Assessment of Ocular Movement Frequency of Adult Zebrafish in response to Optokinetic Visual Stimulus

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Abstract. The Optokinetic Reflex is a combinatorial physiological response of vertebrates to a surrounding visual stimulus in motion and consists of two categorical phases - a slow-phase pursuit in the same direction to that of the movement of the visual surround and a fast corrective saccade in the contralateral direction. Though OKR is a crucial visual function of research, there remains a dearth of commercially available recording devices. Moreover, computerized tracking of the ocular movement in aquatic vertebrates in response to the visual stimulus from videographic recording remains a challenging area of research. In this backdrop, in the present study two-step developmental research has been undertaken to study the OKR in adult wildtype zebrafish model. A cost-effective and customized OKR recording apparatus has been developed in the lab to study the effect of relevant biophysical stimuli on the ocular movement, in near future. In addition, a novel semi-supervised computer tracking algorithm has been developed to track the frequency of the ocular movement of the fish model in response to the visual stimulus. Such free baseline tracking techniques may aid computer scientists to develop more sophisticated yet cost-effective tracker systems in near future for movement analysis and assessment of visual behavior of aquatic animal models like fish.

Keywords: Optokinetic Response, Saccadic Eye Movement, Zebrafish, Tracking, Semi-supervised Technique.

1 Introduction

The optokinetic response (OKR) is a reflexive behavior in which the eyes track the surrounding objects in motion (see Fig. 1) in order to stabilize the image on the retina [1]. The compensatory eye movements usually consist of two components - the first is a slow phase smooth pursuit eye movement (SPEM) that occurs in the same direction as that of the surrounding image in motion and the second is a fast saccadic phase (nystagmus) that occurs in the contralateral direction [2].

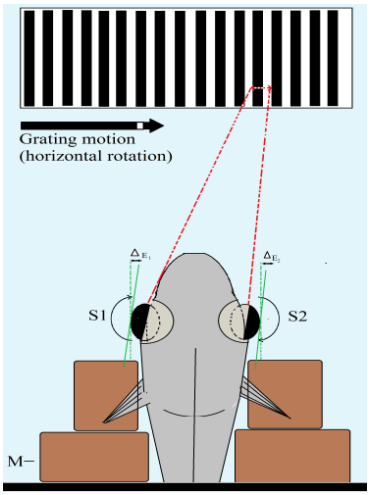

Fig. 1: Illustrated Visualization of OKR
Δ_{E1} and Δ_{E2} – angular eye movement with rotation of surrounding image, S1 and S2 – compensatory (reflexive) eye movements in response to the grating in motion, M – Vise for mounting of fish for fixation of cephalic portion

OKR also serves as a crucial parameter for objectively measuring visual acuity (the ability of the eye to distinguish between two closely spaced objects at a given distance) i.e., the resolving power of the eye and for determining infant visual capabilities [3]. Motion perception is an important sensory modality because motion of the visual image induced by self-motions or by surrounding factors can significantly degrade visual acuity. Therefore, the visual system must compensate for such reduced resolution in order to restore the visual acuity. [4]. The OKR is thus a pivotal neuronal behavior for animals because it helps to maintain the optimal visual acuity, which is of vital importance for orientation in space, hunting for prey, or escaping from predators [5]. To induce OKR in the laboratory the visual stimulus generally used is an alternate black and white striped drum that revolves around the subject [6]. In humans, to record OKR in a laboratory, the subject must be seated in an examination chair, wearing goggles where the visual stimulus is displayed while the surrounding light is turned off. The pupils must be clearly visible in order to record the saccadic eye movement through the examination monitor. The subject is then instructed to fixate the gaze on the target point (around 1 m away). The eye position is then calibrated by instructing the patient to follow the target point as rapidly as possible using only ocular motion; that is, while keeping the head motionless [7]. OKR is robust, highly conserved among vertebrates and develops during early developmental stages and persists till adulthood [8]. It is considered an important response for study because it can be used to assess conscious perception, diagnose lesions of the visual and vestibular pathways and diseases associated with oculomotor deficits like Parkinson's [9]. The first ever recorded application of OKR (1950) in research was that of the pigeon model [10].

1.1 Study highlights

Zebrafish is now considered a standard animal model to study visual physiology owing to the similarity of their retina to other vertebrates [11, 12]. More recently, as adult visual behavior in zebrafish has become an important topic of research, development of research methods to measure specific responses are on the rise [13, 14]. Till date probably, only one type of commercial OKR instrument (VisioBox 2.0, manufactured by Viewpoint Behavior Technology, Europe) is available that can be used to study the response in adult zebrafish [15]. As a result, most of the studies conducted on OKR assessment of zebrafish have developed laboratory-based OKR instruments. In view of this in the present study a cost-effective and customized OKR apparatus has been developed in the laboratory to study the optokinetic response of small-sized fish like wildtype zebrafish. Till date some research works have developed software to automatically record the OKR of mostly larval fish in terms of change in visual angle before and after stimulus presentation [16, 17, 18]. However, in the present study since the broad objective of developing the experimental apparatus is to study the influence of several biophysical stimuli on the OKR of the model fish, quantifying the frequency of the ocular movement in presence of the surrounding visual stimulus remains an important area of research. The presence of tracking software to count the no. of visual saccades in zebrafish is comparatively less [19, 20]. In light of this, in the present study an attempt has been made to quantify the frequency of the oscillatory eye movement of the fish by using a novel semi-supervised object tracing technique. This method incorporates both, region-based segmentation, which focuses on identifying specific areas of interest based on pixel similarities and edge-based segmentation, which detects the boundaries of the ocular region by analyzing changes in pixel intensity. Once the ocular region of the zebrafish was accurately identified, detailed statistical analysis was performed to quantify the frequency of the fish's oscillatory eye movement.

2 Methodology

2.1 Evoking OKR in adult zebrafish using the lab-built OKR apparatus

For the study to induce OKR the lab-built OKR recorder was used (see Fig. 2). The apparatus consisted of a center-fixed surround rotating drum, a fixed platform for placing the mounted fish to be used in experimentation, a stimulus display region for presenting the visual stimulus consisting of alternate black and white stripes (see Fig. 2) and an electrical unit for controlling the speed and direction of the rotating visual stimulus. Adult zebrafish were procured from registered fish breeder and maintained in the institutional Animal House Facility (registration no. 2146/GO/Re/S/22/CPCSEA). After 10 days acclimatization period 3 adult zebrafish were used for the study. For mounting the fish, hypothermia induced anaesthesia was used and the fish were securely placed inside a glass cylindrical chamber that allowed the fish to have a clear visual field of the stimulus (see Fig. 4). Following this the surrounding wall of the drum was rotated using the electrical unit of the apparatus to

induce the characteristic ocular movement in the fish. The visual stimulus was presented for a period of 10 s. The experimental results were digitally recorded using a mobile phone camera (resolution 1080 x 1920 pixels and around 30 frames per second). The experimental protocol was approved by the Institutional Animal Ethics Committee (proposal no. ISI-IAEC/2022/01/01).

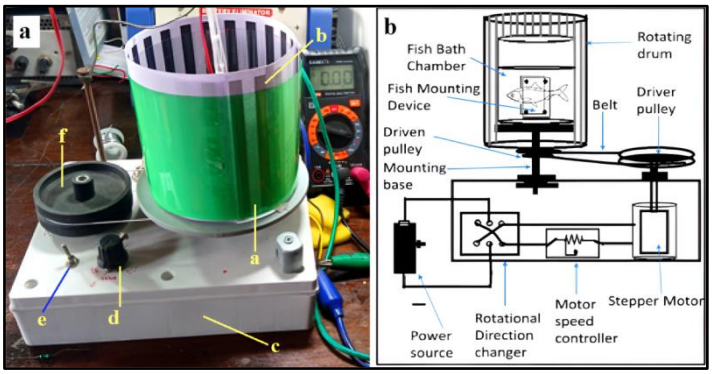

Fig. 2: The lab-built OKR Recorder (a) and the schematic representation of the whole experimental set up (b).
a - rotating drum, b - interchangeable grating pattern, c - electrical unit, d - speed controller, e - direction modulator, f - pulley

2.2 Tracking of the frequency of the ocular movement

In order to carry out the task of quantifying the frequency of the oscillatory eye movement of the fish a novel semi-supervised object tracking technique has been applied. The overall technique is assembled in two parts. The first part consists of computer vision techniques, which deals with the identification of the ocular region of the zebrafish, and the later part deals with the statistical analysis which targets in calculating the total number of oscillatory eye movement. For the first part of the tracking process, both region-based as-well-as edge-based segmentation techniques have been applied for proper identification of the boundary of the region of interest (RoI). The boundary identification of RoI has been carried out in six steps. In the very first step, human interpretation has been incorporated to crop out the region from video containing the zebrafish. Basically, this step has been implemented in the work in order to avoid the computational complexity and to reduce the computational time. In the later step, simple threshold-based segmentation given by,

$$g_i(x,y) = \begin{cases} 1 \; if \; I_i(x,y) \; \leq Th \\ 0 \; if \; I_i(x,y) \; > Th, \end{cases} \tag{1}$$

has been applied on each and every frame extracted out from the cropped video with a view to segment out the zebrafish from the extracted frames. Here, in equation 1, the term $I_i(x,y)$ denotes the pixel intensity of the i *-th* frame at (x,y) coordinates, the

term $g_i(x, y)$ denotes the segmented output and Th indicates the threshold value. Followed by this thresholding technique, area - based image morphological operation has been applied, where image disconnected component was analyzed to remove the redundant regions from the segmented image [21, 22]. That is, if the segmented image component doesn't satisfy the area threshold criteria is removed from the segmented image. In the next stage, the filtered segmented image is divided into four quadrants, and the quadrant with no changes is labeled as zero, to reduce the computational complexity. At the final stage of ocular region's edge detection, binary quadrant with label one is extracted out and the edge of the retained region is detected using canny edge detection technique [23]. Now, at the final phase of tracking of the frequency of the ocular movement, a two-dimensional graph is generated based on the coordinate points of the detected canny edge for each frame of the cropped video, in such a way that x-axis of the graph replicates the pixel coordinate location along x-axis in binary quadrant with label one, similarly, y-axis of the graph replicates the pixel coordinate location along y-axis in the same quadrant image. Then after statistical data analysis has been carried out based on the plotted graph, where variance in the *y* data for each x-coordinate has been calculated using the formulation,

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - \overline{y})^2}{n-1}, \tag{2}$$

where, n denotes the total number of extracted frames. Based on these variance values, the x-coordinate with maximum variance is located. This particular x-coordinate with maximum variance value in turn resembles the point in zebrafish ocular which is having maximum number of movement frequency. Followed by this, Gaussian filter has been applied over the y data for corresponding x-coordinate to eliminate the small fluctuation in *y* value that occurs due to surrounding conditions of the experimental setup, like, movement of water, lighting condition during capturing of video, etc. Thereafter, the frequency of ocular movement has been calculated based on the number of detected peaks in the filtered *y* data using the formulation given by,

$$P = \sum_{i=1}^{n} \mathbb{I}\left(f(i, y_i) > f(i-1, y_{i-1}) \wedge f(i, y_i) > f(i+1, y_{i+1})\right), \tag{3}$$

where, the notion $\mathbb{I}$ resembles the indicator function that is 1 if the condition is true and 0 otherwise. The results obtained using the developed algorithm were validated by comparing the ocular frequency with those obtained through manual counting using the frame-by-frame option (total 300 frames) of the VLC media player. The overall block diagram of the proposed technique of ocular movement frequency tracking is shown in Fig. 3.

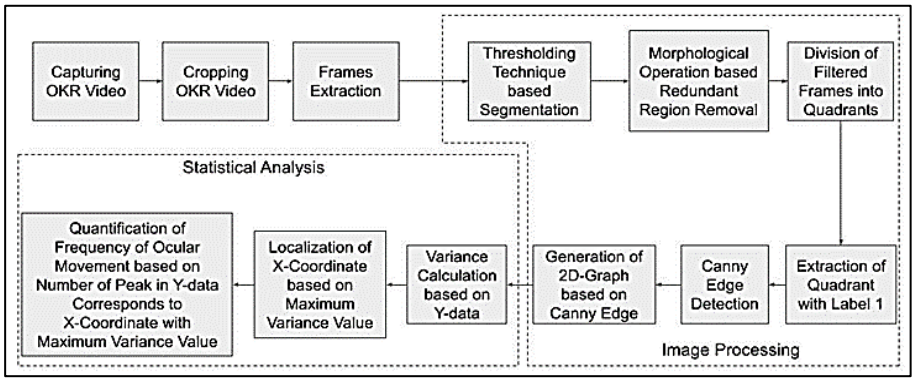

Fig. 3: The block diagram of the proposed technique of ocular movement frequency tracking.

3 Results

In the present study the speed of the rotating drum was kept fixed at 1.05 rad.s^{-1} (10 rpm). The spatial frequency of the visual stimulus was computed to be as 0.2 cpd using the following equation (4).

$$cpd = \frac{1}{(2\ tan^{-1})}\left(\frac{h}{2a}\right), \quad (4)$$

where a is the distance between the center of the eye lens (see Fig. 3) and the grating wall, and h is the length of one cycle of the grating (start of black band to end of white band) at which OKR was observed (see Fig. 4).

In the study the frequency of the ocular movement of the three fish tracked and quantified using the developed algorithm has been graphically represented in the Fig. 5 below. In the graph the no. of peaks represents the smooth pursuit eye movement along the same direction of the rotating drum (visual stimulus) and the no. of valleys represents the corrective saccadic movement in the opposite direction (Table 1). The results has been compared with the output obtained using the classical method of optical flow [24].

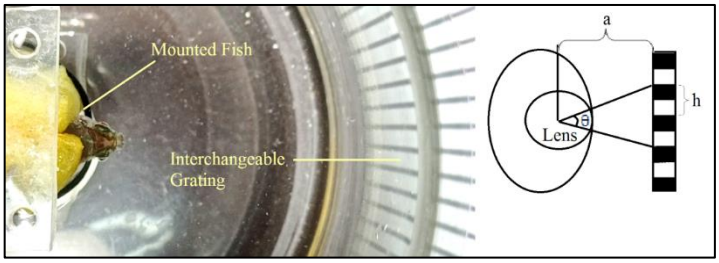

Fig. 4: Top-view of the interior segment of the OKR recorder with the mounted fish placed inside the water filled cylindrical container.

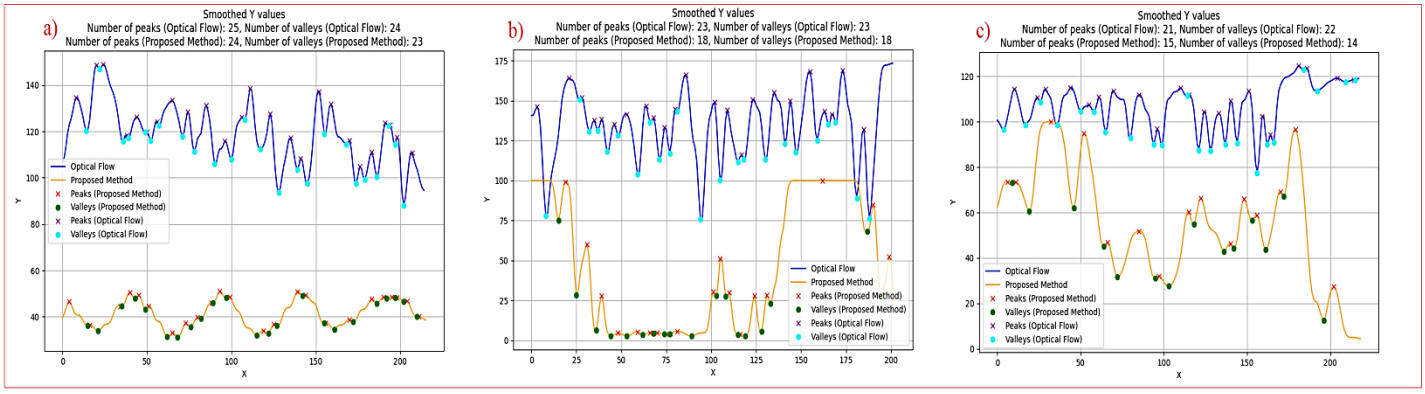

Fig. 5: Comparative graphical representation of the frequency of the ocular movement of a) Fish 1, b) Fish 2 and 3) Fish 3 eliciting OKR in presence of the visual stimulus obtained using the proposed method and the optical flow method

Table 1. Tabular Representation of the number of peaks and valleys obtained from the computerized tracking data of the ocular movement.

N	Actual Count Method	Proposed Method		Optical Flow Method	
	Eye movement Frequency	*No. of Peaks*	*No. of Valleys*	*No. of Peaks*	*No. of Valleys*
1	23	24	23	25	24
2	8	18	18	23	23
3	15	15	14	21	22
AM ± SD	15.33 ± 6.128	19 ± 3.742	18.33 ± 3.682	23 ± 1.633	23 ± 0.816

4 Discussion

The results indicate that the initial attempt to track and quantify the ocular movement frequency using the proposed method may be further used for understanding how different stimuli influence the OKR in adult zebrafish by noting the variation in frequency (no. of peaks and valleys) of the ocular movement. Increasing the sample size in order to improve the tracking accuracy and to further develop the algorithm remains the future goal of the present study. In the present study the frequency of ocular movement computed using the proposed method was validated using the data obtained from manual counting (frame by frame) and was then compared with the classical optical flow method. The comparative results have been represented in Table 1 above. From the resultant graphs it is evident that the proposed technique for ocular movement detection outperforms the existing classical optical flow method.

5 Conclusion

From the study it may thus be concluded that the proposed approach to quantify the ocular movement of zebrafish, generated in response to the visual stimulus may prove to be effective in quantitatively studying the OKR of small-sized fish in terms of frequency of the angular eye movement. The future objective is to implement the convolutional neural network (CNN) based optical flow method for further improvements in quantification of the fish ocular movements.

5.1 Data availability

The three videos used for the study has been uploaded to a repository (link provided below) for future use.
https://github.com/barninibiophysics/Zebrafish.git

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