figure 1: schematic diagram of optical system (see text).
Detail at right shows mirror/aperture MA1.

3. Signal processing

A block diagram of the electronics appears in figure 2. The infrared source LED1 was pulsed at 100kHz, with a duty cycle of 20%. This allowed a higher peak power output that would have been possible with continuous operation. The motivation was to improve signal-to-noise ratio by rejecting dark light noise from the photodetector during the off periods. A

circuit measured the current delivered the the LED, which was assumed to be proportional to light output. This pulse was multiplied with the detector output using 4 quadrant analog multiplier AM1. This signal was then passed through lowpass filter LPF1 to remove the 100kHz pulse frequency.

Figure 2: block diagram of signal processing system (see text).

The scanning mirror SM2 which deflected the measuring beam consisted of a small mirror mounted on a torsion bar, which oscillated at 3.5 kHz, the resonant frequency of the resulting mass-spring system. This scanner incorporated a velocity pickup coil, which proved essential to the operation, because the resonance of the mechanical system was so sharp that it was impossible to drive the mirror with a conventional function generator due to frequency drift in the latter. A special circuit was therefore developed which fed back a suitably phase-shifted version of the velocity signal to the drive inputs, making the whole thing a self-resonant oscillator. A variable gain stage stabilized the amplitude of oscillation to a level set by an external potentiometer.

Signals in the detector output at the scanning frequency were demodulated by multiplying the signal at the output of the first filter LPF1 with the scanner signal in analog multiplier AM2. The phase of the multiplying scanner signal was adjusted by using the scanner velocity signal to trigger a phase-lock function generator with a variable phase delay control. The output of this generator was used as the input to the multiplier. The scanning frequency was then removed by a second lowpass filter LPF2. The final demodulated signal was read by a 12 bit analog-to-digital converter (ADC), and stored by computer.

4. System operation

The subject stabilized his head with a bite bar and fixated a small lamp, placing the stimulus on temporal retina at 10 degrees eccentricity. The checkerboard grating was windowed by aperture A2 so that the subject saw a small patch 3 check wide by 2 check high. Every two seconds scanning mirror SM4 deflected the grating by one check width. Seen through the stationary aperture, the pattern appeared to reverse polarity while remaining fixed in position. The infrared scan was centered on the central horizontal light/dark edge of the visible pattern. The signal was sampled by computer at 4 Hz Data were accumulated for 2 minutes.

5. Results

Repeated runs of the experiment yielded no systematic signals. Based on measurements of the output noise level of the silicon avalanche photodiode and the strength of the reflected signal, we calculated the expected noise level in the digitally averaged signals to have a magnitude equal to the signal expected from a change in reflectance of 0.01%.

6. Discussion

In spite of our failure to measure significant changes in the retina's light scattering properties caused by visual stimulation, we nevertheless hold out some hope that using a suitably modified apparatus it might be yet possible to observe an optoretinogram. Bennett and Clerc[2] observed scattering changes in a suspension of disc membranes, in which the three dimensional orientations of the discs were presumably distributed randomly in a chaotic fashion. It is conceivable that the scattering changes are most pronounced at particular angles of incidence; our apparatus could only measure changes in the direct backscatter from axial incidence.

Advances in CCD camera technology may proved a way to improve sensitivity by collecting data from an array of spatial locations simultaneously. It may also be possible to realize small increases in sensitivity by exploiting the polarization dependence of scattering phenomena by inserting suitable polarizers in the illumination and measuring beams.

References

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