Measurement of the eye pupil response to light stimuli with regulated waveform, wavelength and photopic level

Anna Sobaszek 1, Wioletta Nowak 1, Andrzej Hachoł 1, Zbigniew Moroń 1

1 Wroclaw University of Technology, Institute of Biomedical Engineering and Instrumentation, Wroclaw, Poland

E-mail: Anna.Sobaszek@pwr.wroc.pl

Abstract. All articles must contain an abstract. The abstract text should be formatted using 10 point Times (or Times Roman, or Times New Roman) and indented 25 mm from the left margin. Leave 10 mm space after the abstract before you begin the main text of your article. The text of your article should start on the same page as the abstract. The abstract follows the addresses and should give readers concise information about the content of the article and indicate the main results obtained and conclusions drawn. As the abstract is not part of the text it should be complete in itself; no table numbers, figure numbers, references or displayed mathematical expressions should be included. It should be suitable for direct inclusion in abstracting services and should not normally exceed 200 words. The abstract should generally be restricted to a single paragraph. Since contemporary information-retrieval systems rely heavily on the content of titles and abstracts to identify relevant articles in literature searches, great care should be taken in constructing both.

1. Introduction
The testing of the pupil size (co-called dynamic pupillometry) can be used in different medical studies such Vision and Ophthalmology [4-8], Psychology and Psychiatry [9-15] and Psychopharmacology [16]. The pupil size is controlled by two antagonistic iris muscles: the sphincter (which is under parasympathetic innervations) and the dilator (which is under sympathetic innervations). The most popular types of the pupil dynamic behaviors are the Pupil Light Reflex (PLR), which is the pupil constriction elicited by an increase in retina illumination and the Spontaneous Fluctuation in the Pupil Size (SFPS), which is the small changes of the pupil size under normal conditions. Recently, an accurate measurement of the PLR to chromatic light stimuli (with controlled wavelength and photopic level) has become increasingly important in vision research, since it has been discovered in the human retina a melanopsin-associated photoreceptive system (intrinsically photosensitive retinal ganglion cells-ipRGC) aside from the conventional rod-cone system [17-25]. This system conveys photopic information for ancillary visual functions, such as pupillary light reflex and circadian photoentrainment and there is parallel between the behavior of these retinal ganglion cells and the pupil reflex to chromatic stimuli. The characteristic features of PLR is that it is consensual reaction. When one eye is exposed to light (the direct reaction) the second also reacts in a synchronic way (the consensual/indirect reaction). The PLR reflex pathway includes: afferent way from retina, central processing in the brainstem, the activity of the autonomic nervous system and the iris muscles (Figure 1).
Several commercial dynamic pupillometers based on infrared video systems exist. These systems can measure the pupil size with a sampling rate between 5 Hz and 250 Hz and with a spatial resolution between 0.1 mm and 0.05 mm. Some of them can regulate the level of illumination; however the authors could not find any commercial pupillometers which allow regulating parameters of the light stimuli, such as wavelength, photopic level or waveform.

The objectives of this study were:

- to develop a computer-controlled chromatic stimuli module in Maxwellian–viewing configuration which allow controlling the light stimuli parameters as follow: wavelength, waveform and photopic level,
- to integrate and synchronize the stimuli module designed with dynamic pupillometry system,
- to test the possibilities of the integrated system.

The remainder of this paper is organized as follows: Section 2 describes the pupillometer system, the assumptions, the structure and the operation principle of the stimuli module. Section 3 present the experiment and example results used in this study. Discussion is given in Section 4.

2. The system

The dynamic pupillometry system is composed of PC computer, IR illumination module, recording module and analysis module. These modules are supported on an ophthalmologic slit lamp base equipped with a joystick control. A standard adjustable ophthalmic forehead rest and a chin rest are also fixed to the slit lamp base and provide an adequate stabilization of the subject’s head during the measurement. To avoid changes in gaze position, a red spot (0.5 cm in diameter) is positioned behind the chinrest at the distance of 5 m.

Infra-red illumination, composed of LEDs arrays (\(\lambda=850\) nm, the total illumination of the eye surface is about 3 mW/cm\(^2\) ), is used for imaging the pupil in darkness. Infrared light is reflected by the corneal surface and projected to high-speed high-resolution camera. The camera (Photon Focus, MV-D1024 E), which is focused on an eye of the subject under test by means of an objective with a 75 mm focal length, is a digital monochrome progressive scan camera. The Matrox Solios type frame grabber allows real time storage of the camera output. The PC stores the acquired data (in a gray-scale pupil images in bmp format) and operates, through an ad-hoc software developed by the authors under Visual Builder environment, to get, frame by frame, the measure of the pupil parameters. The camera enable us to record pupil sizes at a sampling rate of at least 100 Hz, which gives time resolution of \(1/100\)Hz = 0.01 sec for 100 Hz. The accuracy of the applied timer is in the order of 0.4 µs, and therefore the error in the time measurement can be neglected.
The spatial resolution of the camera’s sensor is 0.0106 mm. The system resolution was determined experimentally by testing black circle phantoms with known diameters (similar to real pupil). The accurate values of phantoms diameters were measured by using microscope with accuracy better than 0.01 mm, and they were equal 2.69 mm, 4.98 mm and 7.20 mm respectively. The stand with phantoms was placed at a constant distance in front of the pupillometer (the distance corresponds to the cornea position during measurements) and their size was measured in the system. The phantom size (in mm) was divided by the obtained result (in pixels) for each phantom respectively. The ratio values were about 0.0155 mm/pixel for all phantoms. By rounding up this value, the spatial resolution of the proposed system is estimated to 0.02 mm.

The only way to verify the accuracy of the measurement system is to do measurements and then compare the measured values with the values assumed to be accurately known (phantoms used for determining of the system resolution were considered). The system accuracy (i.e. percentage of absolute error in measurement range) was ±1.16%, ±0.63% and ±0.43% for 2.69 mm, 4.98 mm and 7.20 mm respectively. To find the system repeatability one operator measured the same phantom few times without moving it between measurements. The repeatability (i.e. maximum standard deviation in the measurement range) was ±0.01 mm, ±0.03 mm and ±0.03 mm.

Taking into consideration the pupillometry system configuration and requirements to chromatic stimuli for PLR, the proposed stimuli module should fulfil the following assumptions:

- retinal area which is stimulated is constant and independent from pupil size,
- the stimuli parameters are adjusted by operator utilizing the designed software,
- the stimuli parameters are regulated as follow: wavelength (λ=470 nm, λ=534 nm and λ=640 nm), photopic level (from 1 cd/m² to 1000 cd/m²) and waveform (a single pulse and a series of pulses (flicker), time of light ON and light OFF can be set freely),
- the stimuli is synchronized with recording process,
- the stimuli is mounted inside the pupillometery system in such way that enable to measure both direct and indirect pupil light reflex.

The integrated system configuration depends on type of pupil reaction which is measured. Idea how to mount the stimuli module with relation to recording pupillometry module in case of testing direct and indirect pupil light reflex is presented in Figure 2.

![Figure 2. The integrated system configuration.](image)

Figure 2.a. presents the scheme of integrated system configuration for recording direct PLR of the left eye, whereas Fig.2.b. shows the scheme for recording indirect PLR of the left eye.
In case of direct PLR (Fig. 2.a.), the light stimuli module is located in axis of the examined eye while camera is directed to the examined eye with an angle $\theta$ (value of this angle is controlled and known, and is used in procedure of determining pupil size). In case of SFPS and indirect PLR recording, camera is arranged on axis of the examined eye, whereas the light stimuli module is located in axis of the stimulated eye.

IR illuminator is mounted below the camera. To avoid changes in gaze position, a red spot (0.5 cm in diameter) is positioned behind the chinrest at the distance of 5 m. To examine the left eye, subject is requested to fix their gaze on a spot located 10° to the right. To examine right eye, participant should to fix their gaze on a similar spot 10° to the left.

To study PLR, the computer-controlled chromatic stimuli module is designed. The light beam emitted by the diode (LED) is focused by the two lens into a Maxwellian view (Fig. 3.) on the pupil centre of the examined eye. It allows keeping the constant retinal luminance regardless of the pupil size, i.e. a beam of light stimuli falls on the centre of the pupil, and the diameter of the light beam is smaller than the minimum diameter of the pupil. A retinal area with a diameter of about 20° is stimulated.

The light stimuli controller was build using LabJack LV-3 module. The stimuli is synchronized with recording process. The stimuli parameters are adjusted by operator utilizing the designed software and they can be regulated as follow: wavelength ($\lambda=470\text{nm}$ (blue), $\lambda=534\text{nm}$ (green) and $\lambda=640\text{nm}$ (red)), photopic level (from 10 cd/m$^2$ to 1000 cd/m$^2$) and waveform (constant level light (I), a single rectangular light (II), a series of rectangular light pulses (flicker) (III), a positive and negative step (IV)). Time of light ON and light OFF can be set freely. The types of stimulation lights are shown in Figure 4.

3. Experiment and results
The experiment is performed in darkened room and is preceded by few minutes period to allow the subject’s eyes to adapt to the darkness, and to calibrate the instrument. The measurement time can be manually defined. Before the beginning of the experiment, correct adjustment of eye position with respect to the pupillometer measurement head is required. While setting up the pupillometer, a display camera is continually updated, and the operator can view this on the monitor. During recording, a subject is asked to keep his eyes open, to look straight at the fixation point, and to avoid blinking and moving their head.

Figures below are examples of results showing pupil dynamics waveforms recorded for a woman of 34 years of age, who did not have any eye disease. A consensual reaction in the right eye was tested. In this paper, the nine conditions are defined as follow: bh (blue, 1000 cd/m$^2$), bm (blue, 100 cd/m$^2$), bl
(blue, 10 cd/m²), gh (green, 1000 cd/m²), gm (green, 100 cd/m²), gl (green, 10 cd/m²), rh (red, 1000 cd/m²), rm (red, 100 cd/m²) and rl (red, 10 cd/m²).

Figure 5 present PLR following single blue light pulse of different time duration.

When a light is turned on, there is a rapid-onset, high velocity pupil constriction until the pupil reaches a minimum size (the maximum constriction amplitude). This early transient pupil light response is quickly followed by pupillary re-dilation, to a more sustained state of partial pupil constriction that continues for the remainder of the light stimuli. An interesting feature of the light reflex is its tonic nature in bright light: constriction is held steady under continuous illumination.

Figure 6 shows PLR following light stimulus of 10 sec duration, with different photopic levels for short and long wavelengths.

The qualitative analysis of the characteristics presented shows that:

- Blue light stimulation produces a greater pupil constriction amplitude at both intensities than red light stimulation,
- The sustained pupil response to blue light shows no adaptation (escape after an initial transient response) compared to the response to red light, which shows adaptation,
The sustained pupil responses consists of more oscillation at low photopic levels, for both blue and red light, compared to responses at high photopic levels, although it seems that the characteristics for red light at high photopic levels and for blue light at low photopic levels are very similar.

The sustained pupil light response shows more oscillations for red light stimuli, at both low and high photopic levels.

The results confirm the results of similar research published in well-known literature [2]. Figures 7, 8, 9 present PLR following light stimulus of 7 sec duration with different photopic levels and wavelengths. Stimuli starts 0.5 sec after start of recording.

**Figure 7.** Pupil light reflex (noise attenuated) to single light pulse of 7 sec duration, photopic level 1000cd/m² and three different wavelengths (red, blue, green).

**Figure 8.** Pupil light reflex (noise attenuated) to single light pulse of 8 sec duration, photopic level 100cd/m² and three different wavelengths (red, blue, green).
At light ON, the pupil rapidly constricted and the constriction was maintained for the duration of the stimulus. At light OFF, the pupil dilated to the original size. It is worth to emphasize that the ipRGC closely matched the observed pupillary responses in time course.

Figure 9 and Figure 11 presents PLR to series of light pulses. The pupil light reflex to series of blue light pulses of different frequency is presented in Figure 10. PLR to series of blue light pulses of frequency 1Hz with different photopic levels is presented in Figure 11.

When stimulating pupils with a series of pulses, increasing the frequency of the pulses results in a smaller change in the pupil size. In part, this is because the pupil does not have sufficient time to dilate before the next light pulse.

In case of pupil light reflex to series of blue light pulses of different photopic levels, it is observed that the pupillary responses for high photopic level are significantly greater in comparison to low and medium photopic level.
4. Discussion
We have shown the structure and abilities of the stimuli module designed which was integrated with dynamic pupillometry system. The presented system allows the measurement of the direct and indirect Pupil Light Reflex when subjected to pulse or flicker with controlled photopic level, wavelength, time duration (for pulse) and frequency (for flicker). Our results confirm that the proposed stimuli module enable to the detection of difference in the PLR to different wavelengths light stimuli of long (red), medium (green) and short (blue) light in low (10 cd/m²), medium (100 cd/m²) and high (1000 cd/m²) photopic conditions. Further study will aim of testing the repeatability of the PLR in response to chromatic stimuli and assessing those responses taking into consideration the melanopsin - associated photoreceptive system activity.

5. References
[1] S. Peirson, R.G. Foster,” Melanopsin: another way of signaling light”, Neuron, vol. 49, no.3, pp. 331-339, 2006.
[2] P.D. Gamlin, D.H. McDougal, J. Pokorny, „Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells”, Vision Research, vol. 47, no.7, pp. 946-954, 2007.
[3] E. Loewenfeld „The pupil anatomy, physiology and clinical applications”. Ames: Iowa State University Press, 1993.
[4] F.D. Bremner, „Pupill assessment in optic nerve disorders”, Eye, vol.18, pp. 1175-1181, 2004.
[5] H. Wilhelm, B. Wilhelm, „Clinical applications of pupillography”, J. Neuroophthalmol., vol. 23, no.1, pp. 42-49, 2003.
[6] Kawasaki, S. Crippa, S. Anderson, R.H. Kardon, „The Pupil Response to Large Regional Stimuli in Patients with Focal Visual Field Loss”, Neuro-Ophthalmology, vol. 29, no.4, pp. 143-147, 2005
[7] Kawasaki, R.H. Kardon,” Disorders of the pupil”, Ophthalmol. Clin. North Am., vol.14, no.1, pp. 149-168, 2001.
[8] C.A. Girkin, „Evaluation of the pupillary light response as an objective measure of visual function”, Ophthalmol. Clin. North. Am., vol. 16, no.2, pp.143-153, 2003.
[9] P.L. Franzen, D.J. Buysse, R.E. Dahl, W. Thompson, G.J. Siegle, „Sleep deprivation alters pupillary reactivity to emotional stimuli in healthy young adults”, Biological Psychology, vol. 80, pp. 300–305, 2009.
[10] G.J. Siegle, E. Granholm, R.E. Ingram, G.E. Matt, „Pupillary and reaction time measures of sustained processing of negative information in depression”, Biological Psychiatry, vol. 49, no. 7, pp. 624–636, 2001.
[11] B. Wilhelm, H. Wilhelm, H. Lüdtke, P. Streicher, M. Adler, „Pupillographic assessment of sleepiness in sleep-deprived healthy subjects”, Sleep, vol. 21, no. 3, pp. 258–265, 1998.
[12] J.W. McLaren, P.J. Hauri, S.C. Lin, C.D. Harris, „Pupillometry in clinically sleepy patients”, Sleep Med., vol. 3, no.4, pp. 347-352, 2002.
[13] S.L. Merritt, H.C. Schnyders, M. Patel, R.C. Basner, W. O'Neill, „Pupil staging and EEG measurement of sleepiness”, Int. J. Psychophysiol., vol. 52, no.1, pp. 97-112, 2004.
[14] Y. Morad, H. Lemberg, N. Yofe, Y. Dagan, „Pupillography as an objective indicator of fatigue”, Curr. Eye Res., vol. 21, no.1, pp. 535-542, 2000.
[15] W.D. O'Neill, S. Zimmerman, „Neurological interpretations and the information in the cognitive pupillary response”, Methods Inf. Med., vol. 39, no.2, pp.122-124, 2000.
[16] P. Bitsios, E. Szabadi, C.M. Bradshaw, „Comparison of the effects of venlafaxine, paroxetine and desipramine on the pupillary light reflex in man”, Psychopharmacology, vol. 143, no.3, pp. 286-292, 1999.