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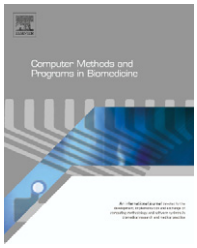

Computerized mouse pupil size measurement for pupillary light reflex analysis

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ABSTRACT

Accurate measurement of pupil size is essential for pupillary light reflex (PLR) analysis in clinical diagnosis and vision research. Low pupil–iris contrast, corneal reflection, artifacts and noises in infrared eye imaging pose challenges for automated pupil detection and measurement. This paper describes a computerized method for pupil detection or identification. After segmentation by a region-growing algorithm, pupils are detected by an iterative randomized Hough transform (IRHT) with an elliptical model. The IRHT iteratively suppresses the effects of extraneous structures and noise, yielding reliable measurements. Experimental results with 72 images showed a mean absolute difference of 3.84% between computerized and manual measurements. The inter-run variation for the computerized method (1.24%) was much smaller than the inter-observer variation for the manual method (7.45%), suggesting a higher level of consistency of the former. The computerized method could facilitate PLR analysis and other non-invasive functional tests that require pupil size measurements.

1. Introduction

Pupillary light reflex (PLR) has been employed in eye disease diagnosis and neurological research [1–8]. PLR requires accurate dynamic measurement of the pupil size (diameter or area) in response to stimulating light under infrared ambient illumination. Traditional measurement by direct observation is not quantitative and pupil diameter changes cannot be easily observed under dim lighting. Infrared imaging systems have been used to capture eye images in darkness for quantitative pupil size measurement [2,3,7–12]. The large quantity of image frames and usually high inter- and intra-observer variations necessitate an automated method for measuring pupil size. The key to such a method is to identify the pupil in an eye image. Pupil identification is also an important step for eye movement measurement, including ocular torsion [8,11,13–16].

The dark pupil in an eye image is usually identified either manually [3] or by a thresholding operation. In a thresholding-based method, pixels below a certain threshold value are classified as pupil pixels [8–15,17]. The pupil center coordinates are then computed as the center-of-mass of the pupil pixels [11,13,14], or determined by fitting a circle [8] or an ellipse [12] to the boundary points of the pupil. The effectiveness of thresholding-based approaches depends on a high pupil–iris contrast. Small laboratory animals often have low pupil–iris contrast and thus a thresholding method may fail to delineate the pupil [7–9]. A "bright pupil" infrared imag-

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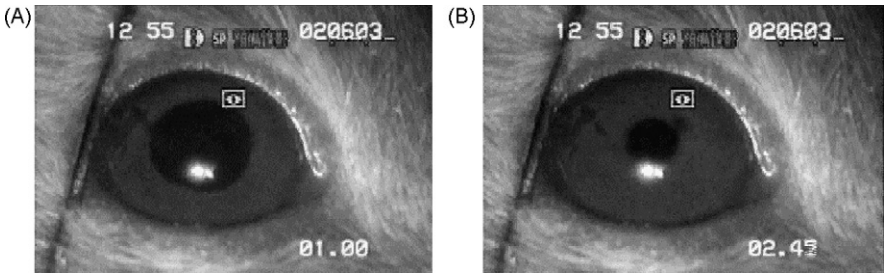

Fig. 1 – Two example mouse eye images: (A) an initial frame before light stimulation, and (B) a frame shortly after the first stimulating light pulse. The time stamp is shown in the bottom-right corner.

ing technique was found to improve the pupil–iris contrast in rabbits, but it required special illumination and collection optics [9]. Furthermore, large errors may occur for eye images containing artifacts, gaps and noises, which arise from eyelids, eyelashes, shadows, and corneal reflections of the light source [8,12,14]. To reduce these errors in thresholding-based methods, superfluous pupil boundary points were identified and excluded from circle fitting [8] or ellipse fitting [12]. In [8], an "interruption exclude algorithm" was used to exclude edge points whose radial distance values were beyond one standard deviation from the mean pupil radius. This algorithm is simple but may fail to exclude a potentially large number of non-pupil edge points [8]. In Ref. [12], four heuristics, based on observed curvature characteristics of the human pupil, were used to eliminate extraneous pupil boundary points. The challenge with this approach is to develop heuristics that are generally applicable.

This paper describes a method for pupil identification in infra-red mouse images based on a region-growing algorithm and an iterative randomized Hough transform (IRHT) [18]. Experimental tests showed that the method is effective and reliable.

2. Methods

2.1. *Experimental animals and image acquisition*

The experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and the protocols were approved by the Animal Care and Use Committee (ACUC) of the University of Missouri, Columbia. Six 8-week-old C57BL/6 wild-type mice and six age-matched mice with retinal degeneration (RD) were used in this research (Jackson Laboratory, Bar Harbor, ME). Retinal degeneration is known to reduce eye responses to light stimulation as indicated by electroretinogram [19,20] and PLR [7]. The mice were kept in darkness for 12 h prior to experiments and were prepared under dim red light. During experiments, the animals were sedated with a mixture of xylazine (10 mg/kg i.m.) and ketamine (50 mg/kg i.m.). The body temperature of the mice was kept between 37 and 38 °C.

Pupil light reflexes were recorded in a dark room (0 lx). Ganzfeld white-light pulses of 10-μs duration were generated with a Grass PS22 Xenon visual stimulator (Grass Instrument Inc. West Warwick, RI). The maximum stimulating light intensity was 0.65 log cd s/m^2 and attenuated with neutral density filters. The luminance was calibrated with an IL-1700 radiometer/photometer (International Light, Newburyport, MA). The flashes were controlled with a UNIBLITZ shutter drive (VMM-T1, Vincent Associates, Rochester, NY) and the stimulus profile was evaluated with a phototransistor.

The mouse eye was imaged with a CCD video camera (Super HAD, Sony, New York, NY) under infrared illumination. A video stopwatch (VS-50, Horita, Mission Viejo, CA) was used to trigger the shutter diver and to start the timing. Video images of the pupil overlaid with timing were recorded with a VCR (SLVN500, Sony, New York, NY) at 30 frames per second. The recorded video was then digitized with a video grabber (Videum 1000 Plus, Winnov, Sunnyvale, CA) into uncompressed three-channel TIFF images of 320 × 240 pixels. The second (green) channel was found to provide the highest pupil–iris contrast and was saved as a monochromatic image for further analysis. Two example mouse eye images are shown in Fig. 1.

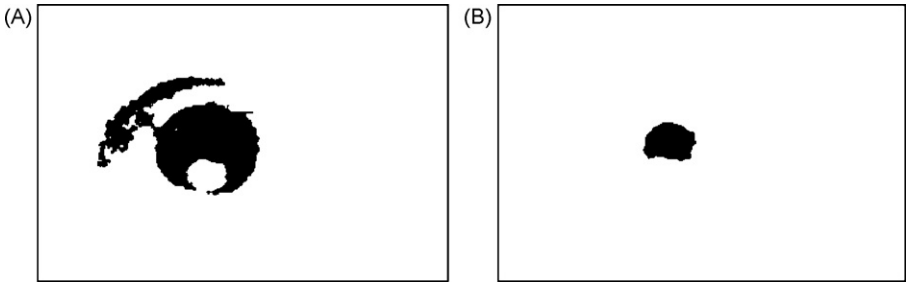

Fig. 2 – Results of the region-growing algorithm: (A) Segmented "pupil region" for Fig. 1A, and (B) segmented "pupil region" for Fig. 1B.