Acquisition, Processing and Analysis of Electrocardiogram in Awake Zebrafish

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***Abstract*—Long-term monitoring of intrinsic electrocardiogram (ECG) in zebrafish plays a crucial role in heart disease studies as well as drug screening. In this work, we developed a polymer-based apparatus with embedded flexible thin-film electrodes to acquire ECG signals of awake zebrafish. The apparatus was made of polydimethylsiloxane (PDMS) using the molding technique with molds formed by 3D printing. A graphical user interface (GUI) was built in *National Instruments* LabView platform for real-time recording, processing and analysis. The program provided important features, such as signal de-noising, characteristic wave detection and anomaly detection. Further, it could operate on both real-time coming signals as well as previously-saved data, facilitating analysis and interpretation. We demonstrated the use of our system to investigate the effects of the anesthetic drug, namely Tricaine (MS-222), on cardiac electrophysiology of zebrafish, revealing promising findings. We speculate that our novel system may contribute to a host of studies in various disciplines using the zebrafish model.**

***Index Terms*— Zebrafish; Electrocardiogram (ECG); Heart Disease; LabView; Automated Processing;**

I. Introduction

THE zebrafish (*Dario rerio*) is an emerging study model for numerous biological investigations in diverse disciplines including pharmacology, toxicology, neurobiology, behavioral and development biology. Unlike mammals', zebrafish hearts fully recover after 20% injury of their ventricle, thereby enabling a crucial model system to study heart regeneration and regenerative medicine [1, 2]. The surgical power of a forward genetic approach in discovering new genes has been demonstrated in lower model organisms such as yeast, Drosophila and C elegans [3-5]. Because of high demand on colony management efforts, this approach appeared to be very difficult in vertebrate, especially on adult phenotypes such as cardiac arrhythmia. During the last decade, Dr. Xu's team at Mayo Clinic has successfully developed a system to conduct insertional mutagenesis screening using a gene-breaking transposon (GBT) cassette, to generate a zebrafish insertional cardiac (ZIC) mutant collection and identify genetic factors of cardiomyopathy [6, 7]. Further, zebrafish have also proven to be an ideal vertebrate model system for phenotype-based screening owing to their physiological similarity to mammals. The major question whether zebrafish models are relevant for human drug screening has been investigated thoroughly and addressed in the past several years, providing evidence-based capability [8].

Along with conventional approaches, zebrafish electrocardiogram (ECG) has been exploited to support studies. During the past decade, our team and others have demonstrated the acquisition of ECG signals from both adult and embryonic zebrafish, revealing a distinct P wave and QRS complex resembling that of humans [9, 10]. In the market, commercially-available systems, *e.g.* the one from iWORX (*Dover, NH*), presented shortcomings that restrain studies, such as *i)* only short period of time can be recorded, which resulted in inconsistent results among different fish; *ii)* the ECG acquisition requires anesthetized animals, rendering it stressful to the fish and inadequate to provide intrinsic cardiac electrophysiological signals; *iii)* due to the high noise, the obtained ECG data is only suitable for manual one-by-one measurement; and *iv)* The T-wave is hardly detectable. To address these, we have been developing various novel tools for acquisition and analysis of zebrafish [11, 12]. For instance, the microelectrode array (MEA) membranes provided ECG with favorable signal-to-noise ratio (SNR), high spatial and temporal resolution [12-14]. However, experiments were still conducted with anesthetized animals. Furthermore, processing and analysis of ECG signals were manually performed, making it difficult for studies as well as high-throughput screenings. Therefore, there is a dire need to establish a system with effective acquisition of awake fish, and ideally automated processing and data interpretation, spearheading a host of important cardiac investigations using the zebrafish model.

In this work, we developed a novel system for ECG acquisition of awake zebrafish including a polydimethylsiloxane (PDMS) housing improved from the previous generation [11] and a LabView graphical user interface (GUI) (*National Instruments, Austin, TX*) for signal processing, feature extraction along with anomaly detection. The small PDMS housings were made using polylactide (PLA)

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3D-printed molds and flexible sputtered-copper strips were embedded as recording and reference electrodes for recording ECG from zebrafish. We successfully demonstrated the use of the novel apparatus and system to investigate cardiac electrophysiology of awake zebrafish under different levels of mild anesthesia.

II. Design, Methods and Implementation

A. PDMS Housing

The apparatus was designed aiming to keep zebrafish comfortable, in order to minimize the unwanted effects and prolong reliable recording. Generally, the length of an adult fish body varies from 17 mm to 33 mm [15], thus the housing was designed with a length of 50 mm, a height of 50 mm and a width of 25 mm. For the ease of demolding after curing PDMS, the mold was split into two pieces as shown in **Fig. 1b.** An additional part (in the middle, blue, **Fig. 1a**) with a height of 43 mm and a maximum width of 20 mm was used to make a tapered shape of the housing to minimize the movement of the fish when loaded. First, the curing agent and PDMS monomer (*Sylgard 184, Dow Corning, Midland, MI*) were mixed together with a ratio 1:10 in weight, respectively. Subsequently, the mixed solution was degassed for approximately 1 hour before being poured into the rectangular mold. Then, the middle part was securely placed. After the PDMS was cured by putting on a hot plate under 100ºC for 5 hours, it was removed from the rectangular box. The middle part was gently pulled out to make sure the apparatus remains the desired shape (**Fig. 1**).

To integrate ECG electrodes, two strips of 125-µm thick polyimide (*Kapton*, *DuPont, Wilmington, DE)* with pre-sputtered Cu electrodes were inserted from the side of the apparatus through two thin-cut slits. The slits were then sealed by applying additional PDMS followed by a post-curing process. The strips were placed so that when the fish is loaded into the housing, the two electrodes would securely position at the chest and abdominal areas, acting as recording (WE) and reference (RE) electrodes, respectively (**Figs. 1c** and **1d**).

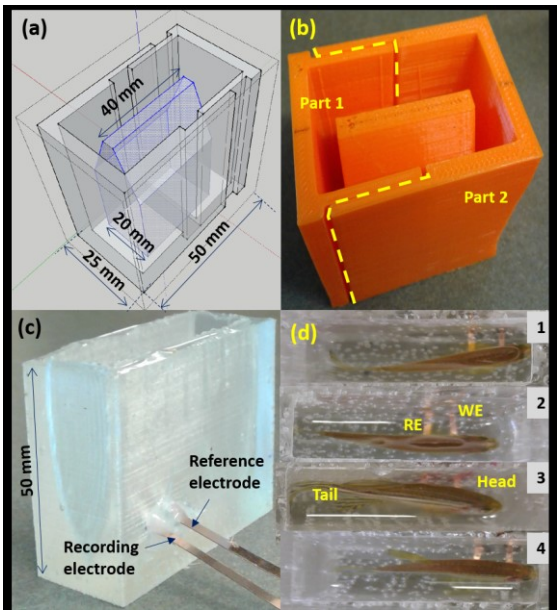

Fig. 1. (**a**) 3D design in SketchUp software; (**b**) 3D-printed mold with parts 1&2 assembled; the dashed line shows the boundary of the two parts; (**c**) PDMS mold with integrated WE and RE electrodes; and (**d**) 4-chamber apparatus with fish.

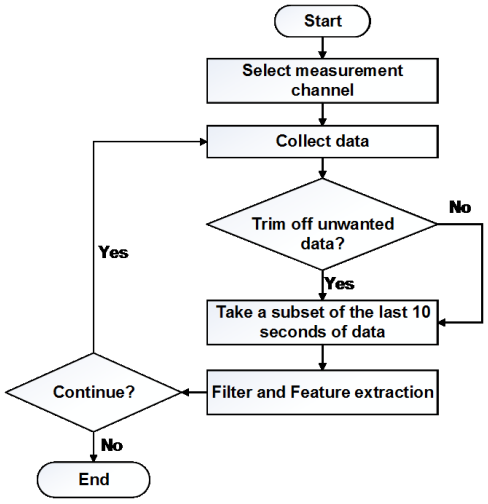

Fig. 2. The flowchart of the LabVIEW GUI.

B. LabVIEW Program for Signal Acquisition and Processing

A LabVIEW GUI was developed to process and analyze zebrafish ECG data in real time [12]. The GUI can also work with saved data. This is to standardize data acquisition and provide automated processing and analyses. An overview flowchart of the software is illustrated in **Fig. 2**.

1) Preprocessing

For real-time recording and processing, first, ECG data are continuously collected at 1000 samples/second. They are checked to look for non-ECG periods, which were due to uncertainties in measurement, such as unexpected strong movement of fish or electrode dislocation, then partitioned into 10-second long segments. Finally, each segment would be de-noised and analyzed along with abnormal detection. The process is iterated until the user stops recording the data.

The method to remove non-ECG sections is illustrated in **Fig. 3**. In every five seconds, the coming data will be checked

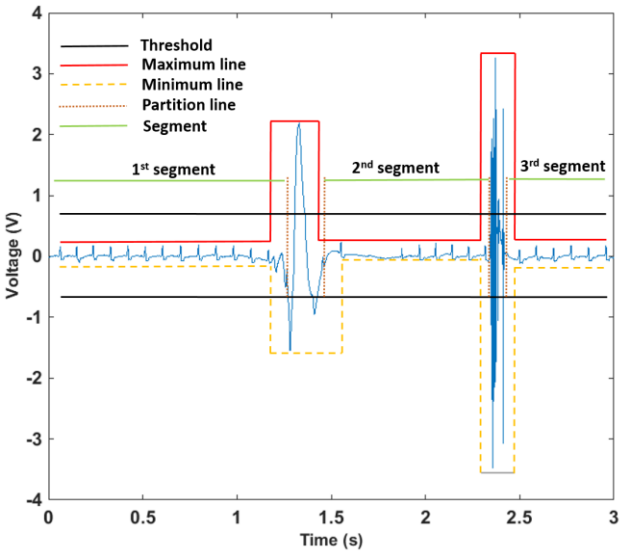

Fig. 3. The illustration of our method to trim off unwanted data.

whether there are the glitches. Specifically, the maximum and minimum values of each interval will be detected (red and dashed orange lines in **Fig. 3**), then two thresholds (black lines) will be set up by taking the average of all maximum points and minimum points in each interval. Therefore, those meaningless points which are higher than the threshold could be found. Then, the distance between two such successive points would be found. If it is greater than 1000 samples, the data will be partitioned into different sub-segments (1st, 2nd and 3rd segments in **Fig. 3**).

Once the non-ECG sections are removed, the data stream would be further processed to remove noise components such as baseline wander (breathing/gill motion interferences) caused by low-frequency and high-frequency noise. First, to remove high-frequency noise from the data, an initial Dolph-Chebyshev low-pass filter is applied using the Bio-signal Filtering VI as shown in Equation (1), which is part of the Biomedical Toolkit of LabView. The Dolph-Chebyshev window filter's Fourier transform is defined by equations reported in [16, 17].

$$W(\omega_k)=\frac{\cos\left\{M\cos^{-1}\left[\beta\cos\left(\frac{\pi k}{M}\right)\right]\right\}}{\cosh\left[M\cosh^{-1}(\beta)\right]} \qquad (1)$$

where

M = Length of the window

α= - Sidelobe attenuation / 20

$$\beta=\cosh\left[\frac{1}{M}\cosh^{-1}(10^{\alpha})\right],\ k=0,1,2,...,M-1$$

Lynch *et al.* [16] comprehensively compared this type of filter with others and the outperforming results were obtained as 1) the computational time was reduced from 6 hours to 3 hours with comparable performance; and 2) the Dolph window gave better attenuation in high-frequency ranges. Here, the Dolph-Chebyshev filter was used with a cutoff frequency of 40 Hz. As a result, the signals greater than 40 Hz were suppressed by a minimum of 40 dB.

The baseline wander was eliminated using the Wavelet Denoise VI, which is a part of Digital Filter Design Toolkit of LabView, with the db06 wavelet. The selection of Daubechies mother wavelet showed the most effective result for de-noising ECG signals [18]. We examined the effectiveness of various mother wavelets by comparing different factors such as root mean square error (RMSE), root mean square bias (RMSB), and L1 norm, and revealed that the Deaubechies mother wavelet outperformed not only in terms of RMSE, but also the preservation of characteristic waves of ECG signals. Once the data were split into several segments with specific interval frequency, a soft threshold would be applied to suppress coefficients which are smaller than the threshold. The new data were then reconstructed based on the new approximation and detailed coefficients.

2) Feature extraction

First, R peaks of the zebrafish ECG signal are detected using the Peak Detector VI within LabVIEW. Specifically, a threshold that equals 50% of the maximum amplitude in the signal is set up and points which are above the threshold value are the R peaks. Second, each R-R interval is used to find the P, Q, S, T wave. The Q-wave and S-wave peaks are searched for as the minimum points 50 ms before and after the R peak, respectively. The T-wave is defined as the maximum value between 15% and 55% of the R-R interval from the first R-wave in the interval. Finally, the P wave is the highest peak 65% to 95% of the R-R interval from the first R-wave in the interval. The R-R interval also is saved for the anomaly detection which is described in next section. Once all the ECG peaks are detected, typical features are calculated and reported in a document as shown in ***Table I***.

To assess the efficacy of the program in de-noising, signal to noise ratio (SNR) calculation was used as reported in [19].

$$SNR=\frac{\sqrt{\frac{1}{b_{s2}-b_{s2}+1}\sum_{k=b_{s1}}^{b_{s2}}\frac{1}{t_{s2}-t_{s1}+1}\sum_{i=t_{s2}}^{t_{s2}}X_k^{\,2}(i)}}{\sqrt{\frac{1}{b_{n2}-b_{n2}+1}\sum_{k=b_{n1}}^{b_{n2}}\frac{1}{t_{n2}-t_{n1}+1}\sum_{i=t_{n1}}^{t_{n2}}X_k^{\,2}(i)}} \qquad (2)$$

where b_{s2}, b_{s1}, t_{s2}, t_{s1} are end beat number, start beat number, end time of the segment and start time for the segment used for calculating the signal, respectively. $X_k(i)$ are the amplitudes. b_{n2}, b_{n1} and t_{n2} are end beat number, start beat number and end time of the segment used for calculating the noise, respectively.

The signal value in Equation (2) was calculated using data from the R-wave of each heartbeat while the noise value was calculated using points from the space between the T-wave and P-wave of each cycle. The time intervals were the same for both the raw and filtered data.

3) Anomaly detection

In addition to finding the ECG features, the program was designed to diagnose various ECG anomalies. We established standards for fish cardiac electrophysiology based on our experience and observations after years working with zebrafish.

TABLE I
SUMMARY OF ECG DATA FEATURES

Feature	Result	Standard deviation	Unit
Number of R peaks	41	N/A	N/A
Average heart rate	73.12	15.09	BPM
Average R amplitude	0.49	0.04	mV
Average normalized P amplitude (in % compared to R amplitude)	0.18	0.11	%
Average RT interval	0.272	0.08	s
RMSSD	0.36	N/A	ms
NN50	29	N/A	N/A
pNN50	74.36	N/A	%

RMSSD: root mean square successive difference of intervals, NN50: The number of pairs of successive R-R interval that differ by more than 50 ms; pNN50: the proportion of NN50 divided by the total number of R-R intervals.

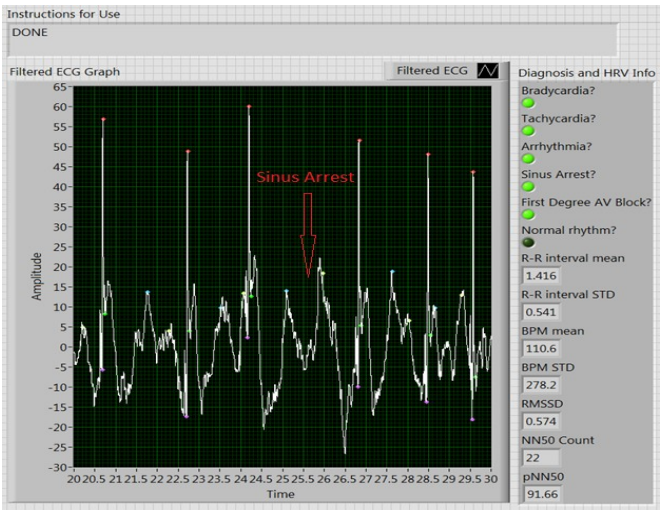

Fig. 4. A zebrafish ECG signal with sinus arrest detected.

Sinus bradycardia which is induced if the heartrate (HR) is less than 90 beats per minute (BPM). The HR value above 150 BPM can be diagnosed as sinus tachycardia. Sinus arrhythmia is a symptom if a difference in R-R interval length between the shortest and longest in the last 10 heart beats is greater than 0.16 seconds. If P-P interval is greater than twice of R-R interval, the signal may be sinus arrest. Finally, the symptom of first-degree AV block is presented when P-R interval is greater than 0.20 seconds long. All anomalies can be detected by calculating the time differences between various ECG features such as R-R, P-R and P-P interval. **Fig. 4** depicts the detection of sinus arrest in a zebrafish ECG signal when comparing the difference between P-P and R-R interval (*see the red arrow*). Similarly, other anomalies can be successfully detected in each R-R interval in the signal as shown in ***Table II.***

TABLE II
ANOMALY ECG DETECTION

R-R Interval Number	Anomaly/ Anomalies
1-2	Bradycardia
3-15	Bradycardia, Arrhythmia
16	Bradycardia, Arrhythmia, First Degree AV Block
17-40	Bradycardia, Arrhythmia

III. EXPERIMENTS AND RESULTS

A. Experimental Setup

All experiments were in compliance with the Institutional Animal Care and Use Committee (IACUC) protocols (#4389-01 at University of Washington and #AUP-18-115 at University of California, Irvine) to minimize the stress to animals. Adult zebrafish are considered under full anesthesia (100%) when they are treated with a buffer solution containing 150-200 mg/l *tricaine methane sulfonate* (Tricaine). An open chest surgery was conducted to form an incision as described in [13] and then the fish would be ready for recording up to several weeks.

Four different zebrafish were simultaneously measured in a 4-chamber apparatus with Tricaine concentration of 20%, 40%, 60% and 80%, respectively. The pairs of electrodes were connected to a differential amplifier (*A-M Systems Inc. 1700 Differential Amplifier, Carlsborg, WA*) with a gain of 10,000. The bandpass filter was set from 0.1 Hz to 500 Hz and a notch filter (60 Hz) also was used. The filtered signals were then digitized at a sampling rate of 1,000 Hz (*National Instruments USB-6251 DAQ device, Austin TX, and LabVIEW 2017*) before being logged, displayed and analyzed using our LabView GUI.

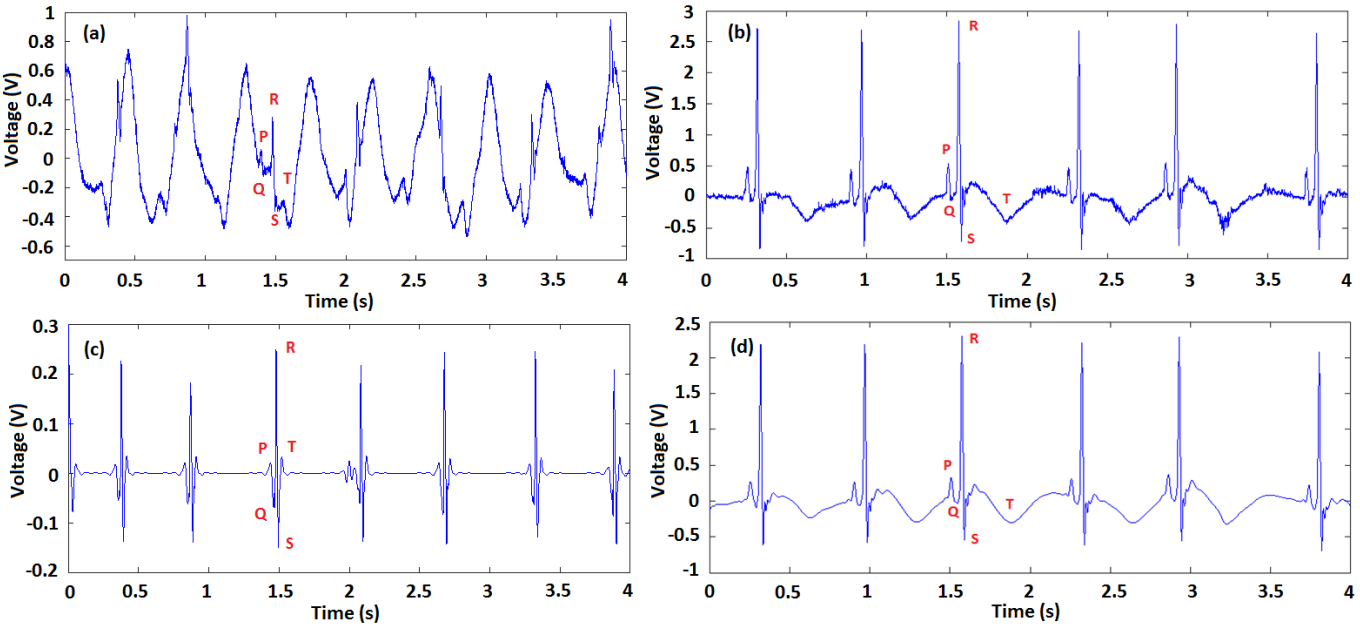

Fig. 5. Zebrafish ECG signals obtained by our novel PDMS housing: (**a**) and (**b**) the measured signals at 20% and 60%, respectively. (**c**) and (**d**) the filtered signals using Wavelet transform of those, respectively.

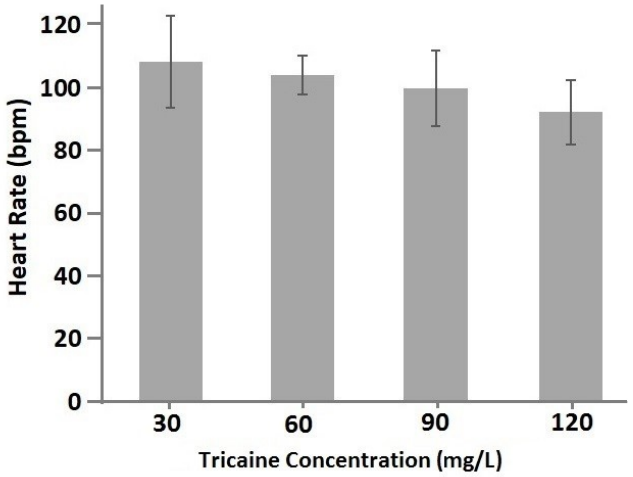

Fig. 6. The heart rate of zebrafish at different Tricaine concentrations.

B. ECG recording with PDMS housing

Fig. 5 shows the ECG signals collected using our apparatus with zebrafish treated at 20% (**5a**) and 60% (**5b**) of Tricaine. A moderate Wavelet de-noising scheme was used to compare among cases, and the filtered signals are plotted in **5c** and **5d,** respectively. We found that when the fish were treated with low concentrations (20% and 40% of Tricaine), the ECG signal was dominated by low-frequency noise due to the gill motion (**Fig. 5a**). In contrast, the fish under 60% and 80% Tricaine provided clear ECG signals with full features (**Fig. 5b**). The effect of anesthesia on cardiac phenotypes of zebrafish was quantified by measuring HR under four different doses of 20%, 40%, 60% and 80% Tricaine. We used 4 subjects for each concentration (n=4). The average HR and error bars were then used to plot and compare (**Fig. 6**). It was found that the lower the Tricaine concentration, the higher HR observed in zebrafish. Specifically, the maximum HR was found with fish treated at 20% Tricaine (30 mg/L), resulting in HR of ~115 BPM. Zebrafish treated with 40%, 60% and 80% Tricaine (60, 90, 120 mg/L) showed HR at ~107 BPM, ~100 BPM, and ~90 BPM, respectively. **Fig. 7** illustrates the SNR of signals corresponded with different Tricaine concentrations. It can be seen that as the Tricaine concentration increases, higher SNR values are achieved. For instance, fish treated with 20% Tricaine (30 mg/L) had SNR of ~30 while SNR values of fish under 60% and 80% Tricaine were approximate 49 and 58 respectively. This could be explained as high Tricaine levels would sedate the fish more, thereby mitigating motion artifacts. In addition, we also characterized the percentage of valid or acceptable ECG data obtained from experiments. Specifically, we collected ECG signals from 4 control subjects in about 1.5 minutes with 5 trials for each fish at each Tricaine concentration. Those signals are considered as valid or acceptable if the QRS complex is clear. For instance, the zebrafish EGG signal as shown in **Fig. 5a**, despite treated by low Tricaine concentrations (*e.g.*, 30 mg/L and 60 mg/L), the QRS complex is still apparent. **Table III** depicts the percentage of acceptable data over total data in 4 different concentrations. It is obvious that the lowest acceptable rate is found with data obtained at the Tricaine level of 30 mg/L with 60 % of usable data. The usable data from other levels are above 80% and the highest one belongs to the group treated with 120 mg/L or 90 % Tricaine.

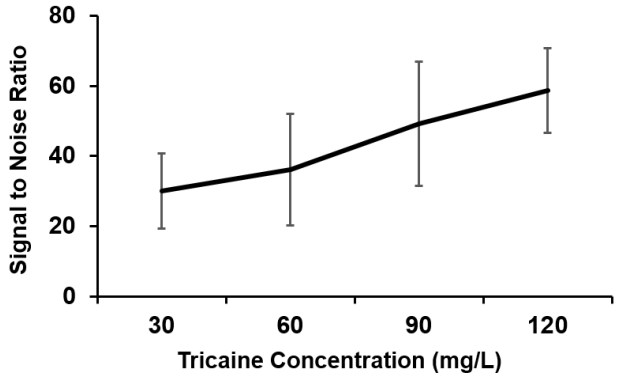

Fig. 7. SNR obtained at different Tricaine concentrations.

TABLE III
THE COMPLETENESS OF FISH DATA MEASUREMENT

Tricaine concentration (mg/L)	The percentage of acceptable data (%)
30	60
60	83
90	87
120	90

IV. DISCUSSION

After conducting various experiments using our novel apparatus, we noticed that the quality of ECG signal is significantly affected by the "*cooperation level*" of the fish. For instance, unexpected strong movement or electrode dislocation due to fish irritation will cause glitches. While mild Tricaine was used to partially sedate the fish, the higher concentrations may interfere the intrinsic ECG significantly. With the lower ones, there will be significant noise in the obtained ECG due to the gill motions. Since the noise is inconsistent and unpredictable, it would be challenging to establish an efficient processing scheme for our LabView program. In the future, we may develop some mild suction force to hold the fish in place, in order to guarantee consistent contacts of the fish body with the recording and reference electrodes.

Currently, the ECG signal is acquired by the A-M system via long-connecting wires. Though all cables were shielded, it still caused complications and high-level noise due to coupling effects. Therefore, we have been developing the next-generation apparatus with embedded electronics and Bluetooth Low Energy (BLE) communication so that data can be sent wirelessly to the GUI connected with a cloud server. By doing so, we significantly eliminate the use of wires in the system, and thus enhancing the signal to noise ratio (SNR). From our past work with the 2-electrode system [11], we revealed that artifacts due to the gill motion are considerable with awake fish thus we would use a 3rd electrode being immersed in the

chamber solution. In fact, when we deployed the ground of the A-M System 1700 as a "pseudo 3rd electrode" and shielded metallic parts, it showed improvement with steady waveforms.

We will also consider establishing cloud connection, storage and computing and implement advanced pattern recognition methods via machine learning [12, 20]. For cloud-based computing, once the LabView program is integrated with this function, the computational time will be significantly reduced, and the processed data can be stored in the cloud, which enables other peripherals such as mobile phone and/or smart devices connected with the Internet to access the data. This integration can be done using the "LabVIEW Cloud Toolkit" [21] which is designed to allow programs developed within LabVIEW to integrate Amazon Web Services data storage and computing. The implementation of machine learning to the current program would help improve analysis and diagnostic capabilities. A LabVIEW Analytics and Machine Learning Toolkit [22] introduced by *National Instruments* is the tool that allows integration of predictive analytics and machine learning algorithms.

Finally, the effect of different levels of anesthesia on HR was revealed. Therefore, we will perform thorough investigations with a large number of fish in order to determine the correlation of the Tricaine concentration as well as ambient temperature with zebrafish cardiac electrophysiology. Machine learning-based programs would significantly contribute to this process. Once done, we can incorporate the correlation factors to our LabView GUI so the measurement data can be automatically normalized accordingly based on additional input parameters, such as Tricaine concentrations and temperature.

V. Conclusion

In conclusion, we have successfully demonstrated a novel system with real-time acquisition, processing and analysis of awake zebrafish ECG. The system was developed successfully, enabling promising opportunities to reveal underlying mechanisms of zebrafish cardiac electrophysiology in numerous studies. Furthermore, it also provides an efficient way to collect large-scale data, providing the chance for data mining, big-data processing and interpretation. Last but not least, our system holds translational implications to realize integrated systems, for both animal models and humans, supporting health monitoring and biological investigations.

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