CG-001

Development of the Method for Estimating Heart Rate in Transparent Fish using Video Imaging

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1. Introduction

Monitoring heart rate in fish plays a crucial role in understanding their physiological state and environmental interactions. Heart rate serves as a key indicator of a fish's health, with abnormal rates often signaling stress or disease. For instance, elevated heart rate may reflect environmental stressors such as poor water quality, sudden temperature shifts, or overcrowding. Continuous monitoring also enables early detection of cardiovascular or systemic disorders, contributing to effective disease management in aquaculture or research settings. In addition to health assessment, heart rate monitoring provides valuable insights into fish behavior and physiological responses. It helps researchers evaluate stress levels during behavioral experiments and understand the fish's reaction to stimuli or environmental changes. Furthermore, in public aquariums or fish farms, heart rate data can be used to improve animal welfare through optimized habitat design and stress mitigation. Traditional methods for measuring fish heart rate often involve implantable devices such as the DST HRT tag—a miniature ceramic data logger that records heart rate and body temperature when implanted in the abdominal cavity. While accurate, these methods are invasive and unsuitable for small or fragile fish species.

In recent years, transparent fish species such as Danionella have emerged as valuable models for non-invasive physiological monitoring, due to their optical clarity [1-5]. Leveraging video imaging techniques offers the potential to estimate heart rate without physical contact, enabling stress-free and continuous assessment. This study aims to develop a method for estimating heart rate in transparent fish using video imaging, with the goal of establishing a non-invasive, accurate, and scalable tool for fish health and behavioral research.

2. Methods

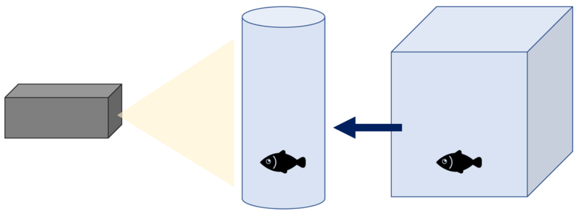
2.1 Video measurement

In this study, we used Danionella—a genus of freshwater fish in the family Cyprinidae—as a transparent model organism for in vivo heart rate monitoring. Due to its remarkable tissue transparency, including the absence of scales and the presence of a fully transparent membrane covering its abdominal organs, Danionella allows for direct visualization of internal organs at a cellular or even subcellular level. These characteristics make it an ideal candidate for physiological imaging and non-invasive cardiac monitoring [1].

Video footage of the fish was recorded using high-definition memory camera (Everio GZ-R470, Japan Victor Company, Tokyo, Japan). The camera features a built-in 32GB memory, approximately 4 hours and 40 minutes of continuous recording time, and a compact body size of 60 × 59.5 × 128 mm with a weight of approximately 302 g. Video was recorded at a frame rate of 30 frames per second (30p), which allowed sufficient temporal resolution to detect heartbeat-induced movements (Fig.1(a)). Each fish was placed individually in a small observation tank with clear water and appropriate lighting to enhance visibility of the heart (Fig.1(b)). The camera was positioned above the tank to capture the heart region from a top-down perspective, minimizing interference from reflections and refractions on the water surface.

Conventional methods mainly involve counting the heart rate by hand using a counter. In this case, the video is recorded in slow motion and the heart rate is counted for one minute. In this study, the conventional method is used as the gold standard and is compared with the proposed method.

(a)



(b)

Fig.1. Measuring transparent fish using a video camera

Transparent fish (Danionella) are usually kept in a 50 cm cube tank. In this experiment, they were moved to a beaker about 10 cm wide and 20 cm long to monitor their heart rates. A video camera was installed near the beaker to record the data. (a) shows the actual shooting, and (b) shows a simplified measurement diagram. (a) shows that the entire shooting was being recorded.

2.2 Automatic measurement using Open CV

To estimate heart rate automatically, we used the OpenCV (Open Source Computer Vision Library), a widely used library in the fields of image processing, computer vision, and machine learning. OpenCV supports pre-trained deep learning models developed using Caffe, TensorFlow, and PyTorch, and includes tools such as cascade classifiers for detecting specific patterns, such as human faces or other objects. For this study, we implemented a custom algorithm to detect the red-colored heart of Danionella and calculate its beating frequency.

The algorithm workflow is described below (Table.1).

Table 1. Flow of automatic heart rate measurement of transparent fish

1	Video Input	The video file containing the transparent fish's heart motion is loaded.
2	Read Frame	Each video frame is read sequentially.
3	Frame Availability	The program checks if the current frame is the last. If so, the process ends.
4	End Process	The program terminates after all frames are processed.
5	Define Red Color Range	Hue, Saturation, and Value thresholds are defined to capture the red hues. Since red spans two distinct HSV ranges (typically 0–10 and 170–180 in Hue), both are included.
6	Create Red Region Mask	A binary mask is generated where pixels within the defined red range are marked, isolating the heart region.
7	Noise Reduction	Optional filtering is applied to further reduce noise.
8	Contour Detection	Contours are extracted from the binary mask.
9	Filter Contours of Appropriate Size (Heart)	Contours are filtered by area and aspect ratio to retain only those that likely correspond to the heart. A Region of Interest (ROI) is defined around the detected heart area to focus the analysis.
10	Set ROI for Heart Region	Calculate Sum or Average of Pixel Values within ROI. For each frame, the sum and average pixel intensity within the ROI is calculated.
11	Record as Time Series Data	The pixel intensity values across frames are recorded as time series data, reflecting rhythmic heart contractions.
12	Heart Rate Counting (Peak Detection)	A peak detection algorithm is applied to the time series to identify individual heartbeats.
13	Display/Save Results	The calculated heart rate is displayed.

The entire process loops from step 2 to 13 for each video frame, enabling continuous tracking and measurement.

The formula for calculating the sum and average pixel intensities within an ROI frame by frame is:

$$S_t = \sum \quad I_t(\mathrm{x}, \mathrm{y}) \qquad (1)$$

S_t: Total pixel intensity (at time t) in the ROI (region of interest) within the frame, I_t(x,y): Intensity of pixel (x,y) within the frame (at time t), all pixels within the ROI (heart region) are (x,y)∈ROI.

The average pixel intensity for each frame is given by the following formula:

$$\mu_t = \frac{1}{n}\sum \quad I_t(\mathrm{x}, \mathrm{y}) \qquad (2)$$

μ_t is the average pixel intensity within the ROI at time t, and N is the number of pixels within the ROI (equal to the number of pixels within the area).

2.3 Analysis method

Common noise removal approaches include filtering (spatial filters, time filters, frequency filters, background subtraction and foreground extraction). This is a method of reducing noise by utilizing the relationship between each pixel in an image and its surrounding pixels. Gaussian filters and median filters are useful for smoothing images and suppressing sudden changes in images (noise). For continuous image data such as videos, methods are also known for identifying and removing noise by analyzing changes in the time direction (between frames), such as moving average filters and Kalman filters. Frequency filters are a method of converting image data into the frequency domain and removing specific frequency components (parts corresponding to noise), using Fourier transforms and other techniques. For videos of moving fish, background subtraction and foreground extraction can eliminate background noise and noise caused by movements other than fish by separating the stationary background from the moving fish (foreground). In this study, we applied a technique to predict and compensate for visual changes caused by objects moving over time in video compression and image stabilization using motion compensation (MC). This is a method of predicting pixel values in the current frame by taking into account the motion of the previous frame. The most basic motion compensation estimates where a block (or pixel) has moved from the previous frame as a "motion vector (MV)" and generates a predicted image based on the motion vector.

$$F_t(x,y) = P_t(x,y) + E_t(x,y) \qquad (3)$$

Predict the pixel value $F_t(x,y)$ at the current frame F_t's position (x,y) from the previous frame F_t-1. $P_t(x,y)$ is the motion-compensated predicted image. This predicted image is generated by moving the pixels of the previous frame F_t-1 by the estimated motion vector $v = (v_x,v_y)$. The predicted image $P_t(x,y)$ means that the pixel at position (x,y) in the current frame Ft is assumed to have been at position $(x-v_x,y-v_y)$ in the previous frame F_t-1, and its value is used as the predicted value.

$$P_t(x,y) = F_t - 1\ (x-v_x,\ y-v_y) \qquad (4)$$

2.4 Ethics Committee

This study was conducted in accordance with the ethical guidelines established by the Mie University Medical Zebrafish Research Center (MZRC, https://zqsp-mie-u.org/mzrc/system/kyo_det.php?no=2652) and the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions, as stipulated by the Ministry of Education, Culture, Sports, Science and Technology (MEXT, https://www.mext.go.jp/b_menu/hakusho/nc/06060904.htm), Japan. Importantly, this research was observational in nature and did not involve any toxicity testing or invasive procedures. Therefore, it complied with all relevant institutional and national ethical standards for the use of aquatic vertebrate models.

3. Results

One example of a recorded transparent fish is shown (Fig. 2). Table 2 shows a comparison table of heart rates in transparent fish Danionella. The conventional method is a manual count, while the proposed method is a measurement by automatic analysis using OpenCV. The results of the proposed method vary within an error range of about ±5 to 10 bpm compared to the conventional method. It can be confirmed that the proposed method shows good agreement with the conventional manual count and can automatically estimate the heart rate within a small error range.

Fig. 2. One example of a transparent fish photographed

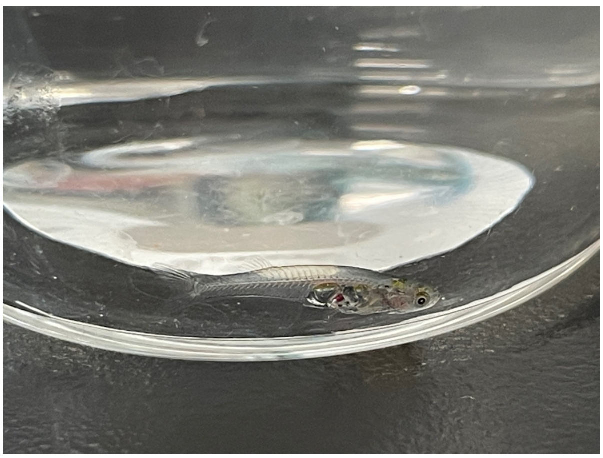


Table 2. Comparison between conventional method (manual counting) and proposed method

Number of measurements	Conventional (bpm)	OpenCV (bpm)
1	158	152
2	162	155
3	155	149
4	152	147
5	148	143
6	159	151
7	147	141
8	166	160
9	153	148
10	145	139

4. Discussion

This study successfully demonstrated that heart rate of the transparent fish Danionella can be automatically measured using image analysis techniques implemented with OpenCV. The proposed method showed good agreement with the traditional manual counting method, with an acceptable deviation of approximately ±5–10 bpm. This result indicates that the image-based approach is a promising alternative for non-invasive and continuous heart rate monitoring in small aquatic organisms.

A major advantage of this approach is its simplicity and scalability. Unlike conventional physiological sensors, the image-based method does not require physical attachment or contact with the subject, minimizing stress and allowing repeated measurements. Additionally, this method enables researchers to visually confirm cardiac motion in real-time, which is a significant innovation in fish physiology and behavioral studies. In actual video compression, motion vectors are typically estimated not only at the pixel level but also at a higher precision level known as subpixel accuracy (e.g., half-pixel accuracy, quarter-pixel accuracy). In this case, the value of $F_t-1(x-v_x,y-v_y)$ is calculated by interpolation (linear interpolation, bicubic interpolation, etc.) if it does not correspond to integer coordinates. In the case of half-pixel precision, since $(x-v_x,y-v_y)$ falls on a non-integer position, it is interpolated from the surrounding integer pixel values.

However, several limitations must be acknowledged. First, we observed that the heart rate of the fish tended to increase temporarily when transferred into a beaker for observation. This stress-induced elevation in heart rate can lead to variability in measurements, especially in the early phase of recording. Ensuring a sufficient acclimation period prior to measurement may help mitigate this issue.

Second, the applicability of the method is currently limited to transparent species such as Danionella, which have gained attention as model organisms due to their optical clarity. In particular, the absence of scales and the transparency of internal organs make it feasible to detect heart movement through video imaging. In contrast, this method is not yet applicable to non-transparent species, as internal structures are not visible through conventional imaging techniques. Developing techniques to extract cardiac signals from opaque fish remains an important challenge for future work. Machine learning and deep learning are one of the most powerful approaches in recent years. It is possible to build a model that learns the pattern of body movement noise from a large amount of data and automatically removes noise based on that pattern. In particular, convolutional neural networks (CNNs) such as U-Net are very effective for image noise removal and image segmentation (separation of fish from noise), while recurrent neural networks (RNNs) and LSTMs are suitable for removing noise from data with temporal continuity such as videos. Therefore, a future challenge is to try machine learning methods. Machine learning techniques are widely used to identify human heart rates, but there are few examples of their application to fish [6-20].

In conclusion, this study presents a novel and effective framework for heart rate estimation using video and computer vision techniques. While improvements are still needed, particularly in reducing stress-related artifacts and expanding applicability to non-transparent organisms, the ability to detect heart rate visually using only video images represents a groundbreaking advance in the field of biological monitoring.

5. Conclusion

In this study, we developed and validated a novel, non-invasive method for measuring heart rate in the transparent fish *Danionella* using video analysis with OpenCV. The proposed system successfully detected cardiac activity and estimated heart rate with accuracy comparable to traditional manual counting. This approach leverages color-based object detection and frame-by-frame analysis, allowing for visual confirmation of heart movement and real-time monitoring. The results demonstrate the potential of computer vision in biological signal analysis without the need for contact sensors. However, current applicability is limited to transparent fish species, and stress-induced heart rate fluctuations during handling remain a limitation. Future research will focus on extending this method to opaque species and improving stability during recording. Our findings highlight the value of image-based physiological monitoring and open new avenues for aquatic animal research.

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